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Influence of *CYP2D6* Polymorphism on the Pharmacokinetic/Pharmacodynamic Characteristics of Carvedilol in Healthy Korean Volunteers

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ABSTRACT

Background: Carvedilol is commonly used to treat hypertension as a β- and α_1-adrenoreceptor blocker, but it is metabolized by *CYP2D6,* and *CYP2D6*10* allele is dominant in Asian population. The objective of this study was to assess the influence of *CYP2D6* polymorphisms on the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of carvedilol in healthy Korean volunteers.
Methods: A PK/PD study for a single and multiple dosing of carvedilol were conducted. All volunteers in 3 genotypic groups received single oral dose of carvedilol 12.5 mg for 3 days, then 25 mg QD for 5 days, and 12.5 mg QD for another 3 days. PK parameters for carvedilol and its three metabolites were determined using non-compartmental analysis. For PD properties, blood pressure, heart rate, and the chronotropic dose 25 (CD25) value were obtained.
Results: The IM_2 group with two **10* alleles (intermediate metabolizers) exhibited lower clearance of carvedilol as well as higher area under the curve (AUC) for O-desmethyl carvedilol. The ratio of CD25 to baseline at multiple dosing was significantly higher in the combined IM group (IM_1 and IM_2) than in the EM group, however, the ratio of CD25 after single and multiple dosing and the other PD markers were not significantly different between the 3 genotypic groups compared with the baseline.
Conclusion: These findings showed that *CYP2D6* genotype influenced the PK characteristics of carvedilol and no differences in PD response were observed in Korean healthy volunteers.

Trial Registration: ClinicalTrials.gov Identifier: NCT02286934

Keywords: Carvedilol; CYP2D6; Genetic Polymorphisms; Pharmacokinetic/Pharmacodynamic; Korean Population

INTRODUCTION

Carvedilol is a β- and α_1-adrenoreceptor blocker, and is currently indicated for the treatment of hypertension as well as chronic heart failure, angina pectoris, and cardiac arrhythmias.[1,2]

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Disclosure
The authors have no potential conflicts of interest to disclose.

Author Contributions
Conceptualization: Seo DW, Lee J, Jeong HS, Kim JM. Data curation: Jung E, Lee J. Formal analysis: Jung E, Lee J. Investigation: Jung E, Ryu S, Park Z, Lee JG, Yi JY, Oh WY. Methodology: Jung E, Ryu S, Park Z, Lee JG, Yi JY, Oh WY. Writing - original draft: Jung E. Writing - review & editing: Jung E, Ryu S, Oh WY.

It is administered as a racemic mixture of the *R*(+) and *S*(−) enantiomers. The enantiomers mediate α_1-blockade, but only the *S*(−) enantiomer mediates β-adrenoreceptor blockade.[3,4] Orally administered carvedilol undergoes stereoselective first-pass metabolism and the plasma concentration of the *R*(+) enantiomer, which has α_1-blocking activity, is approximately 3-fold higher than that of the *S*(−) enantiomer, which has β-blocking activity, in healthy subjects.[5]

Carvedilol is mainly metabolized in the liver, with renal elimination accounting for only 0.3% of the administered dose, and is mostly converted to various active metabolites by *CYP2D6* and *CYP2C9* enzymes.[6] The active metabolites of carvedilol identified in humans are O-desmethyl carvedilol (ODMC), 4′-hydroxyphenyl carvedilol (4OHC), and 5′-hydroxyphenyl carvedilol (5OHC) (**Fig. 1**).[7] Among these active metabolites, only 4OHC appears to contribute to the β-blocking activity of carvedilol based on its relative potency and observed plasma concentration.[8]

Several studies have shown that *CYP2D6* polymorphisms induce individual variations in the ability to metabolize carvedilol. Honda et al.[9] examined the effect of the *CYP2D6* allele on carvedilol pharmacokinetics (PKs) in healthy Japanese volunteers. The clearance (CL) and volume of distribution (V_d) in Japanese subjects with the *CYP2D6*10* allele (*CYP2D6*1/*10*, **2/*10*, **10/*10* genotypes) were significantly lower than those of Japanese subjects with the *CYP2D6*1/*1* or **1/*2* genotypes. These results indicated that the metabolism of carvedilol in the liver is significantly lower in Japanese individuals with the *CYP2D6*10* allele. Similarly,

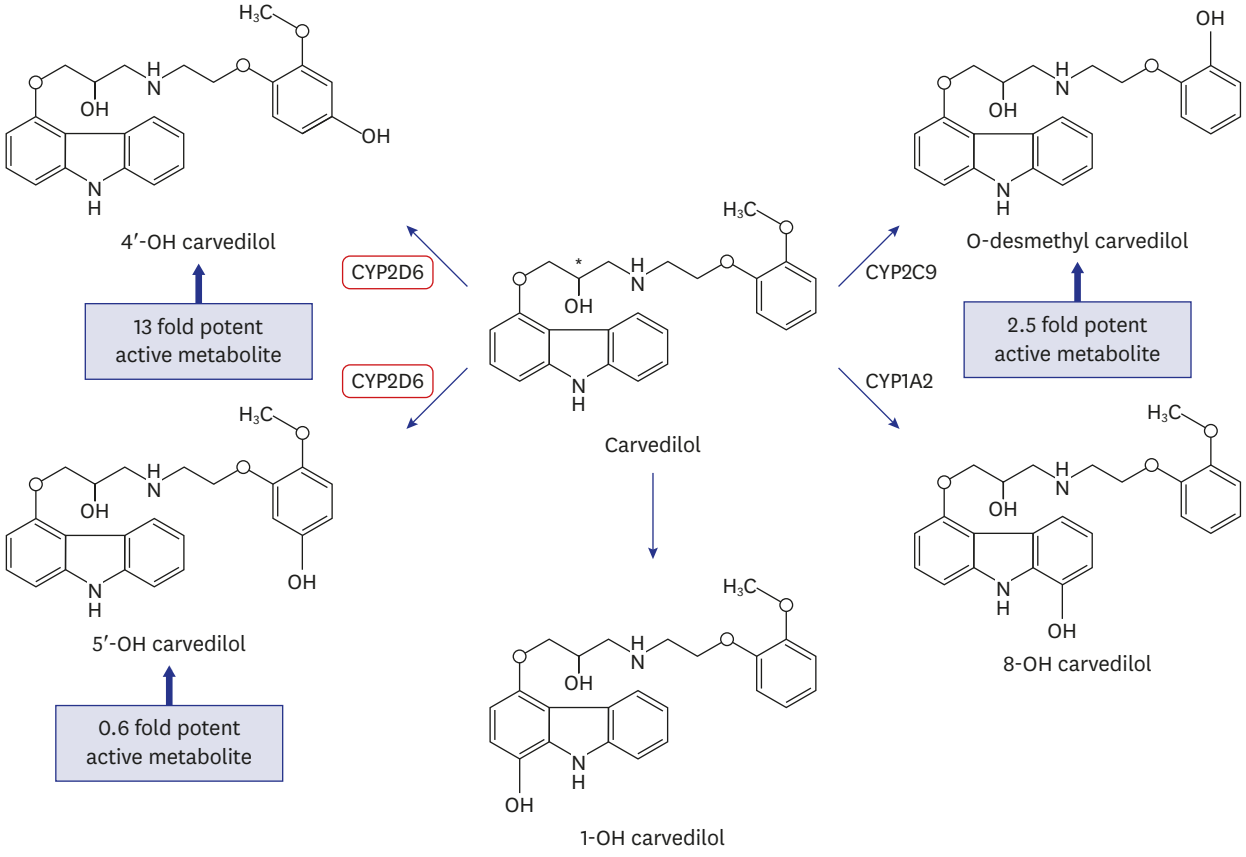

Fig. 1. Structure of carvedilol and its metabolites.

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Saito et al.[10] reported the effect of *CYP2D6* polymorphisms on the population PK of carvedilol in Japanese patients with chronic heart failure (CHF). They showed that the individual oral clearance (CL/F) values for carvedilol were significantly lower in patients with the *CYP2D6* **1/*5*, **5/*10*, and **10/*10* genotypes compared to those with *CYP2D6* **1/*1*, **1/*10*. In a Korean study, Lee et al.[11] assessed *CYP2D6* genotypes in different ethnic groups including the Korean population. The frequency of the *CYP2D6*10* and *CYP2D6*5* genes was 45.00% and 6.13% in 400 Korean subjects and was 43.00% and 4.50% in 206 Japanese subjects.[12] *CYP2D6*1*, **2*, and **10* are the most common *CYP2D6* alleles among extensive metabolizers (EMs) and intermediate metabolizers (IMs) of carvedilol in Asian populations,[13,14] whereas poor metabolizers (PMs) of carvedilol with *CYP2D6*5* alleles comprise less than 1% of the Asian population.[15] Since the frequency of the *CYP2D6*10* allele is high in both Koreans as well as in the overall Asian population, it is necessary to investigate the effect of *CYP2D6*10* genetic polymorphisms on carvedilol and its metabolites in healthy Korean volunteers.

It was reported that there are differences in the pharmacodynamic (PD) properties of carvedilol according to the *CYP2D6* genotypes present. The change in the heart rate (HR) of PMs and EMs with *CYP2D6* was not statistically significant compared with the baseline, but the systolic blood pressure (SBP) of EMs was statistically significantly decreased compared with that of PMs after multi-dose administration of carvedilol in healthy Caucasian subjects.[16] On the other hand, a cohort study of patients with CHF showed that the *CYP2D6* genotypes had no significant effect on the clinical efficacy of carvedilol.[17]

Based on the results of other studies, we investigated the effects of *CYP2D6* genetic polymorphisms on the PK of carvedilol as well as the effects on an individual's response to the drug by evaluating HR, SBP, diastolic blood pressure (DBP), and isoproterenol sensitivity following the initial and subsequent doses of carvedilol in the Korean population.

METHODS

Subjects

Healthy Korean men, aged 20–45 years, with a body mass index (BMI) between 18 and 27 kg/m^2 were enrolled in the study after a medical screening and were separated into 3 *CYP2D6* genotypic groups. Subjects were excluded if they showed clinical evidence of pulmonary, renal, hepatic, cardiovascular, hematologic, endocrine, gastrointestinal, central nervous system, and/or any other acute disease or alcoholism. During screening, subjects with HR less than 50 beats per minute, SBP greater than 140 mmHg or less than 90 mmHg, and DBP greater than 90 mmHg or less than 50 mmHg were also excluded. No subjects were taking any concomitant medication 2 weeks before the study or during the study period. Only male subjects were included in this study because it was difficult to recruit female subjects. All procedures were performed in compliance with the Declaration of Helsinki, and were consistent with Korean Good Clinical Practice guidelines.

All subjects were classified into genotypic groups based on *CYP2D6* alleles, which were determined by the number of *CYP2D6* functional alleles carried by each individual.[18] EMs carried 2 wild type alleles, **1* and **2*, with normal enzymatic activity including *CYP2D6* **1/*1* and **1/*2*. The second group was the IMs with deficient allele (**10*)[19] and this group was divided into 2 subgroups, IM_1 and IM_2 group. IM_1 (**1/*10*, **2/*10*) was the presence of one deficient allele with wild type allele and IM_2 (**10/*10*) had two deficient alleles.

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Study design and data collection

An open-label trial and a PK/PD study for a single dose and multiple doses of carvedilol were conducted. All subjects received oral administration of 12.5 mg of carvedilol tablet (Dilatrend®; Chong Kun Dang Pharm., Seoul, Korea) once daily for the first three days (Days 1–3) and then 25 mg for 5 days (Days 4–8). It has been adapted for a certain period of time for the increase, and the occurrence of adverse reactions can be prevented. In addition, 12.5 mg was given for 3 days (Days 9–11) to prevent the adverse reaction due to sudden stoppage of medication even after the end of the study. The subjects were repeatedly discharged and hospitalized during the study. The profile of the study design is shown in **Fig. 2**.

Blood samples (7 mL) of carvedilol and its 3 metabolites were collected immediately before dosing, then 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after the administration of carvedilol on day 1 and day 8, and stored at −70°C. For PD measurements, blood pressure and HR were measured immediately before dosing and 1, 2, 3, 4, 6, 8, and 12 hours after administration on days 0, 1, and 8. An isoproterenol sensitivity test (IST) was also conducted 1.5 hours after carvedilol dosing on days 0, 1, and 8. The isoproterenol dose versus dose-increase curve is plotted using the highest HR for each dose compared to before dosing. In this curve, the dose of isoproterenol, which increases the HR by 25 beats per minutes (chronotropic dose 25 [CD25]), was calculated using a linear regression.[20] Demographic information for each subject was also collected.

Genotyping analysis

Genomic DNA from the blood samples of subjects was extracted and amplified with a polymerase chain reaction (PCR) to analyze the flanking regions using forward and reverse primers and standard PCR reagents. The genotypes of the *CYP2D6* single nucleotide polymorphisms (SNPs) (**2*; Rs16947 and **10*; Rs1065852) were screened by application of a single base extension assay using the ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA).

Determination of carvedilol and its metabolites

An UPLC-MS/MS method was developed to determine carvedilol, its metabolites (4OHC, 5OHC, ODMC) in human plasma using their deuterated internal standard (IS). Liquid-liquid extraction was used for the plasma sample preparation. Chromatographic separation was achieved on UPLC CSH Phenyl column (100 mm × 2.1 mm, 1.7 μm) using 0.1% formic acid in water, 0.1% formic acid in acetonitrile with gradient mobile phase at a flow rate of 0.4

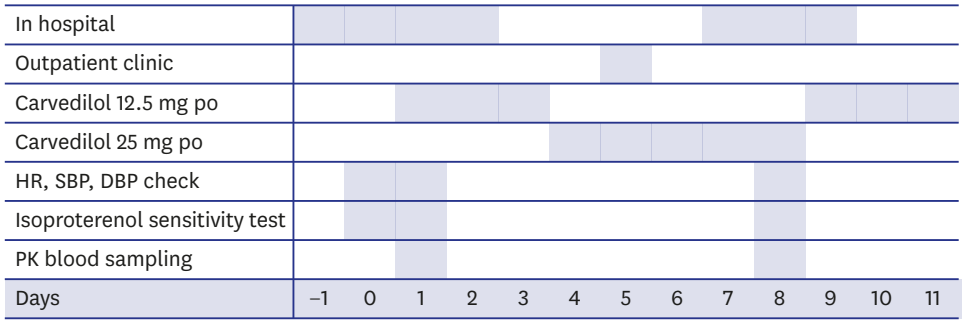

Fig. 2. Study design. The procedures were performed on the days indicated in grey color.
HR = heart rate, SBP = systolic blood pressure, DBP = diastolic blood pressure, IST = isoproterenol sensitivity test.

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mmL/min. A UPLC system (Waters ACQUITY; Waters Corp., Milford, MA, USA) with mass spectrometer (Waters XEVO TQ MS; Waters Corp.) was used. The detection was carried out on a triple quadrupole mass spectrometer with an electrospray ionization source in the positive ion mode. The mass transitions of carvedilol, 4OHC, 5OHC, ODMC monitored in multiple reaction monitoring (MRM) were *m/z* 407.35→100.15, *m/z* 423.30→100.20, *m/z* 423.32→100.15, and *m/z* 393.35→210.25, respectively. The linearity was validated over the analyzed concentration range of 0.05–100 ng/mL for carvedilol and 0.1–100 ng/mL for its metabolites with a lower limit of quantification of 0.5 and 0.1 ng/mL, respectively.

PK/PD and statistical analysis

PK parameters for carvedilol and 3 metabolites were determined with non-compartmental analysis using WinNonlin software (Pharsight Corporation, Mountain View, CA, USA). For the analysis, plasma concentrations below the lower limit of quantitation were excluded. The following PK parameters were calculated from the plasma concentration-time profile after the initial dose (day 1) and at steady-state (day 8) for carvedilol and its metabolites: AUC_{last}, C_{max}, CL/F, Vd/F, $T_{1/2}$ for carvedilol, and AUC_{last}, C_{max}, MR_AUC_{last} (metabolite ratio [MR]), MR_C_{max} for the metabolites. The area under the plasma concentration-time profile from time 0 to 12 or 24 hours (AUC_{last}) was calculated according to the linear trapezoidal rule. C_{max} was determined directly from the concentration-time profile.

For PD analysis, changes in HR, SBP, and DBP were monitored and compared between the baseline (day 0) and after the single dose (day 1), and between the baseline and after multiple doses (day 8, steady-state). Using the results of the IST, CD25 values were obtained for each subject on each day (days 0, 1, 8) and the ratios for the values were calculated ($CD25_{day1}$/$CD25_{day0}$, $CD25_{day8}$/$CD25_{day0}$, $CD25_{day8}$/$CD25_{day1}$). These PK and PD parameters were evaluated to compare the differences between the 3 genotypic groups (EM: extensive metabolizers, IM_1 and IM_2: intermediate metabolizers).

PK and PD parameters were analyzed using SigmaStat 2.03 (Jandel Scientific GmbH, Erkrath, Germany) and the statistical programming language R version 3.2.3 (R Foundation, Vienna, Austria). Differences in the parameters between the 3 genotypic groups were assessed by using the Kruskal-Wallis test, Fisher's exact test, and Man-Whitney U test. The results presented as the mean ± standard deviation or geometric mean ratio (90% confidence interval [CI]). For the statistical test, P value < 0.05 was considered to be significant.

Safety assessment

Safety profile was assessed throughout the study and the assessment included adverse events, physical examinations, vital signs, clinical laboratory parameters, electrocardiographs, and continuous EKG monitoring. The serious adverse events and adverse drug reactions (ADR) experienced until 30-day period following the last dose were reported and evaluated based on the associations with the drug treatment.

Ethics statement

The present study protocol was reviewed and approved by the Institutional Review Board of Ethics Committee of Seoul National University (IRB No. B-1408/263-008). Informed consent was submitted by all subjects when they were enrolled. This clinical trial was registered at a service of the U.S. National Institute of Health (http://clinicaltrials.gov), number NCT02286934.

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RESULTS

Baseline characteristics of subjects

Of the 21 healthy men enrolled in the study, 6 were in the *CYP2D6* EM group (**1/*1*, **1/*2*), 7 were in the IM_1 group (**1/*10*, **2/*10*), and 8 were in the IM_2 group (**10/*10*). The demographics and clinical characteristics of the subjects are shown in **Table 1**. The mean age was 27.3 years, and the mean BMI was 23.1 kg/m^2 with a mean weight and height were 70.4 kg and 174.2 cm, respectively. For baseline characteristics the mean HR was 62.1 beats/min and the mean SBP and DBP were 116.2 and 70.9 mmHg, respectively. In addition, screening test confirmed that baseline of albumin, total bilirubin, aspartate transaminase (AST) and alanine transaminase (ALT), which are indicators of liver function as well as baseline of HR, SBP, and DBP, which are PD markers, are within normal range. Baseline characteristics of the subjects including age, height, HR, SBP, DBP, albumin, total bilirubin, AST, and ALT were similar among the *CYP2D6* genotypic groups, but BMI, and weight showed significant differences between the groups.

PK and PD

The mean plasma concentration-time profiles for carvedilol, ODMC, 4OHC, and 5OHC are shown in **Fig. 3**. PK parameters for carvedilol and its metabolites following single and multiple dosing were calculated using non-compartmental analysis. The differences between the IM_1, IM_2 groups and the EM group were evaluated by calculating the geometric mean ratio (GMR, IM_1/EM, IM_2/EM) and 90% CI at a significance level of 0.05 (**Table 2**). When the 90% CI was included in the equivalence range (80%–125%), no difference between the IM_1, IM_2 groups and EM group was assessed. In the PK parameters of carvedilol, there was no significant difference between EM and IM_1 groups after the single dosing (day 1), but the GMR of CL/F was lower in the IM_2 group than in the EM group. Regarding the GMRs of PK parameters in ODMC for the IM_2 group, the AUC_{last} and C_{max} were significantly increased on day 1 and MR_AUC_{last} (AUC_{last} for ODMC/AUC_{last} for carvedilol) and MR_C_{max} (C_{max} for ODMC/C_{max} for carvedilol) also increased after multiple dosing (day 8) compared with those of the EM group, respectively. The GMRs of AUC_{last} and C_{max} for 4OHC showed no significant

Table 1. Demographics and clinical characteristics of the subjects

Characteristics	Total	EM	IM_1	IM_2	*P* value[a]
No. of subjects	21	6	7	8	-
Age, yr	27.3 ± 4.7	28.5 ± 3.5	29.6 ± 6.1	24.4 ± 2.7	0.07
BMI, kg/m^2	23.1 ± 2.4	25.8 ± 1.1	22.7 ± 2.3	21.6 ± 1.4	< 0.01
Weight, kg	70.4 ± 9.6	79.3 ± 3.9	69.5 ± 10.3	64.5 ± 7.4	0.01
Height, cm	174.2 ± 6.6	175.5 ± 4.1	174.9 ± 9.9	172.6 ± 4.9	0.59
HR[b], beats/min	62.1 ± 9.1	65.3 ± 6.3	58.6 ± 11.2	62.9 ± 9.0	0.36
SBP[b], mmHg	116.2 ± 9.0	117.8 ± 7.9	119.6 ± 10.9	112.0 ± 7.3	2.04
DBP[b], mmHg	70.9 ± 6.6	70.7 ± 7.6	72.3 ± 7.6	69.9 ± 5.7	0.70
Albumin[b], g/dL	4.7 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	0.26
Total bilirubin[b], mg/dL	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	0.9 ± 0.3	0.70
AST[b], IU/L	20.0 ± 6.9	24.0 ± 11.7	19.6 ± 3.4	17.4 ± 2.1	0.43
ALT[b], IU/L	23.2 ± 12.2	33.5 ± 16.8	20.4 ± 7.7	17.9 ± 6.4	0.23
No. of smokers	9	2	2	5	0.47[c]

The data presented as mean ± standard deviation.
BMI = body mass index, HR = heart rate, SBP = systolic blood pressure, DBP = diastolic blood pressure, AST = aspartate transaminase, ALT = alanine transaminase, EM = extensive metabolizer (*CYP2D6*1/*1*, **1/*2*), IM_1 = intermediate metabolizer (*CYP2D6*1/*10*, **2/*10*), IM_2 = intermediate metabolizer (*CYP2D6*10/*10*).
[a]Kruskal-Wallis test; [b]Baseline data; [c]Fisher's exact test.

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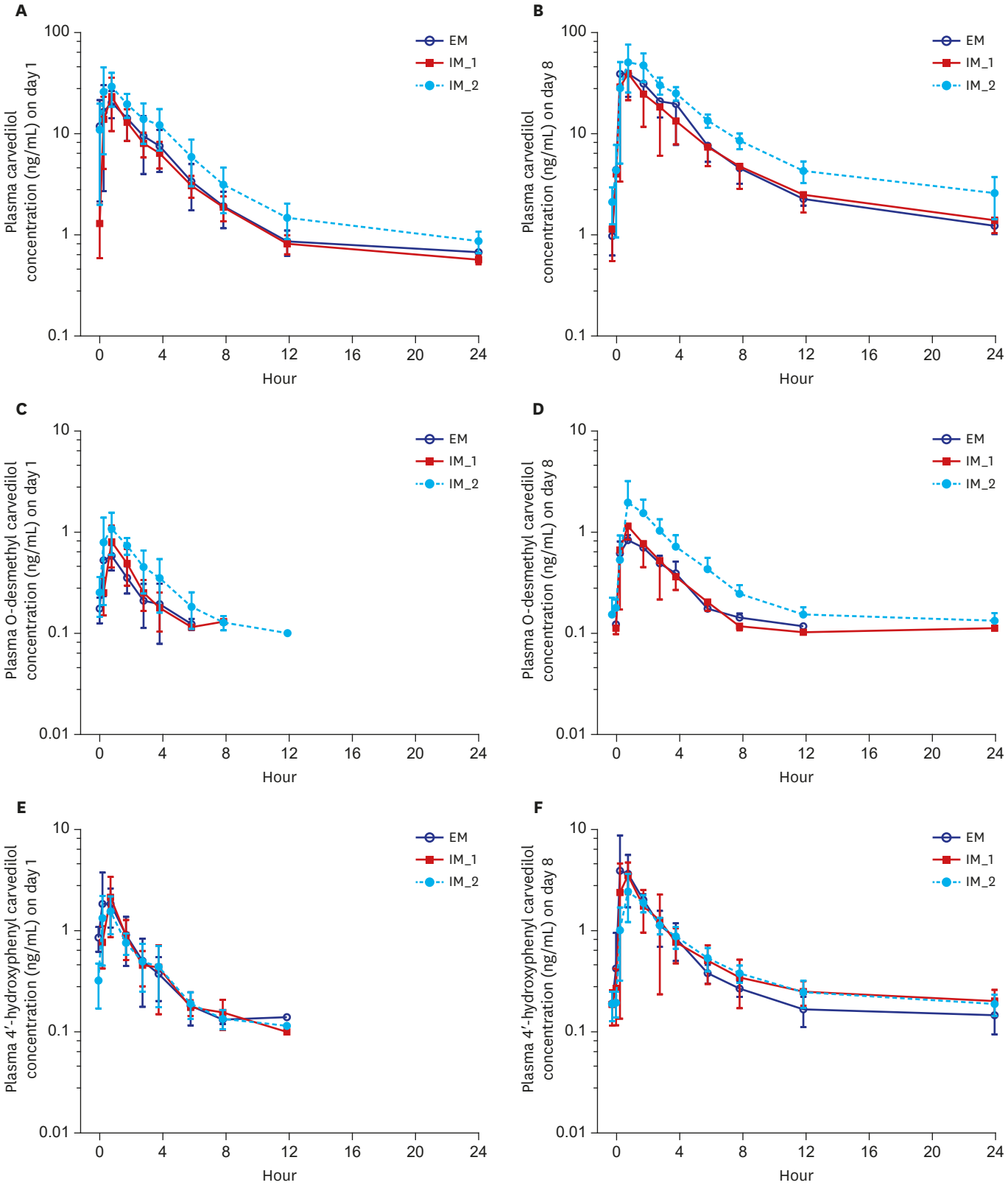

Fig. 3. PK profiles of carvedilol and its metabolites according to *CYP2D6* genotypic groups. Plasma concentration of carvedilol, 4-OH carvedilol, 5-OH carvedilol, and O-desmethyl carvedilol after initial dosing (**A**, **C**, **E**, **G**) and multiple dosing (**B**, **D**, **F**, **H**).
EM = extensive metabolizer (*CYP2D6*1/*1, *1/*2*), IM_1 = intermediate metabolizer (*CYP2D6*1/*10, *2/*10*), IM_2 = intermediate metabolizer (*CYP2D6*10/*10*).

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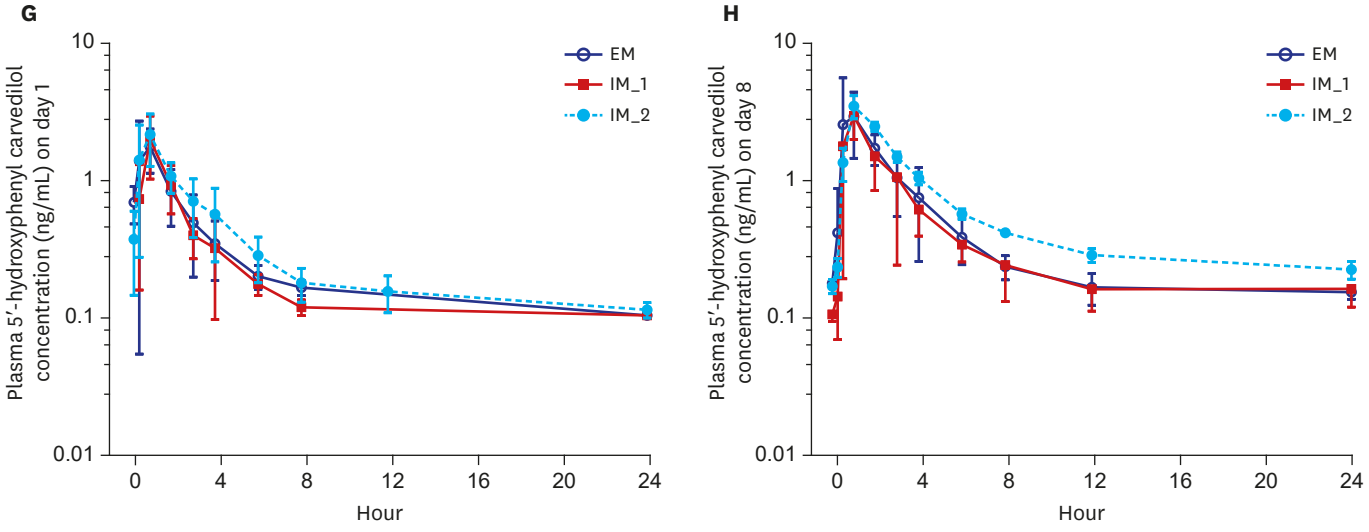

Fig. 3. (Continued) PK profiles of carvedilol and its metabolites according to *CYP2D6* genotypic groups. Plasma concentration of carvedilol, 4-OH carvedilol, 5-OH carvedilol, and O-desmethyl carvedilol after initial dosing (**A**, **C**, **E**, **G**) and multiple dosing (**B**, **D**, **F**, **H**).
EM = extensive metabolizer (*CYP2D6*1/*1, *1/*2*), IM_1 = intermediate metabolizer (*CYP2D6*1/*10, *2/*10*), IM_2 = intermediate metabolizer (*CYP2D6*10/*10*).

Table 2. Geometric mean ratios and 90% CI of PK parameters for carvedilol and its metabolites in the IM_1 and IM_2 groups compared to the EM group following single and multiple dosing

Parameters	IM_1/EM		IM_2/EM	
	Single dosing	Multiple dosing	Single dosing	Multiple dosing
Carvedilol				
AUC_{last}	0.96 (0.72–1.28)	0.87 (0.66–1.14)	1.64 (1.24–2.15)	1.49 (1.14–1.95)
C_{max}	1.00 (0.66–1.49)	0.87 (0.56–1.35)	1.48 (1.00–2.19)	1.13 (0.74–1.73)
CL/F	1.02 (0.76–1.35)	1.08 (0.83–1.40)	0.60 (0.45–0.79)[a]	0.64 (0.49–0.82)
V_d/F	1.38 (0.87–2.20)	1.20 (0.79–1.83)	0.99 (0.63–1.55)	0.65 (0.43–0.97)
ODMC				
AUC_{last}	1.13 (0.87–1.48)	0.92 (0.68–1.25)	2.22 (1.74–2.93)[a]	1.48 (1.10–2.00)
C_{max}	1.32 (0.95–1.84)	0.89 (0.58–1.36)	1.83 (1.33–2.52)[a]	1.09 (0.72–1.66)
MR_AUC_{last}	1.18 (0.88–1.58)	1.35 (0.99–1.84)	1.38 (1.04–1.84)	1.71 (1.27–2.31)[a]
MR_C_{max}	1.33 (0.98–1.80)	1.43 (1.05–1.96)	1.24 (0.92–1.67)	1.79 (1.32–2.42)[a]
4OHC				
AUC_{last}	0.92 (0.64–1.34)	0.97 (0.67–1.41)	1.01 (0.70–1.45)	1.01 (0.71–1.45)
C_{max}	0.96 (0.57–1.63)	0.83 (0.52–1.34)	0.83 (0.49–1.38)	0.63 (0.39–1.00)
MR_AUC_{last}	0.96 (0.73–1.26)	1.12 (0.82–1.54)	0.62 (0.48–0.80)	0.68 (0.50–0.92)
MR_C_{max}	0.97 (0.72–1.29)	0.96 (0.76–1.22)	0.56 (0.42–0.74)[a]	0.56 (0.44–0.70)[a]
5OHC				
AUC_{last}	0.96 (0.70–1.30)	0.92 (0.68–1.25)	1.54 (1.14–2.08)	1.48 (1.10–2.00)
C_{max}	0.99 (0.70–1.55)	0.89 (0.58–1.36)	1.18 (0.77–1.82)	1.09 (0.72–1.66)
MR_AUC_{last}	0.99 (0.76–1.30)	1.06 (0.83–1.35)	0.94 (0.73–1.22)	0.99 (0.78–1.25)
MR_C_{max}	1.00 (0.77–1.30)	1.02 (0.83–1.54)	0.80 (0.62–1.03)	0.97 (0.79–1.19)

The data presented as geometric mean ratio and 90% confidence interval at a significance level of 0.05. Geometric mean ratio (90% CI).
CI = confidence interval, PK = pharmacokinetic, IM_1 = intermediate metabolizer (*CYP2D6*1/*10, *2/*10*), IM_2 = intermediate metabolizer (*CYP2D6*10/*10*), EM = extensive metabolizer (*CYP2D6*1/*1, *1/*2*), ODMC = O-desmethyl carvedilol, 4OHC = 4′-hydroxyphenyl carvedilol, 5OHC = 5′-hydroxyphenyl carvedilol, MR = metabolic ratio, AUC_{last} = area under the plasma concentration-time curve from time zero to last quantifiable concentration, C_{max} = maximum observed plasma concentration, CL/F = apparent clearance, V_d/F = apparent volume of distribution, $T_{1/2}$ = half-life.
[a]It is not included in the 90% CI at a significance level of 0.05.

difference among the 3 genotypic groups, whereas GMR of MR_C_{max} was lower in the IM_2 group than in the EM group on day 1 and 8. All the GMRs of the PK parameters for 5OHC were not significant in both the single and multiple dosing.

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Table 3. Changes in mean and standard deviation of HR, SBP, and DBP over time between single (day 1) and multiple dosing (day 8) for baseline (day 0)

Time, hr	Day 1–Day 0			Day 8–Day 0		
	EM (n = 6)	IM_1 (n = 7)	IM_2 (n = 8)	EM (n = 6)	IM_1 (n = 7)	IM_2 (n = 8)
HR						
1	3.15 ± 9.14	−1.63 ± 9.16	−0.38 ± 7.50	−3.17 ± 5.58	−3.86 ± 9.51	−4.25 ± 10.01
2	2.29 ± 8.19	−5.75 ± 4.26	−0.75 ± 6.67	−0.84 ± 2.97[a]	−6.58 ± 3.54[a]	−1.25 ± 6.14[a]
3	−4.58 ± 4.98	−6.50 ± 7.87	−7.63 ± 7.81	−1.17 ± 5.98	−9.43 ± 6.99	−9.75 ± 12.26
4	−3.72 ± 5.77	−2.25 ± 6.32	−2.50 ± 6.56	−7.50 ± 3.86	−10.58 ± 5.75	−7.00 ± 9.50
6	−1.72 ± 7.46	−2.50 ± 8.77	4.50 ± 8.11	−10.34 ± 5.79[a]	−12.29 ± 4.56[a]	0.13 ± 7.22[a]
8	−1.15 ± 6.77	−4.00 ± 7.42	0.50 ± 9.21	−6.34 ± 8.86	−6.86 ± 7.88	−3.63 ± 15.53
12	−1.29 ± 5.01	−0.25 ± 4.99	0.75 ± 11.2	−3.00 ± 6.19	−3.29 ± 7.44	−5.38 ± 8.59
SBP						
1	0.58 ± 5.31	−6.13 ± 8.13	−9.50 ± 6.52	−5.17 ± 5.67	−12.29 ± 6.13	−4.50 ± 5.00
2	−13.58 ± 8.68	−12.13 ± 6.21	−6.38 ± 7.45	−10.34 ± 7.54	−15.43 ± 8.67	−10.63 ± 4.95
3	−13.58 ± 7.67	−10.88 ± 7.10	−7.38 ± 6.00	−16.50 ± 10.00	−10.43 ± 5.04	−11.75 ± 10.30
4	−4.29 ± 7.70	−7.13 ± 7.22	−10.13 ± 4.81	−11.00 ± 9.09	−14.15 ± 13.4	−4.88 ± 7.83
6	−6.43 ± 11.37	−3.88 ± 10.52	−3.88 ± 9.64	−6.00 ± 6.53	−8.29 ± 5.72	−5.00 ± 8.47
8	−7.43 ± 9.36	−9.50 ± 10.50	−1.88 ± 7.08	−12.34 ± 10.10	−11.29 ± 10.90	−1.88 ± 10.20
12	3.29 ± 6.73	1.50 ± 8.86	2.00 ± 11.26	−1.84 ± 7.08	−5.15 ± 6.81	−3.50 ± 7.14
DBP						
1	0.43 ± 5.58[a]	−8.50 ± 7.05[a]	−7.50 ± 4.21[a]	−3.84 ± 9.15	−10.29 ± 4.86	−6.63 ± 4.82
2	−5.43 ± 8.81	−7.38 ± 8.67	−4.38 ± 8.01	−6.34 ± 14.72	−11.86 ± 4.42	−9.38 ± 5.79
3	−9.29 ± 14.11	−4.75 ± 7.36	−7.25 ± 6.96	−16.84 ± 14.6	−7.00 ± 6.00	−10.75 ± 8.54
4	−5.29 ± 6.63	−5.25 ± 5.67	−3.63 ± 5.00	−10.17 ± 8.25	−9.58 ± 7.67	−3.00 ± 7.18
6	6.43 ± 7.19[a]	−8.50 ± 8.32[a]	1.13 ± 7.72[a]	−1.17 ± 7.06[a]	−11.43 ± 5.90[a]	0.25 ± 4.41[a]
8	−0.43 ± 5.58	−7.63 ± 8.60	−2.38 ± 4.74	−5.50 ± 4.86	−9.29 ± 5.77	−1.00 ± 8.19
12	−0.58 ± 5.75	−1.75 ± 5.83	−0.63 ± 5.17	−5.17 ± 7.01	−7.86 ± 5.51	−4.25 ± 3.80

The data presented as mean ± standard deviation.
HR = heart rate, SBP = systolic blood pressure, DBP = diastolic blood pressure, EM = extensive metabolizer (*CYP2D6*1/*1*, **1/*2*), IM_1 = intermediate metabolizer (*CYP2D6*1/*10*, **2/*10*), IM_2 = intermediate metabolizer (*CYP2D6*10/*10*).
[a]$P < 0.05$ between EM, IM_1, and IM_2.

The mean changes of HR, SBP, and DBP over time are shown in the **Table 3** for each group. The HR of all groups decreased on day 8 compared to the first day and the pattern of the changes for each group was different. Compared with baseline, the significant differences among *CYP2D6* genotypic groups were observed in 2, 6 hours after multiple dosing (day 8). In case of the SBP, the highest decrease was observed at 2–3 hours after administration in all groups but it was not significant in single and multiple dosing compared with baseline. In the DBP, there were statistically significant differences between the genotypic groups at 1, 6 hours after single dosing and 6 hours after multiple dosing. The ratios of CD25 obtained through IST were calculated for each day (days 0, 1, 8). The increase in the ratio of $CD25_{day8}$ to $CD25_{day0}$ was greater than that of the ratio of $CD25_{day1}$ to $CD25_{day0}$. The ratios of CD25 in day 1 and 8 among the *CYP2D6* genotypic groups were no significant ($P_{day1} = 0.59$, $P_{day8} = 0.13$). In addition, the mean ratio of $CD25_{day1}$ to baseline was 7.67 and 28.46 in the EM and combined IM group ($P = 0.38$), respectively, while the ratio of $CD25_{day8}$ to baseline was 18.94 and 117.49 in the EM and combined IM group ($P = 0.05$), respectively. (**Table 4**)

Table 4. The mean and standard deviation of the ratios of $CD25_{day1}$ and $CD25_{day8}$ to $CD25_{day0}$ in each group (EM, IM_1, IM_2) and in the combined IM group (IM_1 + IM_2)

Groups	EM (n = 6)	IM_1 (n = 7)	IM_2 (n = 8)	*P* value[a]	IM_1 + IM_2 (n = 15)	*P* value[b]
$CD25_{day1}/CD25_{day0}$	7.67 ± 8.67	36.38 ± 45.15	15.26 ± 6.87	0.59	28.46 ± 36.03	0.38
$CD25_{day8}/CD25_{day0}$	18.94 ± 12.95	97.21 ± 128.71	137.77 ± 231.93	0.13	117.49 ± 181.43	0.05

CD25 = the chronotropic dose 25, Day1 = single dosing, Day8 = multiple dosing, Day0 = baseline, EM = extensive metabolizer (*CYP2D6*1/*1*, **1/*2*), IM_1 = intermediate metabolizer (*CYP2D6*1/*10*, **2/*10*), IM_2 = intermediate metabolizer (*CYP2D6*10/*10*).
[a]Kruskal-Wallis test; [b]Mann-Whitney U test.

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Safety

Nine adverse events were reported from 6 subjects among the enrolled 21 subjects who were given the drug. The rates of total adverse events were 16.7% (1/6 subjects, 1 case) in the EM group, 14.3% (1/7 subjects, 2 cases) in the IM_1 group, and 50% (4/8 subjects, 6 cases) in the IM_2 group. No serious adverse events were occurred and all the reported events were mild and fully recovered without any action. Of the reported adverse events, only six adverse events (two for nausea, one for dizziness, one for headache, one for heart palpitation, one for excessive sleep) were related to the test drug. In the IM_1 group, nausea and heart palpitation for 2 cases were observed and nausea, dizziness, headache, and excessive sleep for 4 cases were found in the IM_2 group.

DISCUSSION

The aim of this study was to examine the influence of *CYP2D6* genetic polymorphisms on the PK and PD characteristics of carvedilol and its metabolites following single and multiple dosing of carvedilol in healthy Korean volunteers. Carvedilol is mostly metabolized into active 5OHC and 4OHC by *CYP2D6* and into active ODMC by *CYP2C9* and *CYP2D6*.[7]

The difference in the major PK parameters for carvedilol was statistically significant only for the decrease of CL/F in the IM_2 group after single dosing (day 1) compared with the EM group. Several studies have shown that *CYP2D6* activity has a greater effect on the metabolism of *R*(+)-carvedilol, which has low β-blocking activity, than *S*(−)-carvedilol, which has high β-blocking activity.[5,21] However, each of the isomers was not quantified in this study so we could not determine the PK effect of each isomer according to the decrease in *CYP2D6* activity. The lower *CYP2D6* activity in PMs has been reported to cause a decrease in the CL of *R*(+)-carvedilol and an increase in the plasma concentration of *R*(+)-carvedilol more than that of *S*(−)-carvedilol.[9] The PK results obtained in this study were concluded to be consistent with previous studies, suggesting that the plasma concentration of *R*(+)-carvedilol may be increased indirectly.

Compared to the EM group, the exposure to ODMC in the IM_2 group was higher following single dosing. It is well known that the *CYP2D6* enzyme is partly involved in the metabolism of ODMC.[5,7,22,23] In addition, carvedilol is metabolized primarily to 4OHC, 5OHC, and ODMC in human liver microsomes, but the maximum metabolic rate (apparent Km) of ODMC is especially 5–10 times higher than those of 4OHC and 5OHC.[7] The concentration of *R*(+)-carvedilol could be predicted to be increased by considering the maximum metabolic rate of ODMC, which showed high exposure. In this study, the PK results of 4OHC and 5OHC also showed no significant difference between genotypic groups.

No changes in the PD markers (HR, SBP, DBP) were observed before and after administration of carvedilol, depending on the *CYP2D6* genotype (EM, IM_1, IM_2). This indicated that PK changes such as lower CL of carvedilol in the IM_2 group did not cause any PD differences. In previous findings, the *CYP2D6* genotype had a statistically significant effect on the PK of carvedilol but had no significant effects on the PD response to carvedilol.[16,18,24] The results of the IST showed that the ratio of $CD25_{day8}$ to $CD25_{day0}$ was significantly different between the genotypic groups by classifying into EM and combined IM (IM_1, IM_2) groups. It was considered to be due to dose difference between day 1 (12.5 mg) and 8 (25 mg) and drug

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accumulation. Also, the ratios of $CD25_{day8}$ to $CD25_{day0}$ in the 3 genotypic groups did not show any statistically significant difference. Unlike the results of PK, no differences were found for all *CYP2D6* genotype groups in all PD markers.

The present study has several limitations. The results of the IST which performed to identify the PD characteristics were not suitable as a PD marker. IST is an indirect measure of the efficacy of carvedilol and indicates the dose of isoproterenol needed to increase the HR during the administration of carvedilol as a beta blocker. The IST results which related to the β-blocking state were difficult to correlate with the PK result that the α_1-adrenergic antagonist *R*(+)-carvedilol was increased. Also, the effect of β-blocking was relatively negligible because the rapid metabolism of *S*(−)-carvedilol may influence the increase of the plasma concentration in *R*(+)-carvedilol. Another limitation is that only the concentration of the carvedilol racemic mixture was measured without quantitation of each enantiomer. The relationship of PK/PD could not be clearly identified that the increase in the plasma concentration of *R*(+)-carvedilol caused adverse effects due to hyperactivity to the α-receptor blockade. In addition, the PD effect of carvedilol according to *CYP2D6* genotype could not be assessed because subjects were healthy volunteers. Therefore, further studies will be needed to consider other appropriate PD markers for evaluating the PK/PD relationship and to confirm the safety on each enantiomer of carvedilol in IMs with *CYP2D6*10* allele.

Based on the results of the study, it was confirmed that the decrease in *CYP2D6* activity influenced the PK characteristics of carvedilol and no differences in the PD response were observed between the *CYP2D6* genotypic groups. Therefore, findings of this study suggest that the *CYP2D6* genotype is not an important factor for individualizing treatment with carvedilol in the Korean population.

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