Effects of Glipizide on the Pharmacokinetics of Carvedilol after Oral and Intravenous Administration in Rats

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Abstract

This study was designed to investigate the effects of glipizide on the pharmacokinetics of carvedilol after oral or intravenous administration of carvedilol in rats. Clinically carvedilol and glipizide can be prescribed for treatment of cardiovascular diseases as the complications of diabetes, and then, Carvedilol and glipizide are all substrates of CYP2C9 enzymes. Carvedilol was administered orally or intravenously without or with oral administration of glipizide to rats. The effects of glipizide on cytochrome P450 (CYP) 2C9 activity and P-gp activity were also evaluated. Glipizide inhibited CYP2C9 activity in a concentration-dependent manner with 50% inhibition concentration (IC_{50}) of 18 μM. Compared with the control group, the area under the plasma concentration-time curve (AUC) was significantly increased by 33.0%, and the peak concentration (C_{max}) was significantly increased by 50.0% in the presence of glipizide after oral administration of carvedilol. Consequently, the relative bioavailability (R.B.) of carvedilol was increased by 1.13- to 1.33-fold and the absolute bioavailability (A.B.) of carvedilol in the presence of glipizide was increased by 36.8%. After intravenous administration, compared to the control, glipizide could not significantly change the pharmacokinetic parameters of carvedilol. Therefore, the enhanced oral bioavailability of carvedilol may mainly result from inhibition of CYP2C9-mediated metabolism rather than both P-gp-mediated efflux in the intestinal or in the liver and renal elimination of carvedilol by glipizide.

Key Words: Carvedilol, Glipizide, CYP2C9, P-gp, Pharmacokinetics, Rats

INTRODUCTION

Glipizide is a sulfonylurea antidiabetics with the treatment of type 2 diabetes mellitus and has a duration of action of up to 24 hours. The usual initial dose is 2.5 to 5 mg daily given as a single dose about 30 minutes before breakfast (Kradjan *et al.*, 1995).

Glipizide is readily absorbed from the gastrointestinal tract with peak plasma concentrations occurring 1 to 3 h after a single dose. It is extensively bound to plasma proteins and has a half-life of approximately 2 to 4 hours. It is metabolized mainly in the liver and excreted chiefly in the urine, largely as inactive metabolites (Wahlin-Boll *et al.*, 1982).

Furthermore, the effects of glipizide on cytochrome P450 (CYP) 2C9 activity and P-gp activity were also evaluated using CYP inhibition assays.

Carvedilol is an arylethanolamine and has nonspecific β- and α1- adrenergic blocking effects (Bristow *et al.*, 1992). Carvedilol also reduces the release of endothelin and directly

scavenges free radicals of oxygen (Feuerstein *et al.*, 1997). It is used to treat systemic arterial hypertension (Cournot *et al.*, 1992; Lund-Johansen *et al.*, 1992) and congestive heart failure (DasGupta *et al.*, 1991) and is purported to improve exercise capacity (Cleland *et al.*, 1996; Hampton, 1996) and longevity in humans (Bristow *et al.*, 1996).

Carvedilol is well absorbed from the gastrointestinal tract, but is subject to considerable first-pass metabolism in the intestinal and/or liver (McTavish *et al.*, 1993; Morgan, 1994). Carvedilol is more than 98% bound to plasma proteins. Carvedilol is metabolized by both oxidation and conjugation pathways in the liver into some metabolites (Neugebauer *et al.*, 1987; Neugebauer and Neubert, 1991). The oxidation pathways are mainly catalyzed by CYP2C9 enzymes in human (McTavish *et al.*, 1993; Morgan, 1994; Oldham and Clarke, 1997), and then CYP2D6 is responsible for the formation of 4'-hydroxy carvedilol and 5'-hydroxy carvedilol, and both metabolites are excreted into urine (Neugebauer and Neubert, 1991). Since carvedilol is a substrate of both CYP2C9 en-

zymes and P-gp (Bart *et al.*, 2005), the modulation of CYP enzyme activities may cause the significant changes in the pharmacokinetic profile of carvedilol.

There are a few interactions between glipizide and other drugs (Connacher *et al.*, 1987; Arauz-Pacheco *et al.*, 1990; Kivisto and Neuvonen, 1991; Kradjan *et al.*, 1994; Niemi *et al.*, 2001).

Clinically carvedilol and glipizide can be prescribed for treatment of cardiovascular diseases as the complications of diabetes. However, pharmacokinetic interaction between glipizide and carvedilol has not been reported *in vivo.* Therefore, the present study aims to investigate the effect of glipizide on the CYP2C9 activity, P-gp activity and pharmacokinetics of carvedilol after oral and intravenous administration in rats.

MATERIALS AND METHODS

Chemicals and apparatus

Carvedilol, glipizide and nimodipine [an internal standard for high-performance liquid chromatograph (HPLC) analysis for carvedilol] were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC grade acetonitrile was acquired from Merck Co. (Darmstadt, Germany). Other chemicals for this study were of reagent grade.

Apparatuses used in this study were a HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus autosampler and a Waters™ 474 scanning fluorescence detector (Waters Co., Milford, MA, USA), a HPLC column temperature controller (Phenomenex Inc., CA, USA), a Bransonic® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., NY, USA) and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan).

Animal experiments

Male Sprague-Dawley rats of 7-8 weeks of age (weighing 270-300 g) were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Republic of Korea) and given free access to a commercial rat chow diet (No. 322-7-1; Superfeed Co., Gangwon, Republic of Korea) and tap water *ad libitum.* The animals were housed (two rats per cage) in a clean room maintained at a temperature of $22 \pm 2^{o}C$ and relative humidity of 50-60%, with 12 h light and dark cycles. The rats were acclimated under these conditions for at least 1 week. All animal studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) and the Animal Care Committee of Chosun University (Gwangju, Republic of Korea) approved the protocol of this animal study. The rats were fasted for at least 24 h prior to beginning the experiments and had free access to tap water. Each animal was anaesthetized lightly with ether. The left femoral artery and vein were cannulated using polyethylene tubing (SP45, I.D. 0.58 mm, O.D. 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and i.v. injection, respectively.

Oral and intravenous administration of carvedilol

The rats were divided into six groups (n=6, each): an oral group (3 mg/kg of carvedilol dissolved in water; homogenized at 36°C for 30 min; 3.0 ml/kg) without (control) or with 0.1 or 0.4 mg/kg of oral glipizide, and an i.v. group (1 mg/kg of carvedilol, dissolved in 0.9% NaCl solution; homogenized at 36°C for 30 min; 1.5 ml/kg) without (control) or with 0.1 or 0.4 mg/kg of oral glipizide. Glipizide was orally administered 30 min prior to oral or intravenous administration of carvedilol. Oral carvedilol was administered through a feeding tube, and carvedilol for i.v. administration was injected through the femoral vein within 0.5 min. A 0.4-ml blood sample was collected into heparinized tubes from the femoral artery at 0, 0.1, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after intravenous infusion and at 0, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after oral administration. The blood samples were centrifuged (13,000 rpm, 5 min), and the plasma samples were stored at −40°C until HPLC analysis of carvedilol. Rats were infused with approximately 1 ml of whole blood collected from untreated rats via the femoral artery at 0.5, 2 and 8 h to replace the blood loss due to blood sampling.

HPLC assay

The plasma concentrations of carvedilol were determined by the HPLC assay method reported by Zarghi *et al.* (2007). Briefly, 50 μl of dihydroerogostine (20 μg/ml dissolved in methanol; an internal standard) and 0.5 ml of acetonitrile were added to a 0.2 ml aliquot of the plasma in a 2.0 ml polypropylene microtube. The mixture was then stirred for 10 min and centrifuged (13,000 rpm, 10 min). A 0.5 ml aliquot of the organic layer was transferred to a clean test tube and evaporated under a gentle stream of nitrogen gas at 35°C. The residue was reconstituted in a 150 μl of the mobile phase and centrifuged (13,000 rpm, 5 min). The resulting mixture was then vigorously vortex-mixed for 5 min and centrifuged at 13,000 rpm for 5 min. A 50-μl aliquot of the supernatant was injected into the HPLC system. Chromatographic separations were achieved using a Chromolith Performance (RP-18e, 100×4.6 mm) column from Merck (Darmstadt, Germany). The mobile phase consisted of 0.01M disodium hydrogen phosphate (pH 3.5, adjusted with phosphoric acid)-acetonitrile (75.7:24.3, v/v). The flow rate of the mobile phase was maintained at 2.0 ml/min. Chromatography was performed at of 25˚C, which was regulated by an HPLC column temperature controller. The fluorescence detector was operated at an excitation wavelength of 240 nm with an emission wavelength of 340 nm. The retention times at a flow rate of 2 ml/min were as follows: carvedilol at 8.076 min internal standard at 9.305 min. The lower limit of quantification for carvedilol in rat plasma was 10 ng/ml. The coefficient of the variation of carvedilol was less than 14.3%.

CYP 2C9 inhibition assay

The assays of inhibition on human 2C9 enzyme activities were performed in a multiwell plate using CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously (Crespi *et al.*, 1997). Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrates (7-MFC for CYP2C9) were incubated with or without test compounds in the enzyme/substrate contained buffer consisting of 1 pmol of P450 enzyme and NADPH generating system (1.3 mM NADP, 3.54 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase and 3.3 mM $MgCl_2$) in a potassium phosphate buffer (pH 7.4). Reactions were terminated by adding stop solution after 45 min incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Device, Sunnyvale, CA) set at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 μM ketoconazole for CYP2C9 was run on the same plate and produced 99% inhibition. All experiments were performed in du-

plicate, and results are expressed as the percent of inhibition.

Rhodamine-123 retention assay

The procedures was used for Rho-123 retention assay were similar to a reported method (Han *et al.*, 2008). MCF-7/ADR cells were seeded in 24-well plates. At 80% confluence, the cells were incubated in FBS-free DMEM for 18 h. The culture medium was changed to Hanks' balanced salt solution and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20 mM rhodamine-123 for 90 min, the medium was completely removed. The cells were then washed three times with ice-cold phosphate buffer (pH 7.0) and lysed in lysis buffer. The rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and presented as the ratio to controls.

Pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of carvedilol concentration data during the elimination phase, and the terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and the time to reach peak concentration (T_{max}) of carvedilol in plasma were obtained by visual inspection of the data from the concentration-time curve. The area under the plasma concentration-time curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinity ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by Clast/Kel. Total body clearance (CL/F) was calculated by Dose/AUC. The absolute bioavailability (A.B.) of carvedilol was calculated by $AUC_{oral}/AUC_{i.v.} \times Dose_{i.v.}/Dose_{oral} \times 100$, and the relative bioavailability (R.B.) of carvedilol was estimated by $AUC_{with\ glipizide}/AUC_{control} \times 100$.

Statistical analysis

All mean values are presented with their standard deviation (Mean ± SD). Statistical analysis was conducted using one-way ANOVA followed by *a posteriori* testing with Dunnett's correction. Differences were considered significant at a level of *p*<0.05

RESULTS

Inhibition of CYP2C9 activity

The inhibitory effect of glipizide on CYP2C9 activity is shown in Fig. 1. Glipizide inhibited CYP2C9 activity in a concentration-dependent manner, and the 50 % inhibition concentration (IC_{50}) values of glipizide on CYP2C9 activity was 18 μM.

Rhodamine-123 retention assay

As shown in Fig. 2, accumulation of rhodamine-123, a P-gp substrate, was not raised in MCF-7/ADR cells overexpressing P-gp compared to that in MCF-7 cells lacking P-gp. The concurrent use of glipizide did not enhance the cellular uptake of rhodamine-123 in a concentration-dependent manner ranging from 3-30 μM. This result suggests that glipizide could not
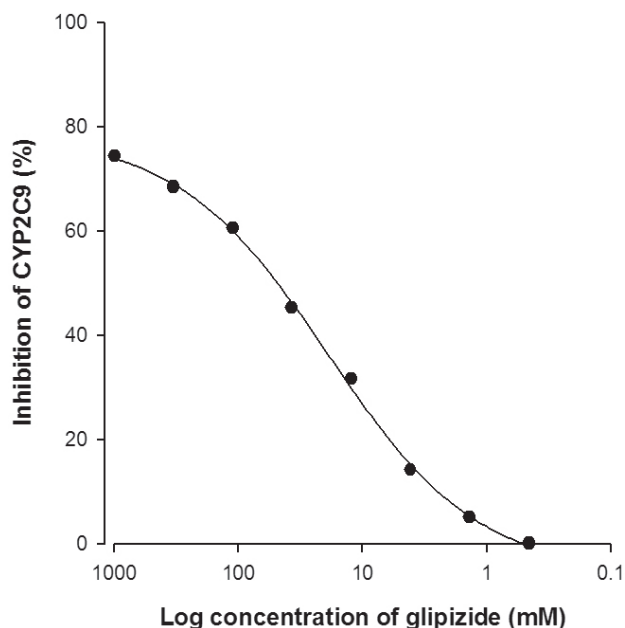

Fig. 1. Inhibitory effects of glipizide on CYP2C9 activity. All experiments were performed in duplicate, and results are expressed as the percent of inhibition.
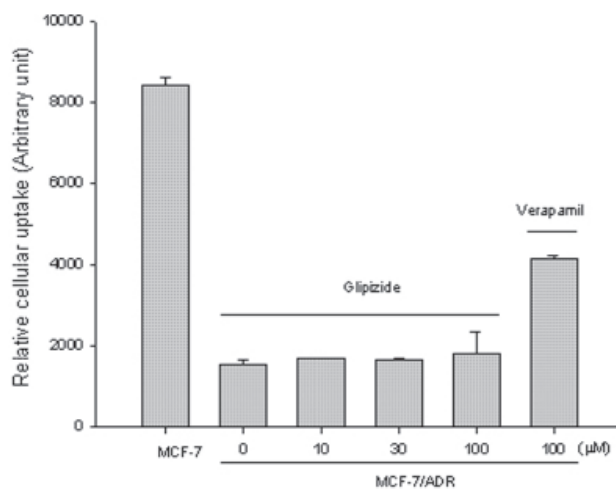

Fig. 2. Effect of glipizide on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD of 6 separate samples (significant versus control MCF-7 cells).

inhibit P-gp activity.

Effects of glipizide on carvedilol plasma concentrations after oral administration

The mean plasma concentration-time profiles of oral carvedilol in the presence or absence of glipizide are illustrated in Fig. 3. The mean pharmacokinetic parameters of carvedilol are also summarized in Table 1. Fig. 3 shows the plasma concentration-time profiles of carvedilol after oral administration of 3 mg/kg of carvedilol in rats without or with glipizide (0.1 or 0.4 mg/kg), and the pharmacokinetic parameters of
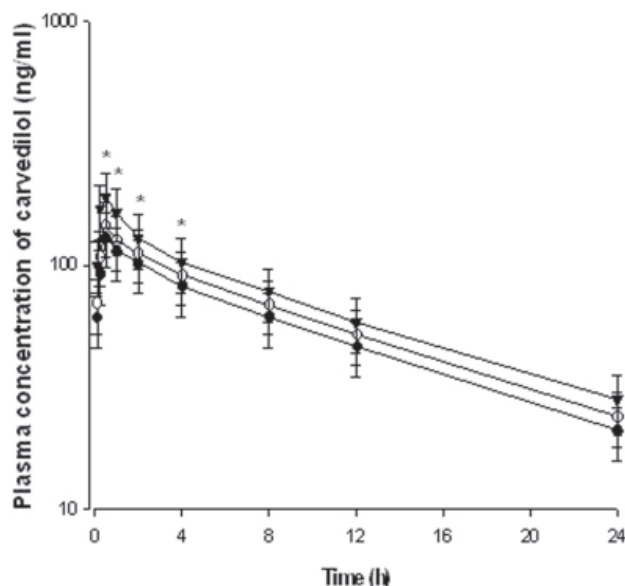
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Fig. 3. Mean plasma concentration-time profiles of carvedilol after oral administration of carvedilol (3 mg/kg) without (●) or with 0.1 mg/kg (○) and 0.4 mg/kg (▼) of glipizide to rats. Bars represent the standard deviation (n=6).

Table 1. Mean (± SD) pharmacokinetic parameters of carvedilol after oral administration of carvedilol (3 mg/kg) in the presence or absence of glipizide to rats

Parameters	Control	Carvedilol+glipizide	
		0.1 mg/kg	0.4 mg/kg
AUC (ng · h/ml)	1,592 ± 285	1,795 ± 339	2,118 ± 421[a]
C_{max} (ng/ml)	130 ± 22	144 ± 26	195 ± 44[a]
T_{max} (h)	0.5	0.5	0.5
$t_{1/2}$ (h)	10.3 ± 1.9	10.5 ± 2.1	11.1 ± 2.3
A.B. (%)	27.5 ± 4.2	31.2 ± 4.4	36.8 ± 4.9[a]
R.B. (%)	100	113	133

Mean ± SD (n=6). [a]$p<0.05$, significant difference compared to the control. AUC: area under the plasma concentration-time curve from 0 h to infinity, C_{max}: peak plasma concentration, T_{max}: time to reach peak concentration, $t_{1/2}$: terminal half-life, A.B. (%): absolute bioavailability, R.B. (%): relative bioavailability compared to the control group.

oral carvedilol are summarized in Table 1. The area under the plasma concentration-time curve (AUC) was significantly (0.4 mg/kg, $p<0.05$) increased by 33.0%, and the peak concentration (C_{max}) was significantly (0.4 mg/kg, $p<0.05$) increased by 50.0% in the presence of glipizide after oral administration of carvedilol. Consequently, the relative bioavailability (R.B.) of carvedilol was increased by 1.13- to 1.33-fold, and the absolute bioavailability (A.B.) of carvedilol in the presence of glipizide was significantly (0.4 mg/kg, $p<0.05$) increased by 36.8%. However, there were no significant changes in the half-life ($t_{1/2}$) and T_{max} of carvedilol in the presence of glipizide.
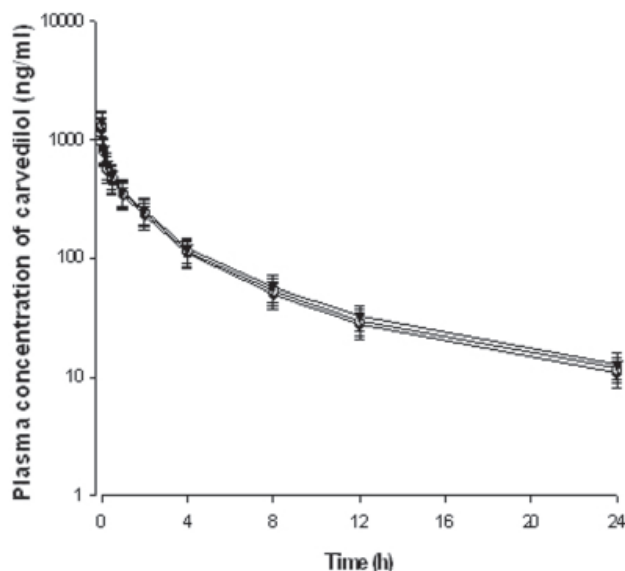

Fig. 4. Mean plasma concentration-time profiles of carvedilol after i.v. administration of carvedilol (1 mg/kg) without (●) or with 0.1 mg/kg (○) and 0.4 mg/kg (▼) of glipizide to rats. Bars represent the standard deviation (n=6).

Table 2. Mean (± SD) pharmacokinetic parameters of carvedilol after intravenous administration of carvedilol (1 mg/kg) in the presence or absence of glipizide to rats

Parameters	Control	Carvedilol+glipizide	
		0.1 mg/kg	0.4 mg/kg
AUC (ng · h/ml)	1,920 ± 371	2,030 ± 382	2,320 ± 419
CL_t (ml/min/kg)	523 ± 131	492 ± 126	472 ± 121
$t_{1/2}$ (h)	7.9 ± 1.6	8.0 ± 1.7	8.1 ± 1.8
R.B. (%)	100	106	111

Mean ± SD (n=6). AUC: area under the plasma concentration-time curve from time 0 to infinity, CL_t: total body clearance, $t_{1/2}$: terminal half-life, R.B. (%): relative bioavailability compared to the control group.

Effects of glipizide on carvedilol plasma concentrations after i.v. administration

The mean plasma concentration-time profiles of i.v. carvedilol in the presence or absence of glipizide are illustrated in Fig. 4. The mean pharmacokinetic parameters of carvedilol are also summarized in Table 2. Fig. 4 shows the plasma concentration-time profiles of carvedilol after i.v. (1 mg/kg) administration without or with of glipizide (0.1 or 0.4 mg/kg) to rats. As shown in Table 2, glipizide did not significantly change the pharmacokinetic parameters of i.v. administration of carvedilol, suggesting that glipizide may improve the oral bioavailability of carvedilol by more increasing the absorption or reducing metabolism in the intestine and/or in the liver than renal eliminatin.

DISCUSSION

CYPs enzymes contribute significantly to the first-pass metabolism and oral bioavailability of many drugs. Moreover, inhibition or induction of intestinal CYPs may be responsible for significant drug interactions which one agent decreases or increases the bioavailability and absorption of a concurrently administered drug (Kaminsky and Fasco, 1991).

Therefore, inhibitors of CYP2C9 activity should have a great impact on the bioavailability of many drugs which is substrat of CYP2C9. Since carvedilol is a substrate of CYP2C9 enzymes (Bart *et al.*, 2005), the modulation of CYP enzyme activities may cause the significant changes in the pharmacokinetic profile of carvedilol.

The hypertension could be induced by the complications of diabetes. Clinically carvedilol and glipizide can be prescribed for treatment of cardiovascular diseases as the complications of diabetes. Therefore, the interaction of the antidiabetic and the antihypertensive drug may be brought out. There are a few interactions between glipizide and other drugs (Connacher *et al.*, 1987; Arauz-Pacheco *et al.*, 1990; Kivisto and Neuvonen, 1991; Kradjan *et al.*, 1994; Niemi *et al.*, 2001). However, pharmacokinetic interaction between glipizide and carvedilol has not been reported *in vivo*. Therefore, the present study aims to investigate the effect of glipizide on the CYP2C9 activity and pharmacokinetics of carvedilol after oral and intravenous administration in rats.

The inhibitory effect of glipizide on CYP2C9-mediated metabolism was confirmed by the employment of recombinant CYP2C9 enzyme. As shown in Fig. 1, glipizide exhibited inhibitory effect on CYP2C9 activity with IC_{50} of 18 μM. Therefore, the pharmacokinetic characteristics of carvedilol were evaluated in the absence and presence of glipizide in rats. Human CYP2C9 and 3A4 and rat CYP2C11 and 3A1 have 77 and 73% protein homology, respectively (Lewis, 1996).

Rats were selected as an animal model in this study to evaluate the potential pharmacokinetic interactions mediated by CYP2C9, although there may be some difference in enzyme activity between rat and human (Cao *et al.*, 2006). Therefore, glipizide might possible increase absorption of carvedilol in the intestine through the inhibition of CYP2C9.

The area under the plasma concentration-time curve (AUC) was significantly increased by 33.0%, and the peak concentration (C_{max}) was significantly increased by 50.0% in the presence of glipizide after oral administration of carvedilol. Consequently, the relative bioavailability (R.B.) of carvedilol was increased by 1.13- to 1.33-fold, and the absolute bioavailability (A.B.) of carvedilol in the presence of glipizide was significantly increased by 36.8%. These results were consistent with report by Choi and Choi (2010), in which ticlopidine significantly increased the AUC and C_{max} of carvedilol, a substrate for CYP2C9.

Studies on drug interactions with grapefruit juice have provided much understanding of the role of intestinal CYP450 in the absorption of orally administered drugs. CYP2C9 is the predominant P450 present in the small intestine (Kolars *et al.*, 1992).

Glipizide did not significantly change the pharmacokinetic parameters of intravenous administration of carvedilol, suggesting that glipizide may improve the oral bioavailability of carvedilol by more increasing the absorption or reducing intestinal metabolism via inhibition of CYP2C9-mediated metabo-lism rather than renal elimination by glipizide.

These results were consistent with report by Choi and Choi (2010), in which ticlopidine did not significantly change the pharmacokinetic parameters of intravenous administration of carvedilol.

Consequently, the increased bioavailability of carvedilol might be mainly due to inhibition of CYP2C9-mediated metabolism of carvedilol in the intestine and/or in the liver rather than both inhibition of P-gp-mediated efflux and renal elimination by glipizide. Therefore, concomitant use of glipizide with carvedilol will require close monitoring for potential adverse interactions such as hepatotoxicity or pruritus by carvedilol (Hagmeyer and Stein, 2001) and hyper-sensitivity reaction or photosensitivity by glipizide (Paice *et al.*, 1985) in the therapy of cardiovascular diseases or daiabetes.

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