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Enantioselective Pharmacokinetics of Carvedilol in Human Volunteers

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Carvedilol is administered as a racemic mixture of the *R*(+)- and *S*(-)-enantiomers, although they exhibit different pharmacological effects. To investigate the stereoselective pharmacokinetics, the enantiomeric separation of carvedilol in human plasma was undertaken using capillary electrophoresis (CE). Resolution of the enantiomers was achieved using 2-hydoxypropyl-β-cyclodextrin as the chiral selector. Phosphate buffer (50 mM, pH 4.0) containing 10 mM of 2-hydoxypropropyl-β-cyclodextrin was used as electrolytic buffer. Achiral separation was carried out with the same electrolytic buffer without chiral selector. Following a single oral administration of 25-mg carvedilol to 11 healthy, male volunteers, stereoselective pharmacokinetic analysis was undertaken. The maximum plasma concentrations (C_{max}) were 48.9 and 21.6 ng/mL for (*R*)-carvedilol and (*S*)-carvedilol, respectively, determined by the chiral method. The profiles of the plasma concentration of (*RS*)-carvedilol showed C_{max} of 71.5, 72.2, and 73.5 ng/mL, as determined by the CE, HPLC/FD methods and calculations from the data of the chiral method, respectively.

Key words: Carvedilol, Pharmacokinetics, Stereospecificity, Human plasma, Capillary electrophoresis, 2-Hydoxypropyl-β-cyclodextrin

INTRODUCTION

Carvedilol, 1-(4-carbazolyloxy)-3-(2-(2-methoxy) ethylamino)-2-propanol (Fig. 1), is used for the treatment of hypertension, ischemic heart disease and congestive heart failure. It is a non-selective, β-adrenergic receptor antagonist and an α_1-adrenoceptor blocker. Carvedilol contains a chiral center in the structure and exists as two enantiomers. The drug is administered as a racemic mixture of the *R*(+)- and *S*(-)-enantiomers which exhibit different pharmacological effects. The β-receptor blocking activity of the (*S*)-enantiomer is about 200-fold higher than that of the (*R*)-enantiomer, whereas both enantiomers show the same α_1-adrenergic antagonism (Nichols *et al.*, 1989; Bartsch *et al.*, 1990). Carvedilol undergoes stereoselective, first-pass metabolism following oral administration in healthy subjects. The apparent, terminal elimination half-life for (*R*)-carvedilol ranges from 5 to 9 h, compared with 7 to 11 h for (*S*)-carvedilol (Paul *et al.,* 2003; Woo *et al.,* 2001).

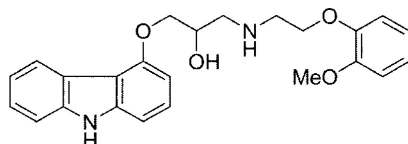

Fig. 1. Chemical structure of carvedilol

To investigate stereoselective pharmacokinetics, it is necessary to measure individual carvedilol enantiomer concentrations. The chiral derivatization method has been used to determine carvedilol enantiomers by HPLC/FD (Lamprecht *et al.,* 2002; Fujimaki *et al.,* 1992; Spahn *et al.,* 1992; Eisenberg *et al.,* 1989). However, this method required off-line derivatization and sometimes an incomplete reaction and multiple derivatization could occur. Meanwhile, capillary electrophoresis (CE) has become a very attractive analytical tool to determine enantiomers and is used for the separation of carvedilol (Clohs *et al.*, 2001). This method is also available for the determination of carvedilol

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even in very small volumes of human plasma (Behn *et al.*, 2001). Several reports comparing the HPLC/FD and CE methods for the analysis of carvedilol enantiomers (Clohs *et al.*, 2003; Oravcova *et al.*, 1996) have shown that the CE method is more effective than the HPLC/FD method in resolution and analysis time, except detectability. The aim of this study was to measure the level of carvedilol in human plasma by CE with increased detectability and to perform the achiral and chiral pharmacokinetic evaluation of carvedilol in humans.

EXPERIMENTAL

Instruments and chemicals

The experiments were performed on a 3DCE (Hewlett Packard, Germany) equipped with a UV detector. Data were collected and analyzed on an HP Vectra computer with HP Chemstation system. Carboxymethyl-, hydroxypropyl- and succinyl-β-CD with average substitution degree of 0.5, 0.9, and 0.4, respectively, were purchased from Wacker Chemie GmbH (Munich, Germany) and dimethyl-β-CD was from Beckman Inc. (CA, U.S.A.). (*RS*)-Carvedilol, a 1:1 mixture of (*R*)- and (*S*)-carvedilol, was kindly donated from Chong Kun Dang Pharm. Co. (Korea) and used as standard. (*S*)-Propranolol (Sigma, USA) was used as internal standard. All other chemicals and solvents were of analytical-reagent or HPLC grade.

Collection of plasma and pretreatment

Eleven healthy, male subjects with a mean age of 25.6±1.7 years and a mean weight of 70.0±8.3 kg took part in this study. None had taken any drugs known to interfere with analysis for 10 days beforehand. Health problems, drug or alcohol abuse, and abnormalities in laboratory screening values were exclusion criteria. The protocol of this study was approved by the local ethical committee. After an overnight fast, all subjects were given a single oral dose of a 25-mg carvedilol tablet. Blood samples (6 mL) were taken before and 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after drug administration, collected in heparin treated tubes, centrifuged immediately at 15,000 g for 10 min (Microspin, Hanil Co., Korea), and then stored at -20 °C until analysis. To 1 mL of plasma, 50 μL of internal standard (4 μg (*S*)-propranolol in 1 mL ethanol) and 2 mL of phosphate buffer (100 mM, pH 8.0) were added. The solutions were mixed for 20 s before extraction with 5 mL diethyl ether. The supernatant was evaporated to dryness under a stream of nitrogen at ambient temperature. The residue was reconstituted in 100 μL of 0.025 M HCl, and centrifuged at 15,000 g for 10 min. The aliquot was analyzed by CE. For the standard calibration racemic carvedilol and (*S*)-propranolol were added to blank plasma and extracted as blood sample.

Electrophoresis

The separation was carried out using a fused silica, uncoated capillary, 80.5 cm in length (72 cm effective length), 50 μm I.D, with extended light path. Phosphate buffer (50 mM, pH 4.0) containing 10 mM 2-hydoxypropyl-β-cyclodextrin (HP-β-CD) was used as electrolytic buffer. The system was programmed to rinse the capillary at the beginning and between runs by alternately flushing with 0.1 M sodium hydroxide, distilled water and running buffer for 3 min each. At the end of the day, the capillary was flushed with 0.1 M sodium hydroxide for 3 min and water for 5 min. Sample injection was performed hydrodynamically at the anodic end of the capillary at 50 mmbar for 40 s. The electrophoresis procedure was developed at high voltage corresponding to an electric field of 360 V/cm with positive polarity. Detection was by UV at wavelength of 200 nm.

HPLC/FD analytical system

The plasma carvedilol concentrations were quantified using HPLC/FD system. The compounds were separated on a reversed-phase column (Spherisorb® S5 C8, 4.6×150 mm, Waters, USA) with an isocratic mobile phase consist of acetonitril and 0.05M-dibutylamine buffer (60% : 40% (v/v), pH 2.5). The mobile phase was eluted using a LC-10AT pump (Shimazu, Japan) at 1.5 mL/min. Quantification was achieved by means of fluorecesence detection at 238 nm (excitation) and 350 nm (emission).

Calculation of pharmacokinetic parameters

Non-compartmental pharmacokinetic analysis was utilized by standard methods. The maximum plasma concentration (C_{max}) and the time of its occurrence (T_{max}) were complied from the concentration-time data. The area under the plasma concentration-time curve from 0 to last sampling time (AUC_t) was calculated using the linear trapezoidal rule and was extrapolated to infinity (AUC_{inf}). The elimination rate constant (K_{el}) was estimated from the slope of the terminal phase of the carvedilol plasma concentration (Milo *et al.*, 1982; Shawn *et al.*, 1998).

RESULTS AND DISSCUTION

Chiral and achiral analysis of carvedilol

The CE method has been used for the determination of carvedilol in plasma with phosphate buffer (50 mM, pH 4.0) as background electrolyte. In preliminary trials, HP-β-CD was found to be an appropriate chiral selector for the separation of carvedilol enantiomers. As shown in Fig. 2a, carvedilol enantiomers were separated from each other with baseline resolution (Rs = 2.34). The optimum concentration of HP-β-CD was 10 mM, which produced a result in good agreement with the reported data (Clohs *et al.*, 2003). Achiral separation of carvedilol was carried out

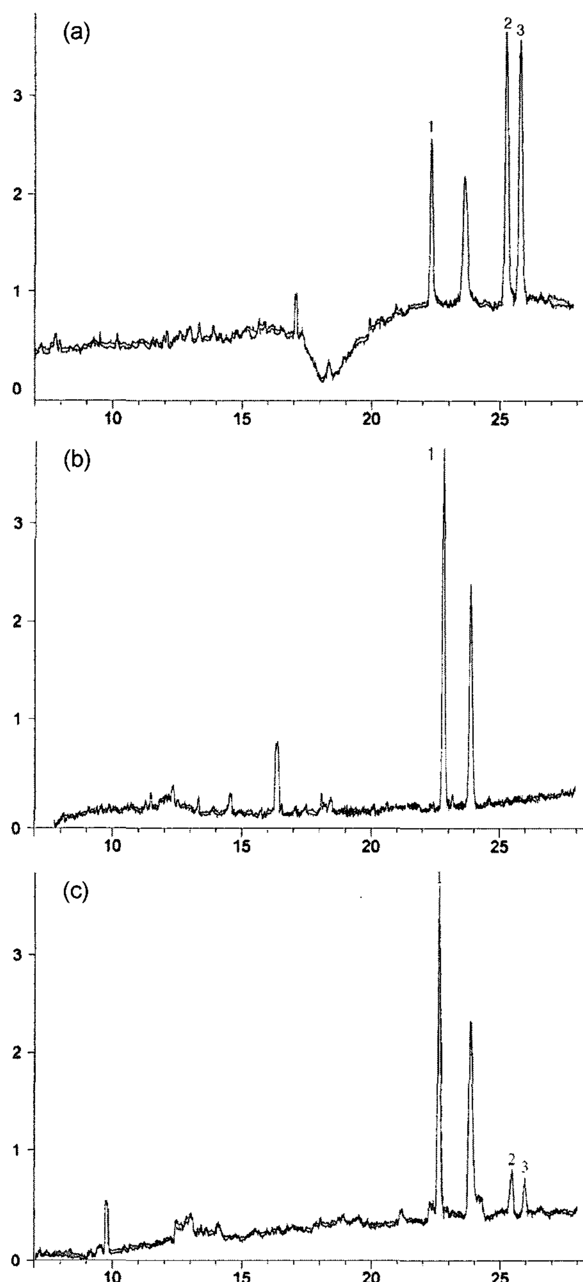

Fig. 2. Electropherograms for chiral separation of carvedilol in human plasma. (a) standard solution spiked to plasma, (b) blank plasma, (c) plasma sample. Electrophoretic conditions: electrolyte; phosphate buffer (50 mM, pH 4.0) with 10 mM 2-hydoxypropyl-β-cyclodextrin, capillary; fused silica, uncoated, 80.5 cm (72 cm effective)×50 μm I.D., electric field strength; 360 V/cm (positive polarity), detection UV at 200 nm. Peaks. 1. internal standard, (*S*)-propranolol, 2. (*R*)-carvedilol, 3. (*S*)-carvedilol.

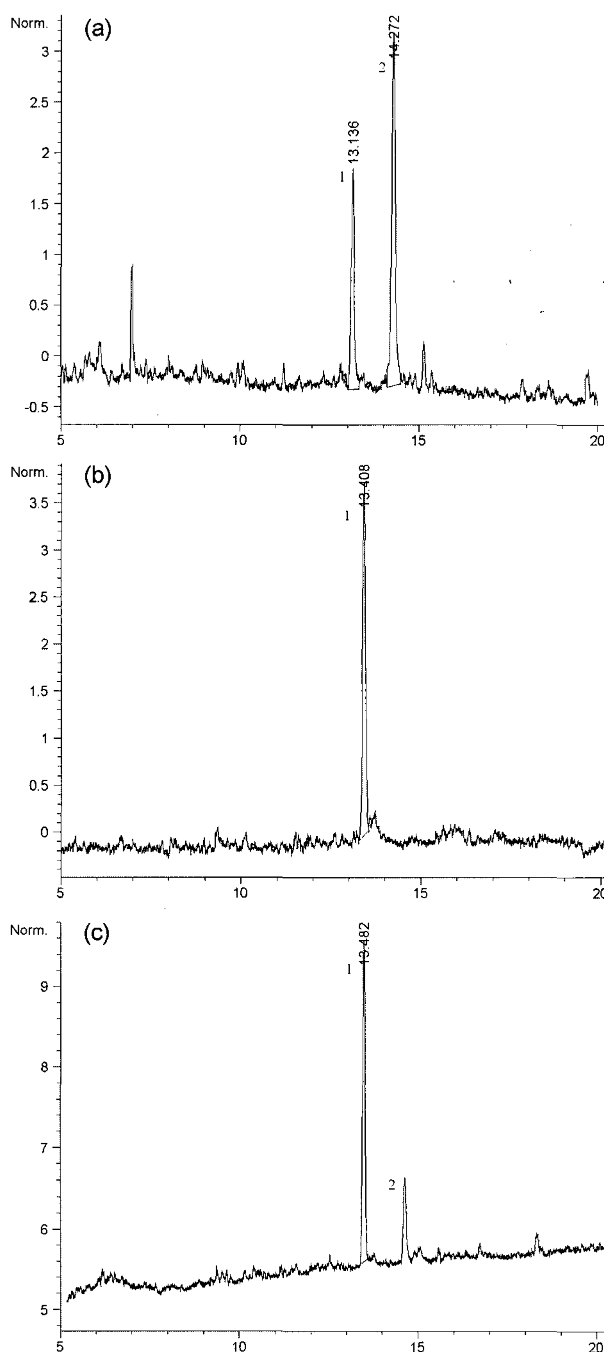

Fig. 3. Electropherograms for achiral separation of carvedilol in human plasma. (a) standard solution spiked to plasma, (b) blank plasma, (c) plasma sample. Electrophoretic conditions: same as Fig. 2, but no chiral selector was added. Peaks. 1. internal standard, (*S*)-propranolol, 2. (*RS*)-carvedilol.

with the same electrolyte used in the chiral method, without addition of the chiral selector (Fig. 3).

Calibration and validation

The method was calibrated by plotting the concentrations versus corrected peak area ratios. The calibration functions for (*R*)- and (*S*)-carvedilol in the range of 7.5 to 250 ng/mL were $y = 0.0083x - 0.0043$ and $y = 0.0073x - 0.0041$, respectively, with a correlation coefficient of 0.999 for both. The accuracy and precision of this method were determined with a plasma-sample-spiked, 50 ng/mL carvedilol.

Table I shows the validation data of this method.

Plasma concentration and pharmacokinetic profiles

The plasma concentration-time profiles of (*R*)- and (*S*)-carvedilol analyzed by CE are shown in Fig. 4. C_{max} was 49.4±16.2 and 21.6±9.3 ng/mL for (*R*)-carvedilol and (*S*)-carvedilol, respectively. C_{max} of (*RS*)-carvedilol, calculated from (*R*)- and (*S*)-carvedilol, was 71.0±22.7 ng/mL. Pharmacokinetic parameters were calculated by non-compartmental analysis and are summarized in Table II. The plasma levels of (*R*)-carvedilol were about two times higher than those of (*S*)-carvedilol, and also AUC_R was about two times higher than AUC_S. The estimated CL/F of (*R*)-carvedilol and (*S*)-carvedilol was 101.8 L/h and 193.1 L/h, respectively. The terminal half-life of (*R*)-carvedilol and (*S*)-carvedilol was 3.30 and 4.64 h, respectively, which appeared to be shorter than those of other studies because of the limited sampling time schedule in this study.

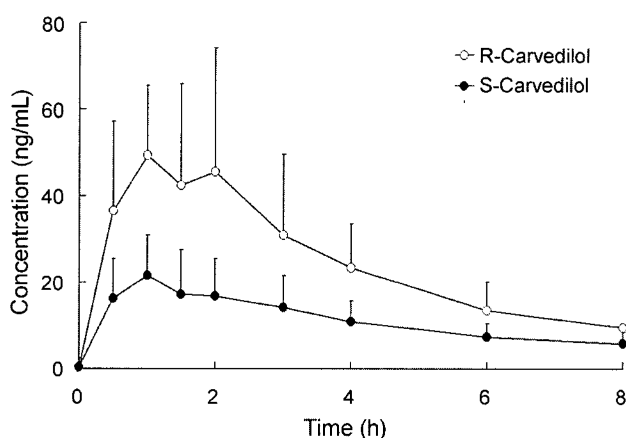

Fig. 4. Plasma concentrations of (*R*)- and (*S*)-carvedilol after an oral administration of a 25-mg carvedilol tablet in human volunteers. Values represent the mean and standard deviation.

Table I. Accuracy and precision of carvedilol spiked 50 ng/ml to blank plasma analyzed by CE method

	R	S	RS
Precision (CV%)			
Within-day (%)[a]	5.7	8.1	8.8
Between-day (%)[b]	11.5	14.8	9.9
Accuracy (%)	107.6	101.8	99.6

[a] n=5 determinations, [b] n=5 days

Table II. Estimated pharmacokinetic parameters of (*R*)-, (*S*)- and (*RS*)-carvedilol after oral administration of a 25 mg carvedilol tablet in human volunteers

Parameters	R	S	RS
K_{el} (1/h)	0.25± 0.02	0.19± 0.01	0.22± 0.01
$t_{1/2}$ (h)	2.75± 0.18	3.71± 0.23	3.18± 0.14
AUC_{8hr} (ng·h/mL)	200.98± 8.86	91.25± 75.43	292.32± 9.89
AUC_{inf} (ng·h/mL)	234.13± 7.98	118.82± 80.41	375.77±10.79
CL/F (L/h)	106.78± 3.64	210.40±142.54	66.53± 1.91
C_{max} (ng/mL)	49.4 ±16.2	21.6 ± 9.3	71.0 ±22.7

The profiles of the plasma concentration of carvedilol as determined by the CE and HPLC/FD methods are shown in Fig. 5. The plasma concentration profiles obtained were similar, with C_{max} values of 73.2±47.1 and 75.4±31.1 ng/mL for the CE and HPLC/FD methods, respectively; as compared with 73.5±9.6 ng/mL calculated from (*R*)- and (*S*)-carvedilol. The calculated pharmacokinetic parameters are summarized in Table III. All the pharmacokinetic parameters, including AUC and elimination rate constants, appeared to be similar in the methods of CE, HPLC/FD, and calculation (*R* + *S*).

In summary, this CE method successfully analyzed the carvedilol racemate and enantiomers stereospecifically without any interference in human plasma. The plasma concentration of (*R*)-carvedilol appeared to be 2~3 times higher than that of (*S*)-carvedilol, and the calculated terminal half-life of (*R*)-carvedilol and (*S*)-carvedilol was

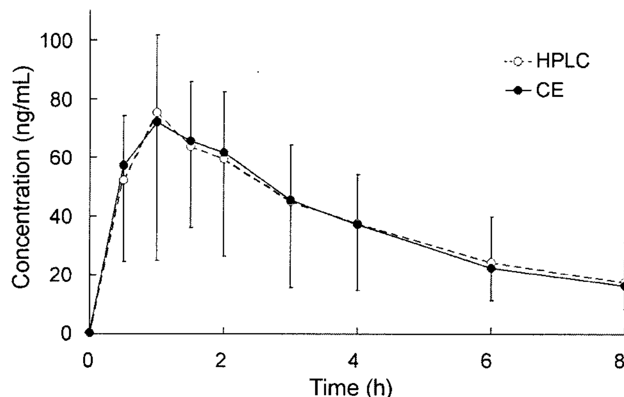

Fig. 5. Comparison of plasma concentration of (*RS*)-carvedilol analyzed by CE and HPLC/FD after an oral administration of a 25-mg carvedilol tablet in human volunteers. Values represent the mean and standard deviation.

Table III. Estimated pharmacokinetic parameters of (*RS*)-carvedilol determined by CE, HPLC/FD method and calculation (*R*+*S*) after an oral administration of a 25 mg carvedilol tablet in human volunteers

Parameters	CE	HPLC/FD	Calculation
K_{el} (1/h)	0.22± 0.01	0.21± 0.01	0.23± 0.01
$t_{1/2}$ (h)	3.18± 0.14	3.37± 0.14	3.04± 0.17
AUC_{8hr} (ng·h/mL)	307.95±11.46	310.02±10.67	292.32±12.83
AUC_{inf} (ng·h/mL)	375.77±10.79	388.71± 8.96	350.23±11.77
CL/F (L/h)	66.53± 1.91	64.32± 1.48	71.38± 2.40
C_{max} (ng/mL)	72.2 ±47.1	75.4 ±31.1	73.5 ± 9.6

3.30 and 4.64 h, respectively.

ABBREVIATIONS

AUC_t, area under the plasma concentration-time curve; AUC_R, area under the plasma concentration-time curve of *R*-form; AUC_S, area under the plasma concentration-time curve of *S*-form; CE, capillary electrophoresis; CD, cyclodextrin; C_{max}, maximum plasma concentration; HP, hydroxypropyl; HPLC, high performance liquid chromatography; FD, Fluorescence detection; K_{el}, elimination rate constant; T_{max}, the time of C_{max}.

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