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Short communication

Rapid and simple micro-determination of carvedilol in rat plasma by high-performance liquid chromatography

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Abstract

We studied the use of high-performance liquid chromatography (HPLC) with spectrofluorometric detection, using a solid-phase extraction for a simple, rapid and sensitive determination of plasma carvedilol levels in rats. Extracted aliquots were analyzed by HPLC, using a reversed-phase octadecyl silica column. The analytical mean recovery of carvedilol added to the blank plasma was 94.2%. The detection limit was 3.6 ng/ml in the plasma. The reproducibilities (C.V.) were 2.7–7.5% for the within-day assay, and 2.6–7.4% for the between-day assay, indicating that the method was effective for the determination of carvedilol plasma levels.

Keywords: Carvedilol

1. Introduction

Carvedilol (±)-1-(carbazol-4-yloxy)-3-((2-(*o*-methoxyphenoxy)ethyl)amino)-2-propanol (Fig. 1), is a nonselective β-blocking agent [1,2] and it also has vasodilating properties that are attributed mainly to its blocking activity at α_1-receptors. Carvedilol is a racemic compound, and the nonselective β-blocking activity resides mainly in the (*S*)-carvedilol, while the α-blocking activity is shared by (*R*)- and (*S*)-enantiomers [3,4], but this drug is used clinically as a racemic mixture of both enantiomers. Carvedilol is used in the treatment of mild to moderate hypertension and angina pectoris [5] and is often used in combination with other drugs.

To evaluate the pharmacokinetic interactions among these drugs in experimental animals, a simple method for the measurement of the drug concentrations in the plasma is desirable. The published methods for the determination of plasma carvedilol have a number of drawbacks. In addition, much time is required during the analysis to condense and dry the extracts obtained using diethyl ether [6].

Accordingly, for the investigation of the pharmacokinetics of carvedilol, we have developed a simple, sensitive method for the determination of carvedilol in rat plasma. It requires only small plasma samples and employs a solid-phase extraction

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(SPE) for easy pretreatment although this method was a nonstereospecific assay.

2. Experimental

2.1. Reagents

Carvedilol was kindly donated by Daiiti Pharmaceutical (Japan). Ethyl *p*-hydroxybenzoate was used as internal standard (I.S.). Methanol was for high-performance liquid chromatography (HPLC) and other reagents were of analytical grade.

2.2. Animals

Twenty male Sprague–Dawley rats (250–310 g) were housed three or four to a plastic walled cage (26×36×25 cm), and had unlimited access to food and water except for 12 h before and during the experiment. The animals were maintained on a 12-h light–dark cycle (light on from 08:00 to 20:00). The ambient temperature and humidity were kept at 22–24°C and ca. 60%, respectively.

The animals used in this study were handled in accordance with the Guidelines for Animal Experimentation of the University of the Ryukyus, and the experimental protocol was approved by the Animal Care and Use Committee of this institution.

2.3. Apparatus for determining the plasma drug level

For plasma separation from the blood sample, a hematocrit-centrifuge (himac CT12, Hitachi) was used. A spectrophotometer (Type RF-540, Shimadzu) was used for selecting the absorption wavelength to detect carvedilol in plasma. The drug plasma concentrations were determined by HPLC (pump-type LC-6A, Shimadzu) with a spectrofluorometric detector (Type RF-530, Shimadzu), and were calculated using a data module (Type C-R3A, Shimadzu). A stainless steel column packed with octadecyl silica (Shim-pack CLC-ODS, 150×6.0 mm I.D., 5-μm particle size, Shimadzu) was maintained at 40°C. Sample were injected with an automatic sample processor (Type SIL-6A, Shimadzu). The mobile phase was methanol–50 m*M* KH_2PO_4, pH 2.5 (60:40, v/v) and the flow-rate was 1.0 ml/min. Measurements were made at an excitation wavelength of 247 nm and emission wavelength of 344 nm.

2.4. Spiking of plasma with drug

For the extraction of carvedilol and the I.S., a Bond Elut (1-ml volume, Lot No. 062217, Varian) SPE column containing octylsilica was used. The column was prewashed twice with 1 ml of methanol, followed by washings with 1 ml of distilled water. Then 500 μl of 20 m*M* KH_2PO_4, pH 4.0, 20 μl of plasma containing carvedilol and 15 μl of 100 μg/ml I.S. solution were added to the column. A 0.5-ml portion of distilled water followed by 0.5 ml of 20 m*M* KH_2PO_4, pH 2.5 were passed through the column, then carvedilol and the I.S. retained on the column were eluted with 500 μl of elution solvent (methanol–20 m*M* KH_2PO_4, pH 2.5, 90:10, v/v). A 20-μl volume was used for injection into the HPLC systems. This volume of elution solvent did not affect the shape of the chromatogram. The chromatographic peaks of carvedilol and the I.S. from plasma samples were coincident with those of the authentic standards.

2.5. Calibration curve

Carvedilol solutions of 15.6, 31.3, 62.5, 125, 250, 500 and 1000 ng/ml were prepared by dilution of stock standard with 20 m*M* KH_2PO_4, pH 4.0. These solutions were injected into the HPLC apparatus after the same pretreatment as for drug-free plasma. The calibration curve for carvedilol concentration was made from the peak-area ratio of carvedilol and the I.S.

2.6. Recovery

To 20 μl of blank plasma, 20 μl of carvedilol solutions at concentrations of 31.3, 62.5, 125, 250, 500 and 1000 ng/ml (dissolved in 20 m*M* KH_2PO_4, pH 4.0) and 15 μl of the I.S. solution were added to the SPE column. These solutions were injected into the HPLC apparatus after the same pretreatment. The recovery of carvedilol was obtained by comparing

with the equivalent amount of carvedilol which was injected directly.

2.7. Precision

To investigate the precision of plasma carvedilol measurements, the present method was used to perform a within-day assay and a between-day assay. Blood samples with carvedilol were obtained from the cervical vein after carvedilol (20 mg/kg) was administered orally (unknown samples I and II) and spiked samples III–VI (1000, 500, 125 and 12.5 ng/ml) were analyzed ten times (within-day assay). For the between-day assay, unknown sample VII and spiked sample VIII (31.3 ng/ml) were analyzed for 7 days.

2.8. Correlation between plasma samples obtained from the cervical vein and tail vein

Blood samples from the tail vein as well as the cervical vein were obtained under laparotomy 1–2 h after carvedilol was administered orally at a dose of 20 or 40 mg/kg. The animals were anesthetized with diethyl ether before the laparotomy operations. Plasma was separated by centrifugation, and the correlation between the two sets of samples was determined as described above.

2.9. Concentration–time profile of plasma carvedilol

To determine the concentration–time profile of plasma carvedilol, blood samples were obtained from the tail vein at 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after oral administration of carvedilol. Carvedilol was administered to the rats at doses of 20 and 40 mg/kg as a 0.5% carboxylmethyl cellulose suspension through a nasal catheter. The animals were anesthetized with diethyl ether before oral administration. The drug concentration in the plasma was measured. The pharmacokinetic parameters were obtained from the carvedilol concentration, using a personal computer program of PHACONET-TWO (System Wave) for non-linear least-squares regression [7]. The maximum plasma concentration (C_{max}) and the corresponding time (t_{max}), the absorption rate constant (K_a), the elimination half-life ($t_{1/2}$) and the area under the plasma concentration–time curve ($AUC_{0\rightarrow\infty}$) were estimated by the computer program.

3. Results

3.1. Chromatography

As shown in Fig. 1, the retention times of carvedilol and the I.S. were 4.8 and 6.5 min, respectively. The I.S. was chosen for suitable fluorometric absorption and its retention time at the detection conditions of carvedilol. No other peaks corresponding to these retention times were noted in the chromatogram of drug-free plasma, indicating that interfering endogenous substances were not present. The analysis method proposed this paper uses a spectrofluorometric detector, therefore if co-administered drugs have no fluorometric absorption, they do not interfere with the carvedilol determination.

3.2. Calibration curve

The standard curve was prepared by analyzing carvedilol plasma solutions at concentrations of 15.6, 31.3, 62.5, 125, 250, 500 and 1000 ng/ml. Between the concentration of carvedilol in blood and the peak-area ratio of carvedilol to I.S., an excellent linear correlation was noted in the range 0–1000 ng/ml. The coefficient of correlation (r) was 0.9995 and the regression equation was $y = 24.297x + 0.2437$. The limit of detection at a signal-to-noise ratio of 3 was 3.6 ng/ml.

3.3. Recovery and precision

The rates of recovery calculated by adding 31.3, 62.5, 125, 250, 500 and 1000 ng/ml carvedilol to blank plasma samples averaged 94.2% (98.5–90.5%) (Table 1).

The coefficients of variation (C.V.) in the unknown samples I, II and spiked samples III–VI were 2.7–7.5% for the within-day assay (Table 2), indicating a good reproducibility. The C.V. in the unknown sample VII was 2.6% (249.0±6.5 ng/ml) and in the

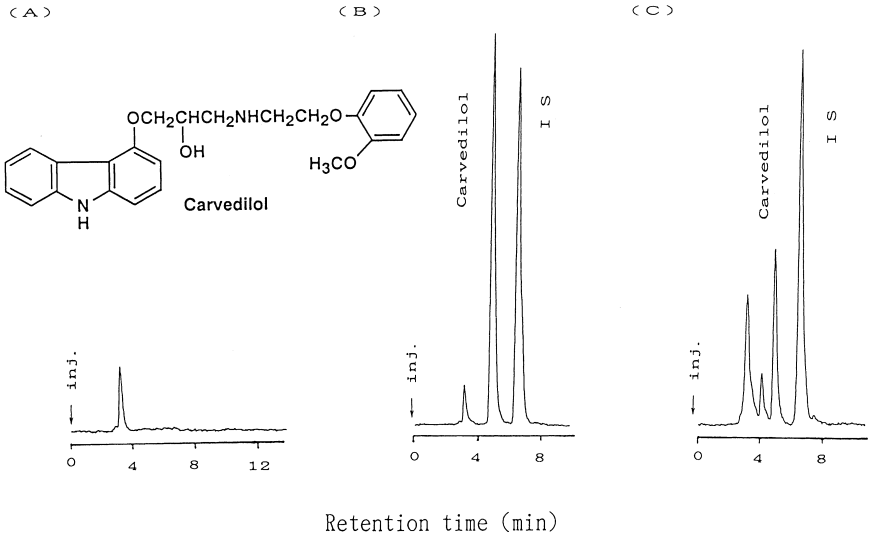

Fig. 1. Typical chromatograms for carvedilol and the internal standard (I.S.). (A) Drug-free plasma (B) spiked plasma (carvedilol 500 ng/ml, I.S. 3 μg/ml) (C) rat plasma 60 min following oral administration of carvedilol at a dose of 10 mg/kg body mass. The rat plasma concentration of carvedilol was 200.6 ng/ml, calculated from peak-area ratio of carvedilol to the I.S.

spiked sample VIII was 7.4% (30.6±2.27 ng/ml) for the between-day assay (7 days).

3.4. Correlation of plasma concentration between blood samples obtained from the cervical vein and the tail vein

In the comparative analysis of the blood samples obtained from the cervical and tail veins in the range 206.2–648.3 ng/ml, the correlation coefficient, r, was 0.965 and the regression equation was $y = 0.9285x + 8.421$, indicating a high correlation between the two.

Table 1
Recovery in proposed method for determination of carvedilol[a]

Added carvedilol to plasma (A) (ng/ml)	Measured (B) (ng/ml)	Recovery (B/A)×100 (%)
1000	984.5±12.96	98.45
500	484.1±18.75	96.83
250	235.8±11.91	94.31
125	116.0±7.43	92.80
62.5	57.8±2.43	90.65
31.3	28.3±2.36	90.50

[a] Each value is the mean±S.D., $n=4$.

3.5. Concentration–time profile of carvedilol

The concentration–time profile for plasma carvedilol following administration of doses of 20 and 40 mg/kg are shown in Fig. 2. The concentration of carvedilol in the plasma increased in a dose-dependent fashion. For each administration group, the values of K_a, t_{max}, C_{max}, $t_{1/2}$ and $AUC_{0\rightarrow\infty}$ are shown in Table 3. The disposition of carvedilol is adequately described by a two-compartment model [7] using

Table 2
Precision of carvedilol in rat plasma determined by HPLC (within-day assay)

Plasma sample with carvedilol	n	Concentration (mean±S.D.) (ng/ml)	C.V. (%)
Sample I (unknown)	10	244.8±13.4	5.5
Sample II (unknown)	10	284.2±18.0	6.3
Sample III (1000 ng/ml)	10	1029.5±27.8	2.7
Sample IV (500 ng/ml)	10	507.8±22.3	4.4
Sample V (125 ng/ml)	10	122.5±9.2	7.5
Sample VI (12.5 ng/ml)	10	12.4±0.8	6.7

Table 3
Pharmacokinetic parameters of carvedilol after oral administration[a]

Carvedilol dose (mg/kg)	K_a (h^{-1})	t_{max} (h)	C_{max} (ng/ml)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	AUC $_{0\rightarrow\infty}$ (μg/ml)
20	2.07±0.50	0.85±0.08	297.7±43.3	0.78±0.15	17.95±4.38	1.46±0.17
40	1.52±0.18	1.02±0.06	491.7±72.3	1.18±0.43	22.33±9.50	2.35±0.37

[a] Each value is the mean±S.E.M.

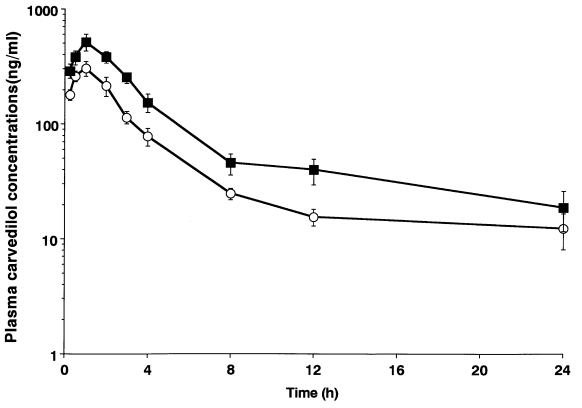

Fig. 2. Plasma carvedilol concentration after oral administration in the rat. Each point represents the mean value for five rats, and vertical lines represents the standard error of the mean; (○) 20 mg/kg; (■) 40 mg/kg.

a personal computer program of PHACONET-TWO. Thus, the pharmacokinetics of carvedilol in small animals can be studied by this method.

4. Discussion

(*RS*)-carvedilol is extensively metabolized and has an absolute bioavailability of about 25%, it undergoes a first-pass effect with hepatic elimination. Both the (*R*)- and (*S*)-enantiomers of carvedilol were metabolized in human liver microsomes, and the (*S*)-enantiomer was metabolized faster than the (*R*)-enantiomer although the same P450 enzymes seemed to be involved in each case. The (*S*)-enantiomers of major carvedilol metabolites (desmethyl- and hydroxy-metabolites) exhibit β-blocking properties, whereas the (*R*)-enantiomers are devoid of β- or α-blocking activities [8,9].

In order to evaluate the drug–drug interactions in small animals, we developed a rapid and simple method for the determination of carvedilol. Although this method is a nonstereoselective assay, it showed a favorable recovery (98.5–90.5%) after pretreatment with Bond Elut, as well as good reproducibility (C.V. 2.7–7.5%). The method also enabled the determination of the plasma concentration using a small volume of plasma (20 μl), such as that obtained from a rat tail vein. In addition, the assay was rapid and easy to perform. Comparison of the plasma concentrations in the cervical and tail veins showed a good correlation ($r=0.995$). By sampling from rat tail veins the time-course of plasma carvedilol following oral administration of 20 and 40 mg/kg could be observed. This method is a rapid and simple way for the micro-determination in plasma and obtaining the plasma carvedilol concentration–time data from a single animal, together with further pharmacokinetic parameters, such as K_a, t_{max}, C_{max}, $t_{1/2}$ and $AUC_{0\rightarrow\infty}$.

It has been reported that clinical doses of carvedilol produce plasma concentrations of 23–79 ng/ml as the C_{max} [7]. The present method may not be adequate to detect concentrations of 2.3 ng/ml (one tenth of the above C_{max}), because the detection limit in this method was 3.6 ng/ml plasma when 20 μl plasma samples were used. However, if the 500-μl of elution solvent are condensed or plasma sample volumes of more than 40 μl are used for detecting plasma carvedilol in patients receiving oral doses of the drug, it may be possible to monitor plasma concentrations of less than 2.3 ng/ml.

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