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Stereoselective Disposition and Tissue Distribution of Carvedilol Enantiomers in Rats

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ABSTRACT After intravenous bolus injection of *rac*-carvedilol at 2 mg/kg to the rat, the (+)-(R)- and (−)-(S)-enantiomer levels in the blood and tissues (liver, kidney, heart, muscle, spleen, and aorta) were measured by stereospecific HPLC assay. As compared with the (+)-(R), the (−)-(S) had a larger Vd_{ss} (3.32 vs. 2.21 liter/kg), MRT (33.4 vs. 25.6 min), and CL_{tot} (96.1 vs. 83.8 ml/min/kg). AUC comparison after iv and po administration showed systemic bioavailability of the (−)-(S) to be about half that of its antipode, explained by the fact that the free fraction of the (−)-(S) in blood was 1.65-fold greater than that of the (+)-(R). Tissue-to-blood partition coefficient values for the (−)-(S) were 1.6- to 2.1-fold greater than those for the (+)-(R) in all tissues, showing that the (−)-(S) accumulates more extensively in the tissues. These results were consistent with the greater Vd_{ss} for the (−)-(S) estimated from systemic blood data. The stereoselective tissue distribution of carvedilol enantiomers results from an enantiomeric difference in plasma protein binding rather than in tissue binding.

KEY WORDS: carvedilol enantiomers, kinetics, tissue-to-blood partition coefficient, plasma protein binding

INTRODUCTION

Rac-carvedilol, (±)-1-(carbazol-4-yloxy)-3[[2-(2-methoxyphenoxy) ethyl]amino]-2-propanol (Fig. 1), is a potent antihypertensive drug with two pharmacological effects, vasodilation and β-adrenoceptor blockade.[1] Both enantiomers are equally responsible for vasodilation, while β-blockade is caused by the (−)-(S)-enantiomer only.[2,3] The disposition of carvedilol enantiomers is stereoselective in humans and monkeys.[4–6] The (−)-(S)-enantiomer is preferentially removed from the blood after oral administration of *rac*-carvedilol in both species. Consequently, the oral bioavailability of the (−)-(S)-enantiomer is about half that of its antipode in humans; this is considered due to stereoselective first-pass metabolism in the liver. On the other hand, the analysis of the pharmacokinetic parameters for both enantiomers after administration of *rac*-carvedilol to humans has shown the distribution volume of the (−)-(S)-enantiomer to be greater than that of its antipode.[4,6] These findings thus imply that carvedilol enantiomers exhibit stereoselective tissue distribution in humans. The composition of the enantiomers in the target organ/tissue, namely the heart and aortic wall rather than in the plasma, should more directly reflect the pharmacological effects of this drug. However, there is little information concerning the tissue distribution of carvedilol enantiomers. As it is impossible to directly prove stereoselective tissue distribution of these enantiomers in humans, we used the rat as an animal model. Unfortunately, pharmacokinetics of carvedilol enantiomers in rat has not been studied. The purpose of this study was to clarify the pharmacokinetics of carvedilol enantiomers in the rat, and to determine whether stereoselective distribution occurs in the organs/tissues following intravenous administration of *rac*-carvedilol to this species.

MATERIALS AND METHODS

Reagents

Rac-carvedilol, [^{14}C]*rac*-carvedilol, (+)-(R)-carvedilol, and (−)-(S)-carvedilol were generously provided by Boehringer Mannheim GmbH (Mannheim, Germany). The optical purities of the (+)-(R)-enantiomer and (−)-(S)-enantiomer were 98.8 and 97.3%, respectively. [^{14}C]Carvedilol enantiomers were prepared by direct resolution of [^{14}C]*rac*-carvedilol on a chiral HPLC column according to our previous report.[7] The radiochemical and optical purities of the (+)-(R)-enantiomer were 96.0 and 100%, respectively, and 96.0 and 99.5% for its opposite enantiomer.

All other chemicals were of HPLC and reagent grade.

Animal Experiments

Male Sprague–Dawley rats weighing 230 to 250 g from Charles River Japan Inc. (Kanagawa, Japan) were used for all experiments. For intravenous study, three animals in each group received a bolus injection of *rac*-carvedilol at 2 mg/kg via the right femoral vein. The dosing solution was prepared according to our previous report.[7] At 0.5, 5, 15, 30, 45, 60, 90, 120, 180, and 240 min after injection, animals were anesthetized with ether and 0.1 ml of heparin 100 units was injected intravenously via the left femoral vein. The abdomens were immediately opened, and blood samples were taken by puncture of the abdominal artery using a syringe; organs and tis-

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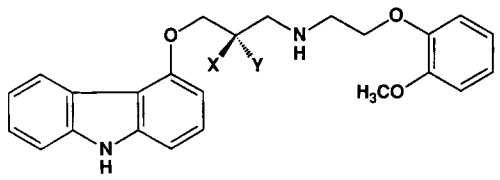

(+)-(R)-carvedilol X=OH, Y=H
(-)-(S)-carvedilol X=H, Y=OH

Fig. 1. Chemical structures of carvedilol enantiomers.

sues including the liver, kidney, heart, muscle, spleen, and thoracic aorta were quickly excised. Portions of the blood samples were centrifuged, and plasma samples collected. These blood, plasma, and tissue samples were stored at −20°C until analysis.

For oral study, all animals were fasted overnight prior to drug administration. Eight animals in each group were given orally *rac*-carvedilol at 10 mg/kg as a suspension in 0.5% aq. sodium carboxymethylcellulose solution. Water was available *ad libitum* during the experiment. Feeding was resumed 4 h after dosing. Blood samples were collected at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h after dosing in the same manner as in the intravenous study, but organ/tissue specimens were not collected. Blood samples were centrifuged and plasma samples collected. These samples were stored at −20°C until analysis.

Analytical Methods

A stereospecific HPLC assay[4] using the diastereoisomeric derivatization of carvedilol with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate as a chiral reagent was used for the quantitation of the (+)-(R)- and (−)-(S)-enantiomers in blood, plasma, and tissue samples.

One milliliter of the blood sample was hemolyzed by the addition of the same volume of water. After measuring the wet weights of the kidney, heart, and spleen, and also its weight of about 1 g of liver and muscle, each tissue sample was mixed with three times 0.1 *M* Britton-Robinson buffer at pH 8.0, and then homogenized with a mechanical homogenizer (Physcotron, Nichion Irika, Co., Japan). Two milliliters of the homogenate was used to determine the concentrations of enantiomers. For the aorta, the tissue was minced in 1 ml of 0.1 *M* Britton-Robinson buffer at pH 8.0, then homogenized with a 2 ml of glass homogenizer. The entire homogenate sample was used for drug measurement. After addition of 250 ng internal standard, 1-(*o*-methoxyphenyl)-4-[3-(naphthyloxy)-2-hydroxypropyl]piperazine, to the hemolyzed blood, plasma, and tissue homogenate samples, the samples were treated according to a previous report.[4] Typical HPLC chromatograms of the blood, plasma, and heart samples are shown in Figure 2.

Calibration curves for the enantiomers were prepared by the addition of 4 to 500 ng of *rac*-carvedilol to each blank sample and obtained by plotting peak area ratios of each enantiomer to the internal standard versus concentrations of the enantiomer. Equations were calculated by the least-squares method using linear regression. All calibration curves for each enantiomer showed good linearity, with correlation coefficients of more than 0.999.

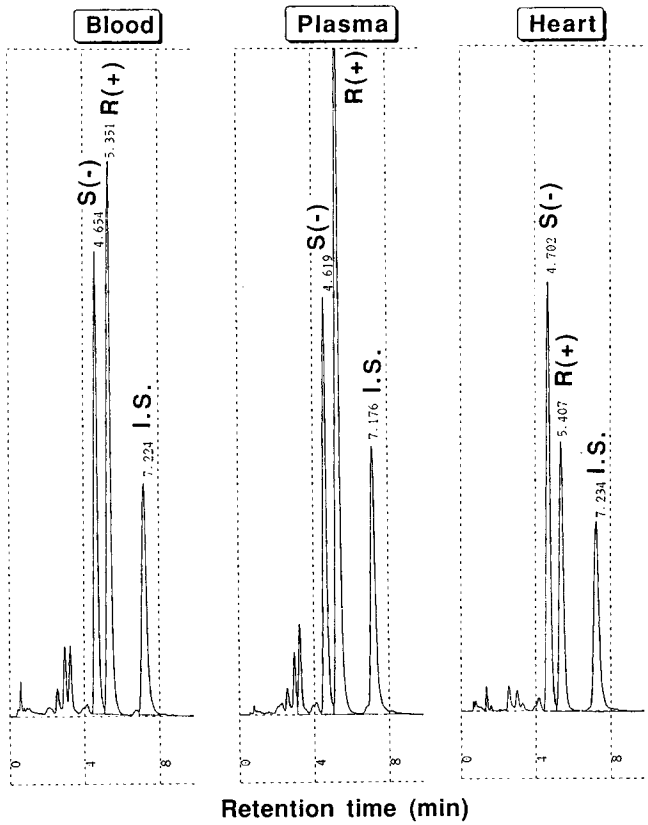

Fig. 2. HPLC chromatograms of the blood, plasma, and heart at 30 min after iv administration of *rac*-carvedilol at 2 mg/kg to rats.

Calculation of Binding Parameters

The blood/plasma concentration ratios (C_b/C_p) for carvedilol enantiomers were determined as follows: each radiolabeled carvedilol enantiomer at 50 ng was added to 1 ml of blank blood collected from each of four rats. After incubation for 30 min at 37°C, the blood samples were centrifuged and plasma samples were collected. An aliquot (200 μl) of plasma was mixed with 12 ml of Aquasol scintillation liquid (NEN, Boston, MA), and radioactivity was measured directly with an LSC-900 liquid scintillation counter (Aloka, Co., Ltd., Tokyo, Japan). C_b/C_p for each enantiomer was determined from the following equation:

C_b/C_p = Concentration in blood/Concentration in plasma.

In vivo plasma protein binding of carvedilol enantiomers was determined by the equilibrium dialysis method. One milliliter of fresh rat plasma containing 50, 150, and 250 ng each of the radiolabeled (+)-(R)- and (−)-(S)-enantiomers was dialyzed in Teflon cells (Spectrum Medical Industries Inc. USA) with a cellulose membrane (molecular cutoff 12,000) (Spectra/Por 2, Spectrum) against 1 ml of 66 m*M* phosphate-buffered saline, pH 7.4, for 3 h at room temperature by rotating the cells. After dialysis, radioactivity in 500-μl aliquots from both sides of the membrane was measured by the same method described above. The free fraction of each enantiomer in plasma (f_p) was calculated from the following equation:

f_p = dpm in buffer/dpm in plasma.

The free fraction of each enantiomer in blood (f_b) was calculated from the following equation[8]:

$$f_b = f_p \cdot \frac{C_p}{C_b}.$$

Calculation of Pharmacokinetic Parameters

AUCs for blood (AUC_b) and plasma (AUC_p) were calculated by the trapezoidal rule using the mean concentration of three or eight rats and extrapolated to infinity. Total body clearance (CL_{tot}) was then calculated as dose(iv)/AUC_b. Distribution volume (Vd_{ss}) was determined by the following equation[9]:

$$Vd_{ss} = \frac{\text{Dose(iv)} \cdot \text{AUMC}}{(AUC_b)^2}$$

where AUMC is the total area under the first moment of the drug concentration curve from zero to infinity.

Mean residence time (MRT) was calculated from the following equation[9]:

$$\text{MRT} = \frac{\text{AUMC}}{AUC_b}.$$

Systemic bioavailabilities (F) of the enantiomers were calculated from the following equation[9]:

$$F = \frac{\text{Dose(iv)} \cdot AUC_b(\text{po})}{\text{Dose(po)} \cdot AUC_b(\text{iv})}$$

AUC_b(po) was determined by AUC_p(po)·C_b/C_p.

Tissue-to-blood partition coefficients (K_p) of the liver and other tissues were calculated from the following equations[10]:

$$K_p \text{ (liver)} = \frac{1}{F_h} \cdot \frac{K_{p(app)}}{(1 + \beta \cdot V_h \cdot K_{p(app)})/Q_h}$$

$$K_p \text{ (other tissue)} = \frac{K_{p(app)}}{(1 + \beta \cdot V_t \cdot K_{p(app)})/Q_t}$$

where $K_{p(app)}$ is the apparent tissue-to-blood partition coefficient and is determined from the tissue/blood concentration ratio in the β-phase, β is the first-order elimination rate constant in each tissue, and V_t and Q_t are volume and blood flow rate in tissue, respectively. Values for V_t and Q_t were quoted from the literature[11–14] (see Table 4). Hepatic blood flow for this experiment was estimated as 111.3 ml/min/kg. F_h is the availability of drug in the liver and obtained from the systemic bioavailability corrected by the intestinal absorption rate of drug.

Free fraction of drug in tissue (f_t) was calculated from the following equation:

$$f_t = q \cdot \frac{f_b}{K_p}$$

where q is a pH-dependent partition coefficient which for carvedilol is 2.08 using $pK_a = 7.8$ from the equation as follows:

$$q = \frac{1 + 10^{pk_a - pH_i}}{1 + 10^{pk_a - pH_e}}$$

where pH_i and pH_e are the intra- and extracellular pH values, respectively. The values for pH_i and pH_e were quoted from the literature[15] at 7.0 and 7.4, respectively.

Statistical Analysis

Statistical significance was assessed by Student's paired and unpaired t test.

RESULTS

Figure 3 shows mean concentrations of the (−)-(S)- and (+)-(R)-enantiomers in blood after intravenous bolus injection of *rac*-carvedilol. Concentrations of both enantiomers declined in a biphasic manner but were not parallel. Until 45 min after dosing, concentrations of the (+)-(R)-enantiomer were higher than those of the (−)-(S)-enantiomer, showing a maximum R/S ratio of 1.48 at 15 min, whereas 90 min after dosing the concentrations of the (+)-(R)-enantiomer were lower than those of its antipode, showing a minimum R/S ratio of 0.45 at 180 min after dosing.

Figure 4 shows mean concentrations of the (−)-(S)- and (+)-(R)-enantiomers in plasma after oral administration of *rac*-carvedilol. Plasma concentrations of the (+)-(R)-enantiomer exceeded those of its antipode until 6 h after dosing. Both enantiomers reached maximum concentration at 30 min after dosing. The C_{max} value was 112.1 ± 9.6 ng/ml for the (+)-(R)-enantiomer and 44.5 ± 5.2 ng/ml for (−)-(S)-enantiomer. As a result, AUC(po) of the (+)-(R)-enantiomer was 2.17 times greater than that of its antipode.

Table 1 lists pharmacokinetic parameters for these enantiomers. These were compared with each other to clarify the disposition of the two enantiomers. Both exhibited relatively high blood clearance values that almost equalled hepatic blood

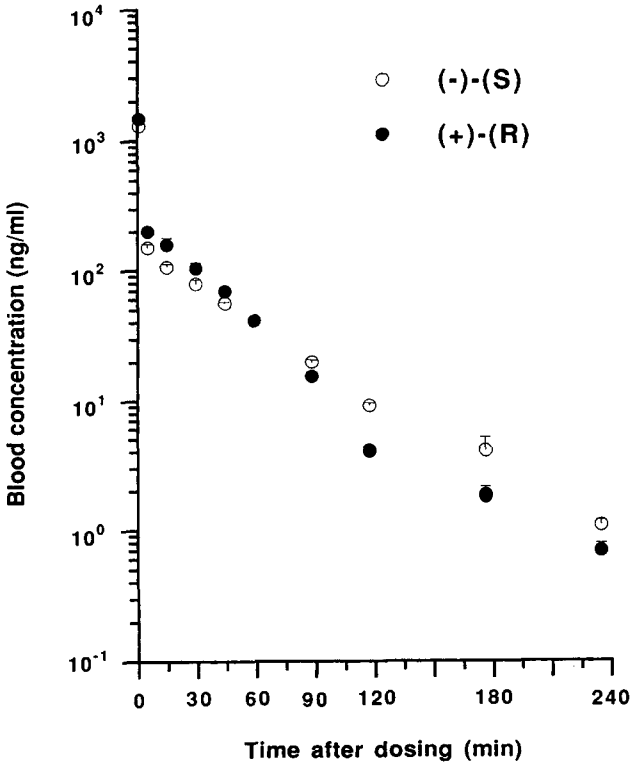

Fig. 3. Mean blood concentrations of (+)-(R)-enantiomer (●) and (−)-(S)-enantiomer (○) in rats after intravenous administration of *rac*-carvedilol at a dose of 2 mg/kg. Mean ± SE; $n = 3$.

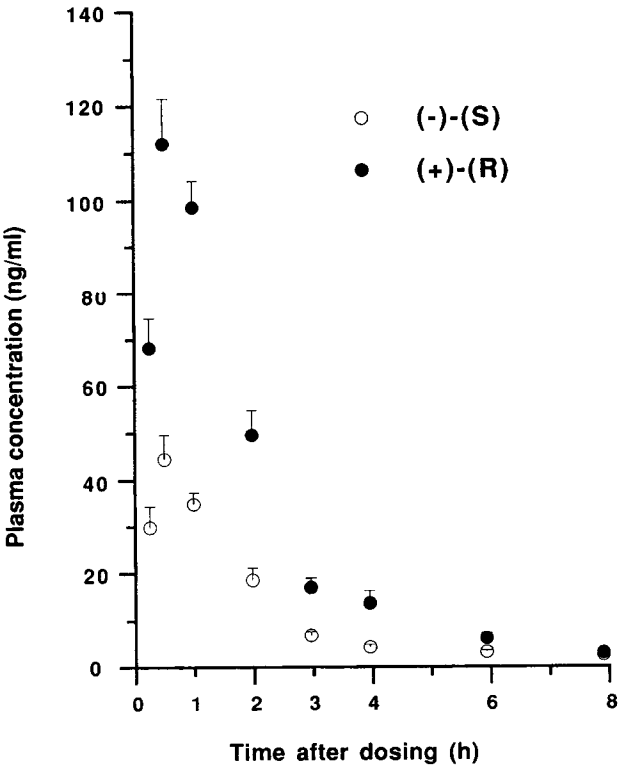

Fig. 4. Mean blood concentrations of (+)-(R)-enantiomer (●) and (−)-(S)-enantiomer (○) in rats after oral administration of *rac*-carvedilol at a dose of 10 mg/kg. Mean ± SE; $n=8$.

flow. Clearance of the (−)-(S)-enantiomer (96.1 ml/min/kg) was slightly greater than that of the (+)-(R)-enantiomer (83.8 ml/min/kg). Further, Vd_{ss} of the (−)-(S)-enantiomer (3.32 liter/kg) was 1.5 times greater than that of its antipode (2.21 liter/kg) and both exceeded total body water (0.70 liter/kg),[16] showing their accumulation in tissues. Consequently, MRT of the (−)-(S)-enantiomer (33.1 min) was slightly greater than that of the (+)-(R)-enantiomer (25.0 min), while systemic bioavailability of the (−)-(S)-enantiomer was 65% that of its antipode.

In vitro plasma protein binding of both enantiomers was extremely high in the 50 to 250 ng/ml concentration range, showing values of 95.4 to 96.9% for the (−)-(S)-enantiomer and 98.1 to 98.2% for the (+)-(R)-enantiomer. The f_p of the (−)-(S)-enantiomer (0.037) was significantly higher than that of the (+)-(R)-enantiomer (0.018) (Table 2). C_b/C_p of the (−)-(S)-enantiomer (1.01) was also significantly greater than that of the (+)-(R)-enantiomer (0.81), suggesting that the extent of distribution of the (+)-(R)-enantiomer to red blood cells (RBC) is lower than that of the (−)-(S)-enantiomer (Table 3). However, there was no enantiomeric difference in the distribution of free drug to RBC; RBC/plasma(free) concentration ratios of the (−)-(S)- and (+)-(R)-enantiomers were almost identical at 27.8 and 28.2, respectively (Table 3). The f_b of the (−)-(S)-enantiomer (0.0366) was significantly higher than that of the (+)-(R)-enantiomer (0.0222).

Figure 5 shows mean concentrations of the (−)-(S)- and (+)-(R)-enantiomers in various tissues after intravenous bolus injection of *rac*-carvedilol. Concentrations for both in the liver, kidney, heart, and aorta declined in a biphasic manner, identical to those in blood. At 0.5 min, the initial sampling point after dosing, the kidney, heart, muscle, spleen, and aorta concentrations of both enantiomers were almost identical. Thus, tissue/

TABLE 1. Pharmacokinetic parameters after intravenous and oral administration of *rac*-carvedilol to rats

Parameter	(−)-(S)	(+)-(R)	(S)/(R)
AUC(iv)[a] (ng·h/ml)	173.5	199.0	0.87
AUC(po)[b] (ng·h/ml)	115.0	204.4	0.56
CL_{tot}[c] (ml/min/kg)	96.1	83.8	1.15
Vd_{ss}[c] (liter/kg)	3.32	2.21	1.50
MRT[c] (min)	33.4	25.6	1.30
F[b]	0.133	0.205	0.65
f_b	0.0366	0.0222	1.65

[a]AUC(iv) was calculated from data based on mean blood concentrations after intravenous administration of *rac*-carvedilol at a dose of 2 mg/kg to rats.
[b]AUC(po) was obtained by multiplying AUC for plasma by C_b/C_p. *Rac*-carvedilol was dosed orally at 10 mg/kg to rats
[c]Parameter based on blood concentrations.

TABLE 2. Unbound fraction of carvedilol enantiomer in rat plasma in vitro[a]

Animal number	(−)-(S)-carvedilol (ng/ml)			(+)-(R)-carvedilol (ng/ml)		
	50	150	250	50	150	250
1	0.050	0.034	0.027	0.025	0.019	0.019
2	0.048	0.036	0.031	0.014	0.019	0.019
3	0.039	0.035	0.027	0.019	0.019	0.020
4	N.T.[b]	0.034	0.039	0.012	0.018	0.019
Mean ± SD	0.046 ± 0.006	0.035 ± 0.001	0.031 ± 0.006	0.018* ± 0.006	0.019* ± 0.001	0.019** ± 0.001

[a]Plasma unbound fraction of each enantiomer was determined by the equilibrium dialysis method. Eqilibrium dialysis of 1 ml of rat plasma to which was added each radiolabeled carvedilol enantiomer in 10 µl solution was performed against 1 ml of isotonic phosphate buffer for 3 h at room temperature.
[b]N.T., not tested.
*$P<0.01$ compared to (−)-(S)-enantiomer.
**$P<0.05$ compared to (−)-(S)-enantiomer.

TABLE 3. The blood-to-plasma partition coefficient and RBC/plasma (unbound) ratio for carvedilol enantiomers in rats

Animal number	C_b/C_p		RBC/plasma$_{(unbound)}$	
	(−)-(S)	(+)-(R)	(−)-(S)	(+)-(R)
1	0.940	0.749	23.0	21.3
2	0.990	0.803	26.3	26.3
3	0.968	0.791	24.9	24.7
4	1.034	0.835	29.1	32.5
5	1.122	0.873	35.7	36.3
Mean ± S.E.	1.011 ± 0.032	0.810* ± 0.021	27.8 ± 2.5	28.2 ± 3.0

*$P<0.01$ compared to (−)-(S)-enantiomer.

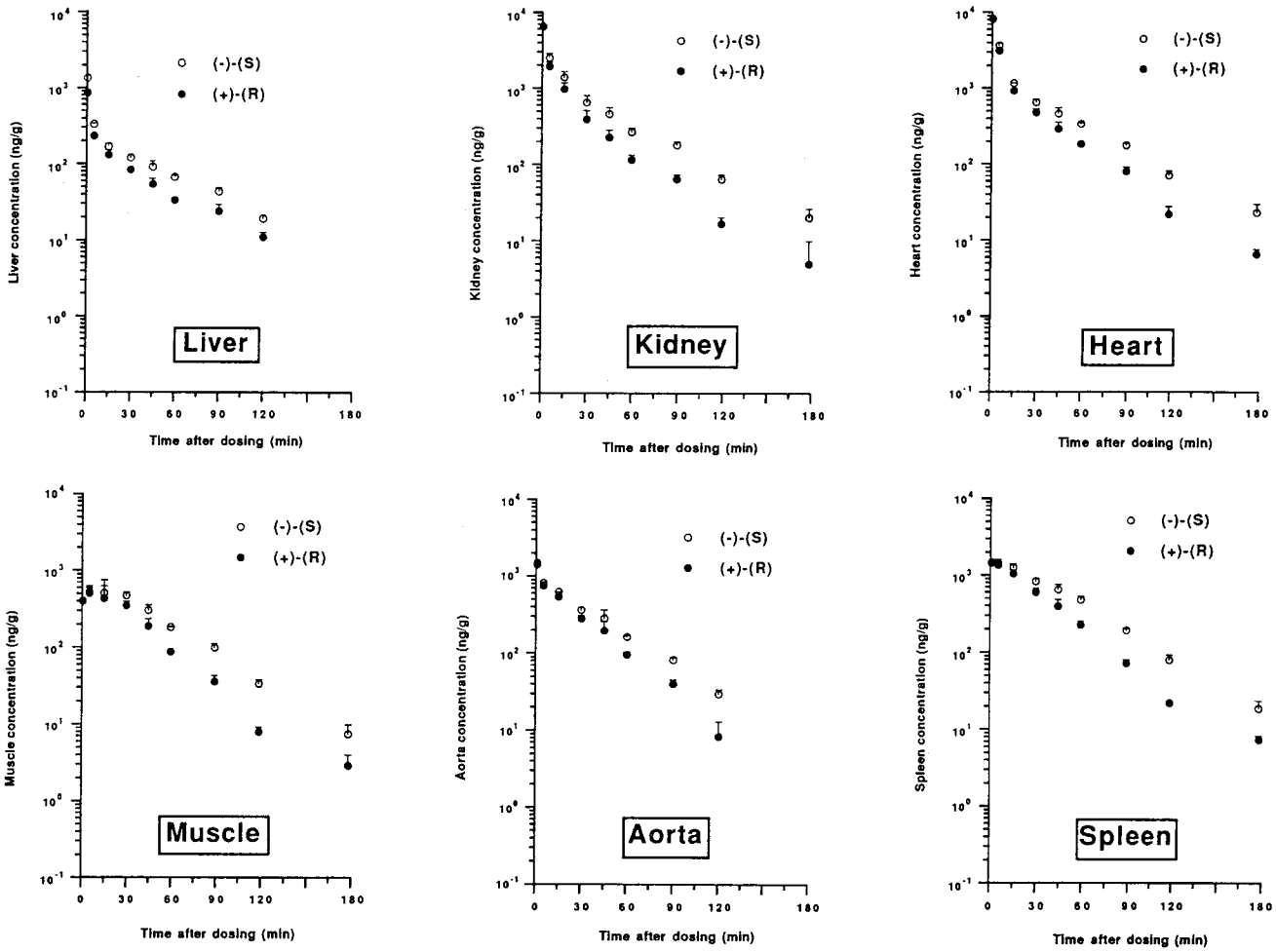

Fig. 5. Tissue concentrations of (+)-(R)-enantiomer (●) and (−)-(S)-enantiomer (○) in rats after intravenous administration of *rac*-carvedilol at a dose of 2 mg/kg. Mean ± SE; $n = 3$.

blood enantiomer concentration ratios for the (−)-(S)- and (+)-(R)-enantiomers were respectively 5.7 and 5.8 in the heart, 4.6 and 4.5 in the kidney, 1.1 and 1.0 in the spleen, 1.0 for both in the aorta, and 0.28 for both in the muscle at 0.5 min after dosing. At 5 min after dosing, however, concentrations of the (−)-(S)-enantiomer in these tissues were higher than those of its antipode, and this tendency accelerated with increasing time after dosing. In the liver, concentrations of the (−)-(S)-enantiomer were higher than those of its antipode throughout the experimental period.

The tissue/blood concentration ratios for both enantiomers were calculated from the terminal phase, in which pseudodistribution equilibrium is attained in each organ/tissue. These ratios were actually determined by calculating mean tissue/blood concentration ratios for both enantiomers from 30 min after dosing to the measurable end point. Apparent K_p values for the (−)-(S)- and (+)-(R)-enantiomers were as follows: 1.76 and 1.29 in the liver, 7.17 and 3.40 in the kidney, 7.67 and 4.47 in the heart, 4.30 and 2.28 in the muscle, 9.25 and 5.05 in the spleen, and 4.03 and 2.66 in the aorta, respectively. K_p values for the (−)-(S)- and (+)-(R)-enantiomers were as follows: 11.42 and 5.42 in the liver, 6.91 and 3.31 in the kidney, 7.36 and 4.34 in the heart, 1.59 and 0.97 in the muscle, and 6.01 and 3.57 in the spleen, respectively (Table 4). These results showed greater accumulation of the (−)-(S)-enantiomer than of the (+)-(R) in these tissues.

Free fractions of both enantiomers in the liver, kidney, heart, and spleen were lower than those in the blood, showing the accumulation of both enantiomers in these organs. In muscle, however, the opposite result was obtained, with both enantiomers showing relatively small accumulations. The free fractions of both enantiomers in the heart, muscle, and spleen were almost identical, with the S/R ratios of 0.97, 1.01, and 0.98, respectively. In contrast, free fraction of the (−)-(S)-enantiomer in the liver and kidney was slightly lower than that of the (+)-(R)-enantiomer, with the S/R ratio of 0.77 in the liver and 0.79 in the kidney (Table 4).

DISCUSSION

Provided that drug transferred into RBC and also drug present in RBC is available for elimination, drug clearance should be calculated according to data based on blood rather than plasma concentrations.[17] The present study showed that carvedilol enantiomers are distributed to the RBC. In addition, the fact that the hepatic extraction ratios for both enantiomers are greater than the fraction of the blood occupied by plasma supports the justification for the use of whole blood concentrations in the calculation of kinetic parameters. Under these cir-

TABLE 4. The tissue-to-blood partition coefficient (K_p) and unbound fraction in tissue (f_t) for carvedilol enantiomers in rats[a]

	$K_{p(app)}$			K_p			f_t		
Tissue	(−)-(S)	(+)-(R)	(S)/(R)	(−)-(S)	(+)-(R)	(S)/(R)	(−)-(S)	(+)-(R)	(S)/(R)
Liver	1.76	1.29	1.36	11.42	5.42	2.11	0.0067	0.0087	0.77
Kidney	7.17	3.40	2.11	6.91	3.31	2.09	0.0110	0.0139	0.79
Heart	7.67	4.47	1.72	7.36	4.34	1.70	0.0103	0.0106	0.97
Muscle	4.30	2.28	1.89	1.59	0.97	1.64	0.0478	0.0474	1.01
Spleen	9.25	5.05	1.83	6.01	3.57	1.68	0.0127	0.0129	0.98

[a]Blood flow rate (Q_t) and volume (V_t) values in each tissue except liver were quoted from the literature[11-14] as follows: Q_K = 11.4 ml/min, V_k = 2.0 ml; Q_H = 4.2 ml/min, V_H = 1.0 ml; Q_M = 6.8 ml/min, V_M = 125 ml; Q_S = 0.4 ml/min, V_S = 1.0 ml. Liver blood flow rate was estimated as 26.3 ml/min and volume was measured as 11.0 ml.

cumstances, blood clearance provides a valid assessment of the clearance of carvedilol enantiomers. If the plasma clearance values as the total body clearance of carvedilol enantiomers are calculated, the total body clearance which should be represented by blood clearance is underestimated for the (+)-(R)-enantiomer. For the (−)-(S)-enantiomer, however, plasma clearance is almost equal to blood clearance.

The extent of the (−)-(S)-enantiomer distribution to the RBC is greater than that of the (+)-(R)-enantiomer. This can be explained by the differences in f_p between the two enantiomers, as there were no enantiomeric differences in the uptake of drug into the RBC.

Biliary excretion of radioactivity following oral and intravenous administration of [^{14}C]*rac*-carvedilol to the bile duct-cannulated rat is reported to amount to about 85% of the dose.[18] Thus, carvedilol is mostly metabolized via carbazole ring hydroxylation followed by glucuronidation in the liver, thereafter being excreted into the bile.[19] For both enantiomers, therefore, blood clearance represents liver clearance. The total body clearance is the same for both enantiomers, because the determining factor is not drug metabolizing enzyme activity but rather drug delivery to the liver, i.e., liver blood flow. The difference between enantiomers in AUC(po) was accelerated in comparison with that in AUC(iv). Considering the intestinal absorption of each enantiomer at 0.878[18] and assuming no intestinal metabolism, the hepatic extraction ratio for the enantiomers (E_h) could be estimated as $E_h = 1 - F/0.878$. Consequently, hepatic blood flow (Q_h) could be calculated from $Q_h = Cl/E_h$ and Q_h for the (−)-(S)- and (+)-(R)-enantiomers being 113.3 and 109.3 ml/min/kg, respectively. These values are slightly greater than the hepatic blood flow which has been reported to be 98.0 ml/min/kg in rats.[20] The relatively good accordance of Q_h estimated from kinetic parameters of both enantiomers implies that the clearance values and extraction ratios for both enantiomers were reasonable. In general, intrinsic clearance of free drug [Cl_{int}(free)] can be calculated according to the following equation[8]:

$$E = \frac{q \cdot f_b \cdot Cl_{int}(free)}{Q + q \cdot f_b \cdot Cl_{int}(free)}$$

where Q is the average value for liver blood flow, estimated in the present study as 111.3 ml/min/kg. As a result, there was no difference in Cl_{int}(free) between the (−)-(S)-enantiomer (6062 ml/min/kg) and the (+)-(R)-enantiomer (5857 ml/min/kg). These findings show that the difference between enantiomers in oral clearance expressed as f_b. Cl_{int}(free) can be simply explained by the difference of f_b. In other words, the difference in AUC between the enantiomers after oral administration of *rac*-carvedilol is ascribed to the difference of plasma protein binding between the enantiomers.

Clearance values for both enantiomers exceeded the volume of free drug delivered by the blood per unit of time. Therefore, both enantiomers belong to the classification of "nonrestricted drugs." For these drugs, the increase in plasma binding results from the decrease in half-life of the drug, which itself is due to the increase in the rate of drug delivery to the site of elimination. Thus, we observed in a previous report[7] that the biliary excretion rate of radioactivity derived from the (+)-(R)-enantiomer is faster than that of its antipode after the iv injection of enantiomerically radiolabeled carvedilol pseudoracemates to bile duct-cannulated rats. Compared to the (−)-(S)-enantiomer, the (+)-(R)-enantiomer exhibits relatively higher plasma protein binding and less distribution volume. This finding implies that the amount of the (+)-(R)-enantiomer delivered to the site of elimination, i.e., liver, is more than that of its antipode, and explains higher biliary excretion rate of the radioactivity derived from the (+)-(R)-enantiomer.

It is generally considered that drugs exhibiting high plasma protein binding have a relatively small Vd_{ss}. However, the Vd_{ss} for carvedilol enantiomers greatly exceeded the plasma volume. These results imply that extravascular binding exists in addition to the lipophilic properties of carvedilol, a basic compound with the relatively great log D = 2.35.[21] There is no enantiomeric difference between enantiomers in the initial uptake of drug represented by the tissue/blood concentration ratio in all tissues except the liver. Elimination rates for the (−)-(S)-enantiomer were smaller than those for the (+)-(R)-enantiomer in all tissues, reflecting their elimination from the blood. The result that tissue concentrations of the (−)-(S)-enantiomer are higher than those of the (+)-(R)-enantiomer were in accordance with the enantiomeric differences in Vd_{ss} calculated from blood concentration data.

The free drug that is associated with pharmacological activity is more important than total drug in the target organ/tissue. Except for muscle, binding for both carvedilol enantiomers is higher in the tissues than in the blood. The results suggested that almost all of both enantiomers present in the tissues bind to tissue components, resulting in the relatively large Vd_{ss}. In the heart, muscle, and spleen, there was no enantiomeric dif-

ference in free fraction of drug, while in the liver and kidney the free fraction of the (−)-(S)-enantiomer was slightly lower than that of the (+)-(R)-enantiomer. Therefore, the enantiomeric difference in K_p values in these tissues can be explained by the difference in plasma binding not tissue binding between the enantiomers. These findings are consistent with a report suggesting that stereoselective tissue distribution of propranolol enantiomers in rats is caused mainly by the difference in plasma protein binding of its enantiomers.[22]

In conclusion, the disposition of carvedilol enantiomers is stereoselective in rats as well as in humans.[4–6] After oral dosing of *rac*-carvedilol to rats, the (−)-(S)-enantiomer, with its greater oral clearance than the (+)-(R)-enantiomer, is subjected to greater first-pass metabolism in the liver. As a result, systemic bioavailability of the (−)-(S)-enantiomer is less than that of its antipode. After reaching the circulating blood, both carvedilol enantiomers are distributed widely to various tissues/organs, and show strong tissue binding. The extent of the (−)-(S)-enantiomer distribution, however, with its greater free fraction in the blood, is greater than that of the (+)-(R)-enantiomer.

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