Carvedilol Stereopharmacokinetics in Rats: Affinities to Blood Constituents and Tissues

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Carvedilol, a lipophilic β-adrenoceptor antagonist with vasodilating activities, is characterized by a high as well as stereoselective metabolic clearance and distribution volume. Tissue distribution of carvedilol enantiomers and their conjugates were determined under steady-state conditions in rats (*p.o.*, 10 mg/kg, repetitive dosage; n = 5) and after single *i.v.* administration in control rats and rats with surgical portacaval shunt (pcs) (10 mg/kg; n = 3 each group). In addition, *in vitro* plasma protein binding was evaluated. - The plasma protein binding of carvedilol in rats is > 98% for total plasma (tp) and > 96% for rat serum albumin (rsa) solution (4%), with enantioselectivity ratios of 1.53 (tp) and 1.27 (rsa). Significantly higher unbound fractions were observed in pcs rats, in part due to reduced protein concentrations. - In contrast to plasma, where a preponderance of the *R*-enantiomer with an *S/R* ratio of 0.6 was found, *S*-carvedilol was predominant in all tissues (heart, liver, kidneys, lung, spleen, muscle, and adipose tissue), with *S/R* ratios of 1.3-1.4 in most of these tissues and 2.3 in liver. This preferential tissue partitioning of *S*-carvedilol was in accordance with its higher unbound fraction in plasma. Carvedilol accumulated predominantly in the highly perfused and/or eliminating organs liver, kidneys, and lung (tissue/plasma ratios; lung: *S* 76, *R* 34; liver: *S* 21, *R* 5; kidney: *S* 8, *R* 3). A similarly enantioselective distribution into the heart of control as well as pcs rats was observed, where the *S*-enantiomer concentrations exceeded the plasma concentrations 7-fold. Probably because of the impaired liver function in pcs rats with increased importance of the renal route, kidney concentrations were higher in these rats. The kidney/plasma ratio was elevated approximately 2-fold for the parent compound (control: *S* 7, *R* 2; pcs: *S* 14, *R* 4) and 4-fold for the *R*-carvedilol conjugate (control: *S* 2, *R* 1; pcs: *S* 3, *R* 4).
The conjugates of carvedilol were detectable in all organs, with significantly smaller concentrations than those of the aglycones and with varying stereoselectivities.

Stereopharmakokinetik von Carvedilol bei der Ratte: Affinität zu Blutbestandteilen und Geweben

Carvedilol, ein lipophiler β-Adrenozeptor-Antagonist mit zusätzlicher vasodilatierender Wirkung, ist charakterisiert durch eine hohe und stereoselektive metabolische Clearance sowie ein hohes und stereoselektives Verteilungsvolumen. Die Verteilung der Enantiomere von Carvedilol und der entsprechenden Konjugate in verschiedene Gewebe bei der Ratte wurde unter steady-state-Bedingungen (*p.o.*, 10 mg/kg, Mehrfachgabe; n = 5) und 5 h nach einmaliger *i.v.*-Gabe bei Kontrolltieren sowie bei Ratten mit operativ angelegtem portakavalem Shunt (pcs) (10 mg/kg; je n = 3) untersucht. Zusätzlich wurde die *in-vitro*-Plasmaproteinbindung von Carvedilol bestimmt. Sie betrug > 98% für Gesamtplasma (tp) und > 96% für Rattenserumalbumin (rsa) in 4proz. Lösung mit einem Enantioselektivitätsverhältnis der Bindung von 1.53 (tp) sowie 1.27 (rsa). Signifikant höhere ungebundene Fraktionen als bei Kontrolltieren wurden nach pcs beobachtet. Dies ist z.T. durch eine reduzierte Gesamtplasma- bzw. Albuminkonzentration zu erklären. - Im Gegensatz zu Plasma, wo die Konzentration des *R*-Enantiomers überwog und ein *S/R*-Verhältnis von 0,6 gefunden wurde, dominierte *S*-Carvedilol in allen untersuchten Geweben (Herz, Leber, Nieren, Lunge, Milz, Muskel und Fettgewebe) mit einem *S/R*-Verhältnis von 1.3-1.4 außer in der Leber, wo es mit 2.3 deutlich höher lag. Die Anreicherung von *S*-Carvedilol im Gewebe ist u.a. zu erklären über die höhere ungebundene Fraktion im Plasma. Carvedilol kumuliert überwiegend in gut perfundierten Organen und/oder Eliminationsorganen wie Leber und Nieren sowie in der Lunge (Gewebe/Plasma-Verhältnisse; Lunge: *S* 76, *R* 34; Leber: *S* 21, *R* 5; Niere: *S* 8, *R* 3). Die Enantioselektivität der Verteilung in das Herz ist bei Kontroll- und pcs-Ratten ähnlich, dabei wird das *S*-Enantiomer gegenüber dem Gehalt in Plasma 7fach angereichert. Wahrscheinlich aufgrund der reduzierten Leberfunktionen bei pcs-Ratten mit gleichzeitiger Erhöhung der renal ausgeschiedenen Fraktion waren die Konzentrationen in den Nieren bei den pcs-Ratten deutlich höher. Der Nieren/Plasma-Quotient der Muttersubstanz war 2mal (Kontrolle: *S* 7, *R* 2; pcs: *S* 14, *R* 4) und der für das *R*-Carvedilolkonjugat (Kontrolle: *S* 2, *R* 1; pcs: *S* 3, *R* 4) 4mal so hoch bei den pcs-Tieren. - Die Konjugate des Carvedilols sind in allen Organen meßbar, allerdings mit signifikant niedrigeren Konzentrationen als ihre Aglyca und auch mit variierenden Stereoselektivitäten.

With respect to its pharmacokinetics the lipophilic carvedilol (Fig. 1) is characterized by a high and enantioselective metabolic clearance (with preferential extraction of the *S*-enantiomer) and a rather high and enantioselective distribution volume. Clearance is mainly oxidative for carvedilol with formation of several different desalkyl- and hydroxy derivatives and subsequent phase-II metabolism[1,2]. Although its urine concentrations are rather low, the glucuronic acid conjugate of parent carvedilol represents one of the 'major' metabolites in man (approximately 20% of total

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radioactivity in plasma and 5% in urine[2]). In rats the clearance to form this conjugate is only up to 2% of total clearance with a similar stereoselectivity as observed in man[3,4]).

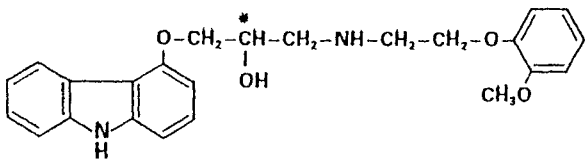

Figure 1: Carvedilol, a lipophilic β-adrenoceptor antagonist with α_1-antagonistic properties (The asterisk denotes the chiral carbon).

The steady-state volume of distribution for the parent compound was calculated as 192 L for *R*- and 289 L for *S*-carvedilol in man and 6.3 L/kg for *R*- and 9.5 L/kg for *S*-carvedilol in rats[3,5]). These values are indicative of a considerable affinity of parent carvedilol to tissues.

In order to be able to use a physiological pharmacokinetic model for simulation studies, data about tissue distribution, blood and plasma concentrations and binding are of significant importance.

The aims of the present studies in rats were therefore

- the determination of the protein binding of carvedilol and its enantioselectivity ratio for albumin and total plasma proteins *in vitro* and the quantification of the binding to various tissues (as well as its stereoselectivities) *in vivo*,
- the comparison of the steady-state tissue and erythrocyte concentrations of the respective conjugates with those of parent compound, and
- the comparison of the concentrations in plasma and tissues after a single carvedilol dose in untreated control rats with that in rats with experimental liver cirrhosis (caused by a surgical portacaval shunt).

Results and Discussion

In order to determine the tissue partition coefficients of carvedilol, the present studies were performed at steady-state. When the studies were designed it was assumed that the extent of accumulation in plasma equals that in tissues. Very recently published studies by *Fujimaki*[6]) confirm this hypothesis. This author found a rapid distribution of carvedilol into the tissues, and his data also indicate that the decrease of the concentrations in the terminal section of the curve parallels that in plasma in most tissues. Thus, the dosage regimen in the present study was calculated on the basis of an average plasma half-life of 3 h. After an initial dose of 20 mg/kg, rats were administered repetitive doses of 10 mg/kg in 3 hourly intervals, in order to reach steady-state. Blood and organ samples were taken and assayed for unconjugated and conjugated carvedilol employing a procedure that is based on chiral derivatization with phenylethyl isocyanate[5]). In the second part of the study tissue samples were analyzed that had been obtained in a single dose study in rats with portacaval shunt (pcs) 5 h after a 10 mg/kg i.v. dose[4]).

Plasma concentrations, affinities to blood constituents

While in plasma the concentrations of *R*-carvedilol always significantly exceeded those of *S*-carvedilol in the steady-state study, more *S*- than *R*-carvedilol was detectable in blood cells, the respective *S/R* ratios being 0.6 (plasma) and 1.7 (blood cells) (Tab. 1). Yet, the concentrations in blood cells were smaller than those in plasma (Fig. 2). Although the two diastereomeric conjugates were present in plasma as well, their concentrations were significantly below those observed in plasma of humans. They reached up to 1% of those of the aglycone enantiomers and showed a similar enantioselectivity (*S/R* ratio 0.3). In blood cells only traces of carvedilol conjugates were found.

The data obtained in the single-dose study are not readily comparable with those from the steady-state study due to

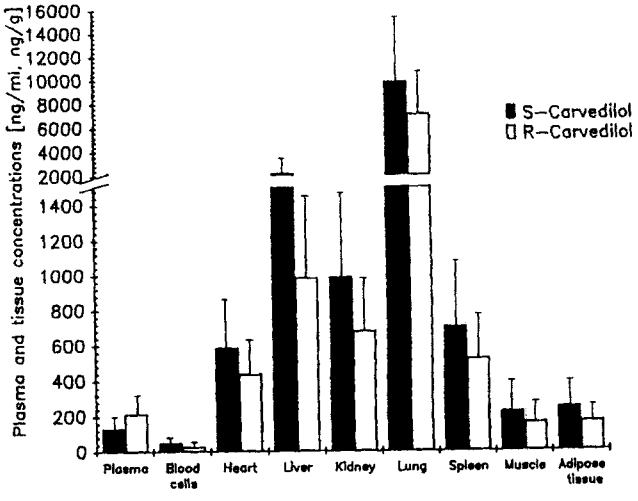

Figure 2: Concentrations of *S*- and *R*-carvedilol (arithmetical means + SD) in various tissues and blood constituents after repetitive dosage of *rac* carvedilol.

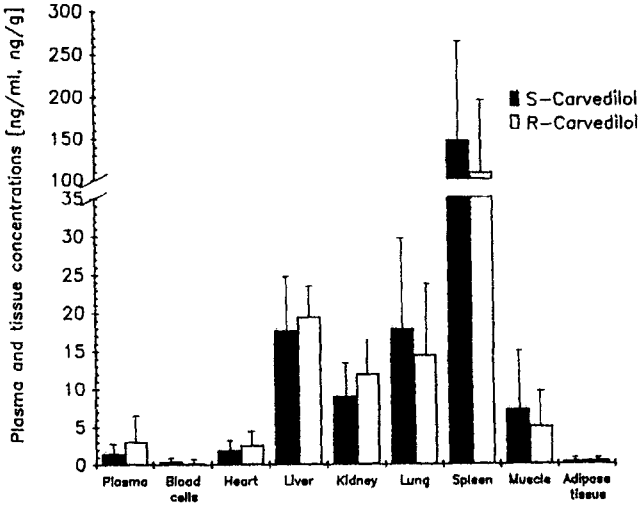

Figure 3: Concentrations of *S*- and *R*-carvedilol conjugate ($\bar{x}$ + SD) in tissues and blood constituents after repetitive administration of *rac* carvedilol.

Table 1: Arithmetical means (± SD_{n-1}) and medians of the *tissue/plasma ratios* as well as of the individual *S/R ratios of the tissue concentrations* of the carvedilol enantiomers and their conjugates in rats (n = 5) under steady-state conditions.

		Carvedilol			Carvedilol conjugates		
		S-(-)	R-(+)	S/R	S-(-)	R-(+)	S/R
Heart	$\bar{x}$	4.7 ± 0.9	2.2 ± 0.5	1.30	0.5 ± 0.5	0.5 ± 0.1	0.69
	$\tilde{x}$	4.8	2.1	1.44	0.5	0.5	0.64
Liver	$\bar{x}$	20.5 ± 10.4	5.4 ± 2.7	2.26	6.5 ± 2.1	5.3 ± 4.2	0.90
	$\tilde{x}$	20.8	6.0	2.18	7.7	3.1	0.88
Kidney	$\bar{x}$	7.9 ± 1.2	3.3 ± 0.6	1.42	3.7 ± 1.6	4.1 ± 1.7	0.77
	$\tilde{x}$	7.4	3.1	1.34	3.7	4.1	0.72
Lung	$\bar{x}$	75.6 ± 17.0	34.0 ± 8.6	1.34	7.0 ± 5.1	3.4 ± 0.5	1.12
	$\tilde{x}$	77.0	32.4	1.35	9.5	3.4	1.21
Spleen	$\bar{x}$	5.7 ± 1.5	2.6 ± 0.6	1.31	45.6 ± 37.7	21.2 ± 8.1	1.26
	$\tilde{x}$	4.9	2.3	1.28	60.1	17.8	1.35
Muscle	$\bar{x}$	1.8 ± 0.6	0.8 ± 0.3	1.35	3.6 ± 3.2	1.5 ± 0.3	1.24
	$\tilde{x}$	1.7	0.7	1.36	4.8	1.4	1.37
Adipose	$\bar{x}$	1.9 ± 0.2	0.8 ± 0.2	1.41	0.2 ± 0.01	0.1 ± 0.04	0.88
tissue	$\tilde{x}$	1.9	0.8	1.41	0.2	0.1	1.00
Blood	$\bar{x}$	0.4 ± 0.1	0.1 ± 0.04	1.66	0.1 ± 0.1	0.04 ± 0.1	-
cells	$\tilde{x}$	0.3	0.1	1.67	0.07	0	-

the different experimental set-up (steady-state *vs.* single dose, diethyl ether anesthesia throughout the whole sampling period in the single dose study only). Yet here as well, the plasma concentrations of *R*-carvedilol were significantly higher than those of *S*-carvedilol in the controls. In rats with portacaval shunt plasma concentrations of both carvedilol enantiomers were significantly elevated when compared to the respective controls[4]. The *S/R* ratio, too, increased to some extent due to the reduced hepatic extraction.

Interestingly, the concentrations of both conjugates were elevated in the pcs rats, yet, the enantioselectivity was decreased and even less pronounced than for parent carvedilol in pcs rats.

Table 2: Summary of protein binding data *ex vivo* and *in vitro*. Average percentages bound and free, found with plasma from control and pcs rats and with rat serum albumin
(ER = enantioselectivity ratio, n = 3 for each value)

	S-Carvedilol	R-Carvedilol	ER*
Control plasma			1.53
bound	98.96	99.32	
free	1.04	0.68	
Pcs plasma			1.27
bound	97.63	98.13	
free	2.37	1.87	
Rat serum albumin (4%)			1.28*
bound	97.10	96.33	
free	2.90	3.67	

* The given ratios represent *S/R* for total plasma, where higher binding was detected for the *R*-enantiomer. In the given ER value for albumin the affinity constant for *S*-carvedilol is the denominator.

A considerable binding to plasma proteins was detected (Table 2). Since for propranolol[7], the major binding protein (yet not responsible for the enantioselectivity in plasma protein binding) is albumin, additional *in vitro* studies with albumin were performed, in order to determine the respective enantioselectivity.

Slightly lower values than for total plasma protein were found for a 4% rat serum albumin solution with a binding of 97.1% for *S*- and 96.3% for *R*-carvedilol. Hence, the enantioselectivity ratios, ER, were 1.53 for rat plasma and 1.28 for rat albumin with inverse enantioselectivities.

In *ex-vivo/in-vitro* studies the unbound fraction of plasma carvedilol at steady-state amounted to 1.84% for *S*- and 1.25% for *R*-carvedilol (ER 1.48). The respective values found for the control rats after a single dose are comparable. A significant increase of the unbound fraction was detected for plasma samples originating from pcs rats, where the free fractions amounted to 2.4 (*S*) and 1.9% (*R*) (ER 1.3). The binding data are summarized in Table 2.

The enhanced free fraction in pcs rats goes along with – and should at least in part be due to – the decrease in the concentration of plasma proteins. The average concentrations of total protein and albumin, respectively, amounted to 5.6 and 3.0 g/dl in the rats used in the steady-state studies, to 5.5 and 3.0 g/dl in the control rats in the single dose studies and to 4.1 and 2.0 g/dl in the pcs rats.

Tissue concentrations

As depicted in Fig. 2 carvedilol concentrations in tissues were always higher than those in plasma. In all tissues the concentrations of *S*-carvedilol exceeded those of *R*-carvedilol, with *S/R* ratios of 1.3-1.4 in most of the tissues and 2.3

in liver tissue (Tab. 1). The tissue/plasma ratios for carvedilol enantiomers under steady-state conditions are summarized in Table 1. In particular, carvedilol partitioned into the highly perfused and/or eliminating organs liver and kidneys as well as into the lung (tissue/plasma ratios at steady-state; lung: *S* 76, *R* 34; liver: *S* 21, *R* 5; kidney: *S* 8, *R* 3). In the tissue sample from the heart the concentration of *S*-carvedilol exceeded the plasma concentration seven-fold.

Table 3: Tissue/plasma ratios of the carvedilol enantiomers and their conjugates in controls and pcs rats 5 h after an i.v. dose of 10 mg/kg *rac* carvedilol

	Carvedilol				Carvedilol conjugates			
	Control		PCS		Control		PCS	
	S-(-)	R-(+)	S-(-)	R-(+)	S-(-)	R-(+)	S(-)	R-(+)
Heart	6.9	2.4	7.6	2.6	0.1	0.2	0.1	0.3
Liver	1.2	0.6	1.9	0.6	0.7	0.5	0.3	0.7
Kidney	7.0	2.1	14.6	3.9	1.8	0.9	2.7	3.9
Blood cells	0.4	0.2			0.1	0.1		

The tissue enantioselectivity in pcs rats was rather similar to that in the respective controls, although the extent of protein binding and the differences between the plasma concentrations of the enantiomers are reduced in the pcs rats. Furthermore, the data in Table 3 illustrate that the tissue/plasma ratio in the kidney is doubled for the pcs rats. This can be explained by the fact that the kidney contributes to a significantly higher extent to the overall drug and metabolite elimination or excretion in rats with impaired liver function (kidney/plasma ratio, controls: *S* 7, *R* 2; pcs rats: *S* 14, *R* 4).

Regarding the concentrations in the heart tissue it was found that the tissue/plasma ratio as well as the *S*/*R* ratio were virtually identical for the two groups.

Surprisingly, very high carvedilol concentrations were found in the lung, and high conjugate concentrations were detected in the spleen under steady-state conditions.

With respect to the conjugate concentrations in tissues (Fig. 3) it can be stated that - unlike for parent carvedilol, where the *S*-enantiomer concentration is enhanced - the concentrations of the *R*-carvedilol conjugate are higher, except in lung, spleen, and muscle. The conjugates of carvedilol were detectable in all organs (Fig. 3), yet, their concentrations were significantly smaller than those of the aglycone enantiomers. Interestingly, a significant increase of the kidney concentration of the *R*-carvedilol conjugate was detectable in the pcs rats (ratio, controls: *S* 1.8, *R* 0.9; pcs rats: *S* 2.7, *R* 3.9). The detected glucuronides may either partition into the tissue and/or be formed there, since UDPGTs (Uridine diphosphoglucuronosyl transferases) are located in various tissues[8].

As found in our previous studies with prenylamine[9], carvedilol is "concentrated" in the major eliminating organs. This may be explained by its preferential uptake into these organs. The preferential partitioning of the *S*-enantiomer in tissues is in accordance with its higher unbound fraction in plasma. However, it may in part also be due to the higher affinity of the *S*-enantiomer to tissue binding sites, e.g. β-adrenergic receptors that are located in the respective tissues.

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Experimental Part

Enantiospecific assay for carvedilol in biological material

Tissues were homogenized prior to extraction. They were cut into small pieces, and 0.4 g of these pieces were homogenized (ice cold) with 1.0 ml 0.05 M phosphate buffer (pH 7.4, containing 0.9% NaCl) using an Ultra-Turrax^R. This mixture was handled as described for plasma and urine[5], but for adjustment to pH 9.8 a mixture of M NaOH and 0.75 ml 0.1 M carbonate buffer (pH 9.8) were used instead of just carbonate buffer.- Carvedilol conjugates were enzymatically hydrolyzed prior to determination[5].

Protein binding

In vitro (*ex vivo*) protein binding was determined by equilibrium dialysis of 1 ml plasma or a 4% solution of rat albumin (Sigma Chemicals, D-Deisenhofen) against 0.1 M phosphate buffer (pH 7.4, containing 0.9% NaCl). Equipment: Dianorm^R apparatus with 1.0 ml cells and Diachema membranes No. 10.17 (G. Maierhofer, D-Munich). Equilibrium was reached after 4 h (at 37°C).- Total protein content as well as albumin amounts were determined using standard laboratory methods, in order to evaluate the possible influence of an altered protein concentration. A decrease in protein concentration would cause an altered free fraction in plasma from rats with experimental liver cirrhosis (caused by a surgical portacaval shunt). Due to the observed volume shifts the following correction was made when calculating the bound fraction:

$$\% \text{ bound} = (F \times 100)/(F + C_{buf})$$

where F represents $[(C_p - C_{buf}) \times (2 \times Vol_p)/(Vol_p + Vol_{buf})]$.

(C_p = concentration in plasma, C_{buf} = concentration in buffer, Vol_p = volume in plasma compartment of dialysis cell, V_{buf} = volume in buffer compartment of dialysis cell).

The enantioselectivity ratio (ER), a measure for binding site enantioselectivities, was calculated from the association constants, k_a, of the enantiomers, where the higher value is the numerator and the lower value the denominator. The association constant, k_a, represents the ratio of the bound to the free fraction. When comparing plasma or blood with tissue concentrations, it was assumed that the specific mass of the respective tissue was 1 and that 1 mg/ml can directly be compared with 1 mg/g. The tissue/plasma ratio represents a measure for the affinity of the compound to tissues.

Animal studies[*]

Male Sprague-Dawley rats (n = 5) with an average weight of 280 ± 11 g were included into the steady-state studies. After overnight fasting they were administered 20 mg/kg racemic carvedilol *p.o.* as initial dose and a maintenance dose of 10 mg/kg 3, 6 and 9 h thereafter. The drug was administered as solution prepared by dissolution in small amounts of dimethylformamide/acetic acid (1 + 1) and dilution with 2 ml of 5% glucose. 1 h after the last dose the animal was sacrificed under ether anesthesia. A blood sample and various organs (heart, liver, kidney, lung, spleen, hind leg muscle, and adipose tissue) were taken. Blood cells were briefly washed with physiological saline. Liver, kidney, lung, and heart were

[*] The studies were performed at the University of Freiburg. Permission to perform these studies had been obtained from the local animal research committee.

immediately perfused (retrograde) with physiological saline. Organs were immediately frozen in liquid N_2. The samples were kept frozen at -20°C until analysis. Two control rats were treated in a similar way.

In the second part of the study (single-dose study) male Sprague-Dawley rats (210-350 g) were used. One group consisted of 3 animals, which were investigated 6 weeks after surgical portosystemic shunting[4], the second group consisted of 3 untreated control rats. The rats were administered 10 mg/kg *rac* carvedilol *i.v.* as a short infusion over 1 min under diethyl ether anesthesia. Plasma, red blood cell, and tissue concentrations (heart, liver, and kidney) were studied 5 h after a single i.v. dose. The samples had been obtained during the previously performed pharmacokinetic studies in rats with portacaval shunt[4].

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