Saturable Enantioselective First-pass Effect for Carvedilol after High Oral Racemate Doses in Rats

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Carvedilol shows a highly enantioselective first-pass extraction after therapeutic *p.o.* doses with preferential extraction of the *S*-enantiomer. To investigate, whether the enantioselective first-pass metabolism is saturable, male Sprague-Dawley rats were administered increasing single doses of *R/S*-carvedilol (*p.o.*, 5-30 mg/kg; *i.v.*, 5 and 10 mg/kg), and the individual stereopharmacokinetics were studied. - Like in humans the plasma concentrations of *R*-carvedilol exceeded always those of *S*-carvedilol. As expected, a dose-dependent reduction in oral clearance was observed, while the total clearance after the *i.v.* doses was constant. Beyond 20 mg/kg an increased plasma half-life was found for both enantiomers, which is due to a reduced plasma clearance.

Sättigbarer First-pass-Effekt bei Carvedilol nach hohen Dosen von Carvedilol-Razemat bei der Ratte

Peroral appliziertes Carvedilol unterliegt bei therapeutischer Dosierung einem deutlich enantioselektiven First-pass-Effekt mit bevorzugter Extraktion des *S*-Enantiomers. Um zu prüfen, ob dieser First-pass-Effekt sättigbar ist, wurde männlichen Sprague-Dawley-Ratten razemisches Carvedilol in steigenden Dosen (5-30 mg/kg *p.o.*; 5 und 10 mg/kg *i.v.*) appliziert und die individuelle Pharmakokinetik untersucht. - Wie beim Menschen lagen auch bei der Ratte die Plasmakonzentrationen von *R*-Carvedilol nach peroraler Gabe höher als die von *S*-Carvedilol. Wie zu erwarten, waren die oralen Clearances mit zunehmender Dosis geringer, während die systemische Clearance bei *i.v.*-Gaben von 5 und 10 mg/kg identisch war. Bei einer Dosis von 20 bzw. 30 mg/kg *p.o.* waren die Plasmahalbwertszeiten für beide Enantiomere erhöht. Dies läßt sich durch eine reduzierte Plasmaclearance erklären.

In rats - similar as in humans - the vasodilating non-selective β-adrenoceptor antagonist carvedilol (Fig. 1) is subject to an expressed and enantioselective first-pass effect with preferential extraction of the *S*-(-)-enantiomer when dosed perorally. The systemic availability amounts to 16% for the *S*- and 39% for the *R*-enantiomer in humans and 29% for the *S*- and 37% for the *R*-enantiomer in rats[1,2].

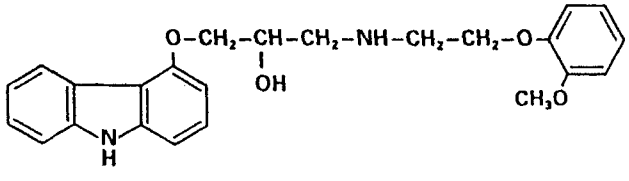

Figure 1: Chemical structure of carvedilol

With respect to β-adrenoceptor antagonism the *S*-enantiomer is the eutomer with a eudismic ratio of 100, while the vasodilation *via* α_1-adrenoceptor antagonism is mediated by both enantiomers to a similar extent[3].

The lipophilic compound is extensively metabolized *via* phase-I- and phase-II-metabolism. Important metabolic pathways are oxidation to *O*-desmethylcarvedilol and various hydroxylated metabolites and subsequent conjugation to the respective glucuronides[4,5]. Only a small percentage of the dose (≤ 5%) is excreted unchanged via the renal and biliary route. Yet, excretion with the bile represents the major elimination route for the metabolites[5,6].

In one of our recent publications we described the reduction of the systemic clearance of carvedilol as well as of the stereoselectivities of the investigated hepatic clearance processes (except for the biliary clearance) for *p.o.* dosage in rats with portacaval shunt[2]. From the obtained data we concluded that - as for propranolol[7] - certain hepatic processes are capacity-limited and hence saturable. Therefore, we hypothesized that also with normal liver function the total clearance as well as its stereoselectivity should be reduced at higher doses of the drug.

Thus, the aim of the present studies with racemic carvedilol was to investigate the changes in stereopharmacokinetics with increasing doses and the influence of the dose on the extent of first-pass effect and systemic availability in healthy rats.

An enantiospecific carvedilol assay was used that is based on chiral derivatization with *R*-(+)-phenylethyl isocyanate and chromatographic separation on a silica gel column with fluorescence measurement of the eluate. The carvedilol conjugates were quantified after enzymatic cleavage with β-glucuronidase.

Results and Discussion

For both intravenous and peroral dosage the plasma concentrations of the *R*-enantiomer exceeded those of the *S*-enantiomer significantly (Fig. 2).

Yet, with increasing *p.o.* doses the difference between the two enantiomers decreased. As expected, the apparent oral clearances were reduced with higher doses, while the systemic clearances calculated for the *i.v.* administration of 5 and 10 mg/kg, respectively, remained constant.

When doubling the *p.o.* dose from 5 to 10 mg/kg the respective AUCs were no longer proportional to the dose but higher, which may be explained by a reduced first-pass effect. The AUC/dose relationship is depicted in Fig. 3. For

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Table: Average pharmacokinetic parameters for carvedilol after racemate dosage [n = 4 for 5 and 10 mg/kg, n = 2 for 20mg/kg, n = 1 for 30 mg/kg]

		p.o.												i.v.					
		5 mg/kg			10 mg/kg			20 mg/kg			30 mg/kg			5 mg/kg			10 mg/kg		
		S-(-)	R-(+)	S/R	S-(-)	R-(+)	S/R	S-(-)	R-(+)	S/R	S-(-)	R-(+)	S/R	S-(-)	R-(+)	S/R	S-(-)	R-(+)	S/R
C_{max}	[ng/ml]	8	25	0.32	83	225	0.35	219	316	0.72	731	1300	0.59	624	822	0.75	907	1060	0.84
$C_{max.con}$	[ng/ml]	1.1	0.0		3.5	6.3	0.51	5.6	3.8	1.47	28.0	8.8	3.18	7.6	6.0	1.49	18.5	33.9	0.82
t_{max}	[h]	8.0	1.4	5.60	1.3	1.2	1.05	1.1	0.6	1.50	1.0	1.0	1.00						
$t_{max.con}$	[h]	8.0	0.0		1.0	1.6	0.59	1.0	1.0	1.00	2.0	3.0	0.67						
$t_{1/2}$	[h]	6.7	2.7	2.50	3.9	4.6	0.98	12.5	13.4	0.91	34.8	31.2	1.25	4.8	4.0	1.26	2.4	3.0	0.82
$AUC_{0\to\infty}$	[ng·ml^{-1}·h]	93	186	0.50	420	1227	0.34	3851	6080	0.65	15276	17943	0.85	1538	2440	0.65	2121	3630	0.58
MRT	[h]	11.2	7.7	1.44	6.1	7.2	0.96	19.1	20.3	0.93	49.4	43.6	1.13	6.7	5.8	1.19	3.6	4.4	0.83
$MRT_{.con}$	[h]													3.6	4.3	1.57	4.4	3.8	0.92
$Ae_{0\to\infty}$	[%]	0.010	0.011	0.88	0.006	0.007	0.82	0.006	0.003	2.19	0.163	0.098	1.66	0.042	0.039	1.15	0.078	0.058	1.34
$Ae_{0\to\infty,con}$	[%]	0.006	0.004	1.30	0.002	0.001	1.70	0.001	0.001	0.39	0.001	0.001	0.42	0.002	0.002	1.24	0.005	0.009	0.45
CL	[ml/min]													10.9	6.9	1.65	11.7	6.6	1.76
CL_0	[ml/min]	183.0	91.9	1.99	73.3	22.5	3.13	17.0	11.1	1.55	3.27	2.79	1.17						
CL_R	[ml/min]	0.018	0.010	1.76	0.004	0.001	2.46	0.001	0.0003	3.60	0.005	0.003	1.96	0.005	0.003	1.56	0.009	0.004	2.34
$CL_{R.con}$	[ml/min]	0.092	0.0		0.016	0.013	3.51	0.020	0.100	0.20	0.003	0.032	0.09	0.022	0.020	0.75	0.026	0.044	0.74
V_{ss}	[L]													4.1	2.1	1.97	2.4	1.7	1.43
F		0.058	0.073	0.79	0.186	0.318	0.59												

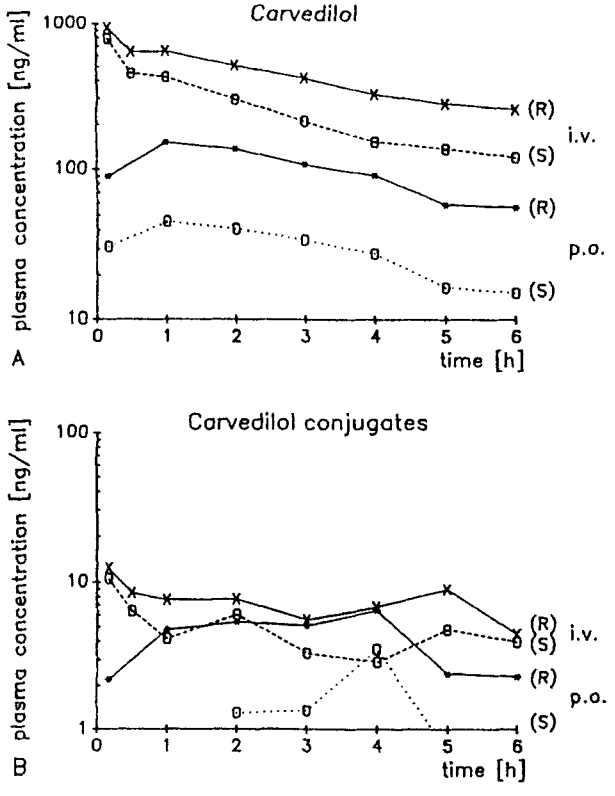

Figure 2: Median plasma concentration *vs.* time curves for the carvedilol enantiomers and their glucuronides after a single *i.v.* or *p.o.* dose of 10 mg/kg racemic carvedilol

the 20 mg/kg dose an increased plasma half-life was found for both enantiomers, which is due to a reduced plasma clearance.

In contrast to the observations made for unchanged carvedilol, the *S*/*R*-ratio for the conjugates in plasma increased after *p.o.* administration due to a smaller raise of the *R*-carvedilol conjugate than of the *S*-carvedilol conjugate concentrations.

The renal excretion of both carvedilol and its glucuronides were low. The renal clearances of the carvedilol enantiomers and the respective glucuronides were in a similar range for the two *i.v.* doses, yet they were significantly lower for the high *p.o.* doses indicating that active renal processes are contributing to the clearance, which are saturated when the systemic concentrations are above a certain level. All pharmacokinetic parameters are given in Table 1.

The nonlinear pharmacokinetics of carvedilol, *i.e.* the higher than proportional increase in systemic availability and the fact that the enantiomer ratio in plasma approaches 1, are supporting the hypothesis of a dose-dependent (saturable) first-pass effect for carvedilol. In addition, the systemic clearance is decreased with high doses. However, it remains to be investigated, which role the various phase-I- and phase-II-routes are playing with respect to saturability of clearance and its stereoselectivity.

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Experimental Part

Pharmacokinetic study in rats

To male Sprague-Dawley rats (average weight, 331 g) different doses of racemic carvedilol were administered intravenously (5 and 10 mg/kg) and perorally (5, 10, 20, 30 mg/kg). Each 5 and 10 mg/kg dose was given to a group of 3 animals. Two rats were administered 20 mg/kg racemic carvedilol, while one rat received the very high dose of 30 mg/kg.

Blood samples of 0.25 to 0.5 ml were drawn from the femoral vein and the respective volume substituted by *Ringer* solution. Sampling times were

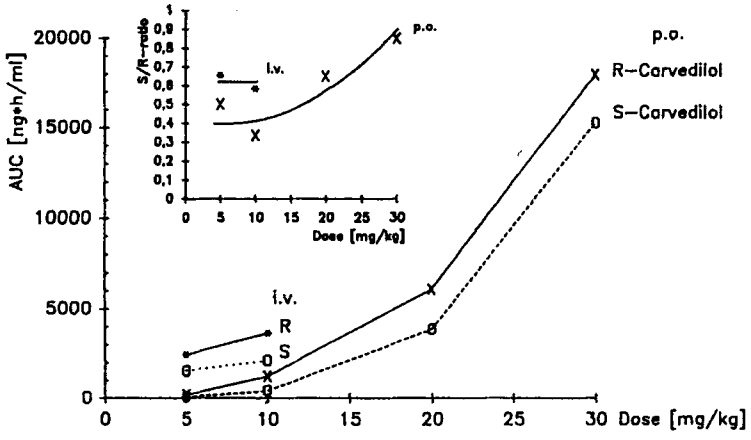

Figure 3: AUC/Dose dependency for carvedilol as well as *S/R* ratios as obtained in this study

at 0, 0.17, 0.5, 1, 2, 3, 4, 5, and 6 h post-dose. Urine was fractionally collected in 1-hourly intervals up to 6 hours.

Enantiospecific analytical procedure for carvedilol in plasma and urine

The mixture of a 0.1-ml aliquot of plasma or urine, respectively, 1.0 ml 0.1M carbonate buffer (pH 9.8) and 0.5 g NaCl was extracted with 5.0 ml diisopropyl ether. The org. layer (4.0 ml) was transferred into a second tube and evaporated using a vacuum centrifuge. To the dry residue 0.1 ml methanolic triethylamine solution (0.2%) and *R*-(+)-phenylethyl isocyanate reagent (0.4 mg dissolved in methanol) were added and the mixture was kept at room temp. for 0.5 h. The reaction was terminated by addition of 0.2 ml methanolic ethanolamine solution (0.2%). After evaporation of the solvent(s), the remaining residue was reconstituted in 250 µl of the mobile phase. - The resulting solution (100 µl) was injected into the HPLC system. A silica gel column (Waters Resolve®) was used as stationary phase, a mixture of diisopropyl ether, dichloromethane and methanol (95:5:2, v/v) as mobile phase, which was delivered by a Knauer model 64 HPLC pump at a flow rate of 1 ml/min resulting in an average pressure of 2.8 MPa. The fluorescence of the eluate was monitored at 280/340 nm.

Release of the aglycone from carvedilol glucuronides

The samples, from which carvedilol had been extracted, were extracted 3 more times with diisopropyl ether, in order to remove unconjugated carvedilol completely from the samples and tubes. Then the pH value was adjusted to pH 5 and 20 µl β-glucuronidase solution (44 000 *Fishman* units) were added. The mixture was kept at 37°C for 4 h. Then the pH was readjusted to pH 9.8 by addition of 35 µl 1M NaOH and 0.5 ml pH 9.8 carbonate buffer and the extraction of released carvedilol enantiomers performed as described above. - Concentrations of carvedilol glucuronides are given as the respective carvedilol enantiomer equivalents.

Pharmacokinetic definitions and calculations

The C_{max} value represents the maximum concentration in plasma at time t_{max}. The plasma half-life was determined from the terminal log-linear phase of the concentrations *vs.* time curve and included at least three data points. The area under the concentration-time curve ($AUC_{0-\infty}$) was determined up to the last measured concentration (y at time t) by the linear trapezoidal rule, and it was extrapolated to infinity ($AUC_{t-\infty} = y/\lambda_z$).

The mean residence time, MRT, was calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$, where the AUMC represents the area under the first-moment curve.

The total amounts excreted into urine ($Ae_{0-\infty}$) were calculated by dividing the amount excreted until time t by ($1 - e^{-kt}$).

The systemic or the oral clearances (CL or CL_0 = CL/F), respectively, were determined as $Dose/AUC_{0-\infty}$, the renal clearances (CL_R) as $Ae_{0-\infty}/AUC_{0-\infty}$ for carvedilol enantiomers as well as their glucuronides (con).

The noncompartmental approach was used to determine the steady-state volume of distribution V_{ss} (V_{ss} = Dose · $AUMC/AUC^2$). The absolute systemic availability (F) was estimated by comparing the AUC values obtained after *p.o.* and *i.v.* doses in the range, where the clearance was independent of dose, *i.e.*, where the first-pass effect was not yet saturated.

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