Rats With Portacaval Shunt as a Potential Experimental Pharmacokinetic Model for Liver Cirrhosis: Application to Carvedilol Stereopharmacokinetics

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ABSTRACT As an experimental model for reduced liver function rats with surgical portacaval shunts (pcs) may be used. Carvedilol, a nonselective β-adrenoceptor antagonist with vasodilating activity, is extensively metabolised by phase I as well as phase II pathways. In order to study the stereoselective pharmacokinetics of carvedilol in liver disease, pcs and control rats were given rac-carvedilol intravenously and p.o. The carvedilol enantiomers and their conjugates were assayed in plasma, urine, and bile. Carvedilol was highly bound to plasma proteins; binding was reduced by pcs. In all groups, the plasma concentrations of (R)-carvedilol exceeded those of (S)-carvedilol significantly. In comparison to the control group the plasma concentrations of both enantiomers increased after pcs, while the difference between the stereoisomers decreased. The total clearance decreased proportionally to the decrease in liver weight (30%). Both the apparent oral clearance, as well as its stereoselectivity were reduced, by up to 90 and 43%, respectively. The biliary clearance of the parent drug after i.v. dosage increased in rats with pcs due to the reduced hepatic metabolism.

KEY WORDS: enantiomers, conjugates, protein binding, tissue binding, stereoselective pharmacokinetics, first-pass effect, drug metabolism, liver disease

INTRODUCTION

Carvedilol (Fig. 1) was recently introduced as a non-selective β-adrenoceptor antagonist with vasodilating activity via an α_1-adrenoceptor antagonism. At higher doses it is also a calcium channel antagonist.[1] The affinity for β_1-adrenoceptors is at least 100 times higher for the (−)-(S)-enantiomer than for the (+)-(R)-enantiomer, while no significant difference is observed between the enantiomers in the α_1-adrenoceptor antagonistic activity.[1]

The lipophilic compound carvedilol is extensively metabolised by phase I and phase II pathways yielding its O-desmethyl-, various hydroxy derivatives, and the respective conjugates.[2,3] Only a small percentage of the dose is found as the parent drug in urine and bile.[3] The metabolites are mainly excreted into bile (85% of the radioactive dose within 48 h[4]).

In humans a relatively high systemic plasma clearance is observed (589 ml/min)[5] with a slight stereoselectivity for the (−)-(S)-enantiomer after i.v. administration [CL, (S) 718 vs. (R) 541 ml/min].[6] Oral administration results in an extensive first-pass extraction predominantly for the (S)-enantiomer [apparent oral clearance, (S) 5500 vs. (R) 1382 ml/min].[6–9] This is, like for xibenolol,[10] the opposite of that observed with most other β-adrenoceptor antagonists of the phenoxypropanolamine type, which exhibit a more rapid clearance of the (+)-(R)-form.

Although oxidative metabolism in rats is qualitatively and quantitatively different from that observed in humans, the stereoselectivity of the clearance is similar in rats (including the pcs rats) and humans, while smaller differences between the enantiomers were detected in monkeys.[8,11,12]

As can be expected for a drug with relatively high hepatic extraction, Neugebauer et al.[13] found in cirrhotic patients that liver cirrhosis has a significant impact on the pharmacokinetics of carvedilol, although the study was performed after dosage of the racemate with nonenantiospecific assays. These data indicate that the absolute bioavailability increases tremendously in cirrhosis (control 19%; cirrhosis 83%). Verapamil is another example in which liver disease decreases the (stereoselective) first-pass effect in humans.[14]

It is, however, difficult to perform systematic pharmacokinetic studies in cirrhotic patients. As an experimental model for reduced liver function, such as chronic liver disease, rats with surgical portacaval shunts (pcs) may be used.[15,16] In previous studies it had been found that the important changes which occur in liver morphology, physiology, and biochemis-

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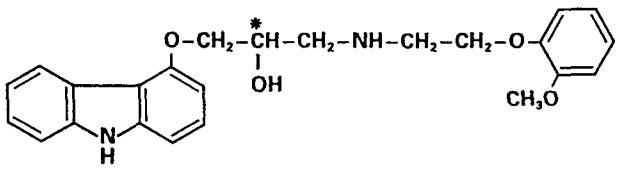

Fig. 1. Chemical structure of carvedilol. The asterisk denotes the chiral carbon.

try caused by portacaval anastomosis may be summarized as follows: fibrosis, decrease of liver weight (up to 60%[15]) related to a decreased number of hepatic cells, growth of a second cell barrier leading to a less intense contact between blood and hepatocytes, altered intracellular structure indicated by large vacuoles in the hepatocytes, proliferation of the bile ducts, unchanged structure of the liver, and similar microcirculation with pcs as seen in liver cirrhosis (reduced hepatic blood flow, intrahepatic shunts and capillarisation of the sinusoids).

For a highly extracted drug, maximum bioavailability (F_{max}) may be increased in this experimental model due to the reduction of flow-dependent hepatic clearance. Furthermore, because of the decreased number of intact hepatocytes the total amounts of enzymes are reduced, which may result in a lower intrinsic hepatic clearance and the potential for saturation of certain metabolic processes.

Therefore, the purposes of the present studies were to evaluate the pharmacokinetics of the carvedilol enantiomers and their conjugates, especially the extent of stereoselectivity of certain clearance processes (including biliary excretion) in rats, and to investigate the influence on clearance of decreased liver function after shunt surgery.

MATERIALS AND METHODS

Chemicals

Carvedilol reference compounds (racemate and enantiomers) were kindly donated by Boehringer Mannheim GmbH (Mannheim, FRG). All reagents and solvents were of analytical or HPLC grade. Ethanolamine and (+)-(R)-phenylethyl isocyanate were purchased from Fluka (Buchs, CH), triethylamine from Aldrich (Steinheim, FRG), β-glucuronidase (*E. coli*) from Boehringer Mannheim (Mannheim, FRG), and rat albumin fraction V from Sigma (St. Louis, MO). Anesthetic diethylether was purchased from Hoechst (Frankfurt, FRG). All other reagents were from Merck (Darmstadt, FRG).

Animal Studies

The studies had been approved by the local Animal Research Committee of the Government of Freiburg.

Male Sprague–Dawley pcs rats (210–350 g) were used 6 weeks after surgical portosystemic shunting.[15,17] All animals (controls and pcs rats) had a light–dark rhythm of 12 h (07–19 light and 19–07 dark). They were fasted overnight for 12 h prior to drug administration. Within this period water was available ad libitum. Diethylether anesthesia was maintained during surgery and treatment. Similar to what was observed with antipyrine and paracetamol under diethyl ether,[18] anesthesia slightly raised the plasma concentrations of both carvedilol enantiomers, while no significant change in stereoselectivity was observed (Stahl, unpublished data). The right femoral vein, the bladder, the bile duct, and the duodenum were all cannulated. All animals were given a single dose of 10 mg/kg racemic carvedilol as a short infusion into the femoral vein over 1 min (3 rats per group) or by direct administration through the cannula into the duodenum (4 control and 6 pcs rats). In each of the groups, bile was collected for both i.v. and p.o. administration in 2 animals. In a preliminary study no significant difference had been detected between the kinetic parameters without bile collection and with bile collection, indicating that no significant enterohepatic recirculation occurs for parent carvedilol and its glucuronides. Venous blood samples (0.25–0.5 ml) were taken before administration and at 0.17, 0.5, 1, 2, 3, 4, 5, and 6 h afterwards with heparinized syringes. Plasma was separated by centrifugation. Urine was collected before administration of carvedilol and hourly thereafter. Bile was collected before and after drug administration at the following intervals: 0–0.17, 0.17–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–5, and 5–6 h. The withdrawn blood volume (<4.5 ml) was substituted with Ringer solution and the withdrawn bile volume by infusion of pooled rat bile into the duodenum through the cannula. All samples were stored at −20°C until analysis. To study tissue concentrations of drug and conjugates, organs were perfused retrograde with NaCl solution (0.9%) through the caval vein. Liver, kidneys, and heart were excised, weighted, placed into liquid nitrogen for immediate freezing, and stored at −20°C until analysis.

Protein Binding

Protein binding was determined in vitro by equilibrium dialysis (Dianorm™ apparatus with 1.0 ml cells, and Diachema membranes No. 10.17, G. Maierhofer, Munich, FRG) for 4 h with pooled spiked rat plasma (400 ng/ml rac-carvedilol or 400 ng/ml enantiomer) against an isotonic 0.1 *M* phosphate buffer pH 7.4 at 37°C.

To evaluate the possible influence of altered protein content, and hence altered free fraction in plasma from control and pcs rats, total protein and albumin were determined in plasma samples obtained within the first 10 min after drug dosage employing routine laboratory methods.

HPLC Equipment

The HPLC system consisted of a Knauer HPLC pump model 64 (Berlin, FRG), a Waters Resolve™ column (5 μm spherical silica; Waters-Millipore, Milford, MA), a Shimadzu RF 535 fluorescence detector (Kyoto, Japan), a Spectra Physics autosampler SP 8880 with a sample cooler SP 8760 (18°C, to prevent solvent evaporation), and a ChromJet Spectra Physics integrator (San Jose, CA).

Assay Methods

Carvedilol was determined by HPLC after derivatisation to its diastereomers (modified from ref. 19) as previously described for other β-adrenoceptor antagonists.[7,20,21] Briefly, aliquots of plasma, urine (0.2 ml), and bile (0.05 ml) were adjusted to pH 8.9 with 1.0 ml 0.1 *M* carbonate buffer pH 9.8, and extracted with 5.0 ml of diisopropylether (30 min horizontal shaking). In the case of plasma samples, 0.5 g sodium chloride was added in order to improve the separation of the

layers and to aid the release of carvedilol from plasma proteins. After centrifugation (15–30 min, 10°C, 1,500 g), 4.0 ml of the organic layer was evaporated to dryness. The residue was reconstituted in 100 µl of 0.2% methanolic triethylamine. Two hundred microliters of 0.2% methanolic (+)-(R)-phenylethyl isocyanate (freshly prepared) was added, the derivatisation reaction performed at 30°C and stopped after 30 min by adding 200 µl of 0.2% methanolic ethanolamine (10 min, 30°C). The mixture was evaporated and the residue was reconstituted in 250 µl of the mobile phase (diisopropylether–dichloromethane–methanol, 95/5/2, v/v). One hundred microliters of this solution were injected on to the Waters Resolve™ column. The mobile phase was delivered at a flow rate of 1 ml/min (2.8 MPa ≈ 400 psi). The carvedilol derivatives were detected via their fluorescence, excitation 280 nm, emission 340 nm. The detection limit was below 1 ng/ml of each enantiomer. The linear peak area–concentration relationship was confirmed up to 1,500 ng/ml per enantiomer in biological material (correlation coefficient 0.999). The coefficients of variation were evaluated at different concentrations, and were 3.8 and 2.6% at 5 ng/ml, 8.1 and 7.2% at 50 ng/ml, and 6.2 and 7.6% at 150 ng/ml for S and R, respectively.

The conjugates were cleaved with β-glucuronidase (50,000 Fishman units, pH 5, 4 h, 37°C) after 3-fold extraction of the parent compound, prior to hydrolysis. Neither prolongation of incubation time nor increase of enzyme concentration or temperature or pH variation increased the yield of aglycone. The released carvedilol was extracted and derivatised as described above. The carvedilol conjugate concentrations are given as carvedilol equivalents. Coefficients of variation for the conjugate assay were < 10% in the range of 5–500 ng/ml.

Pharmacokinetic Calculations

The kinetic parameters were calculated using noncompartmental analysis applying standard pharmacokinetic procedures.

The fraction of the dose excreted in urine (R) or bile (B) until time t, $Ae_{0\to t}$, was extrapolated to infinity for carvedilol (c) and its conjugates (con) $\to [Ae_{0\to\infty,\text{c or con}} = \to Ae_{0\to t}/(1 - e^{-\lambda z t})]$. Due to the high first-pass extraction after p.o. administration to rats with normal hepatic blood supply, the biliary clearance calculated from the excreted amount and plasma AVC is overestimating the true value, and the obtained value is apparent. Assuming that the conjugates are formed in the liver only and all conjugates are excreted into bile and urine, the clearance of carvedilol enantiomers to their conjugates, $CL_{c\to con}$, was calculated as $Ae_{0\to\infty,con}$(urine + bile)/$AUC_{0\to\infty,c}$.

The absolute bioavailability (F) was estimated from the arithmetical means of the areas under the plasma concentration time curves of the i.v. and p.o. data for each group. The blood to plasma ratio (C_b/C_p) was evaluated according to the equation: $C_b/C_p = 1 - H + (C_{bc} \cdot H/C_p)$, where C_{bc} represents the concentration of carvedilol in blood cells, C_b in blood, C_p in plasma, and H is the hematocrit fraction, i.e., the packed cell volume as determined from the volume ratio after centrifugation. CL divided by the blood to plasma ratio yields the systemic blood clearance ($CL_{sys.b}$). F_{max} of the drug, the maximum bioavailability after p.o. dosage, can be predicted after i.v. administration as the difference between 1 and the extraction ratio (ER). ER was determined as CL_H/Q_H. When CL_R is negligible, the hepatic clearance (CL_H) is equal to $CL_{sys.b}$. The hepatic blood flow (Q_H, in healthy rats 92 ml min^{-1} kg^{-1} [22]) was assumed to be proportional to the bile flow, which was assumed to be unchanged in the pcs rat.

Statistical Methods

All data are given as arithmetical means ($\bar{x}$) and standard deviations (SD_{n-1}). Differences between control and pcs rats were tested for statistical significance using the nonparametric rank sum test of Wilcoxon, Mann, and Whitney (U-test) ($P \leq 0.05$ is significant).

RESULTS AND DISCUSSION

Several publications have described the influence of propranolol on hemodynamics in rats with portal hypertension (of different origin).[23–25] Pharmacokinetic data for β-adrenoceptor antagonists are available, for [^{3}H]propranolol, for example, which was studied in isolated perfused rat livers obtained after partial portal vein ligation and in which it was found that the intrinsic hepatic clearance of propranolol was significantly reduced.[26] Detailed pharmacokinetic data for chiral β-adrenoceptor antagonists in rats with surgical portacaval shunts are not available. In order to evaluate pcs rats as a model to study stereopharmacokinetics in early stage liver cirrhoses, carvedilol was chosen as model compound. During the course of the studies the animals were examined for potential changes in organ morphology and biochemical parameters. These observations were similar to those described previously.[27,28]

Influence of Surgical Portacaval Shunting on Liver Function

Shunt surgery was performed 6 weeks prior to the pharmacokinetic study. Due to decreased blood supply, certain changes occurred in the liver leading to decreased liver function. Rats with pcs had a significantly lower liver weight (40% decrease; $P < 0.05$) and a slightly higher kidney weight (10% increase; n.s.). Bile flow was variably reduced after shunt surgery, ranging from a slight change to a reduction of up to 53%. This indicates that total blood flow is not significantly different from the controls provided that the correlation between hepatic blood flow and bile flow is similar in the two groups of animals. (These findings may support the assumption that anastomoses exist.)

Urea, creatinine, serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase concentrations determined in plasma using standard laboratory procedures, were similar to the control values. The bilirubin concentrations as an indicator of liver disease increased 4-fold after surgical portosystemic shunting.

Plasma Proteins and Protein Binding of Carvedilol

When incubating carvedilol with either control plasma or plasma obtained from pcs rats it was found that the unbound fraction was higher in plasma from pcs rats [control plasma: (S) 98.9, (R) 99.3%, pcs: (S) 97.6, (R) 98.1% bound]. In vitro binding to rat albumin (4% in phosphate buffer) was

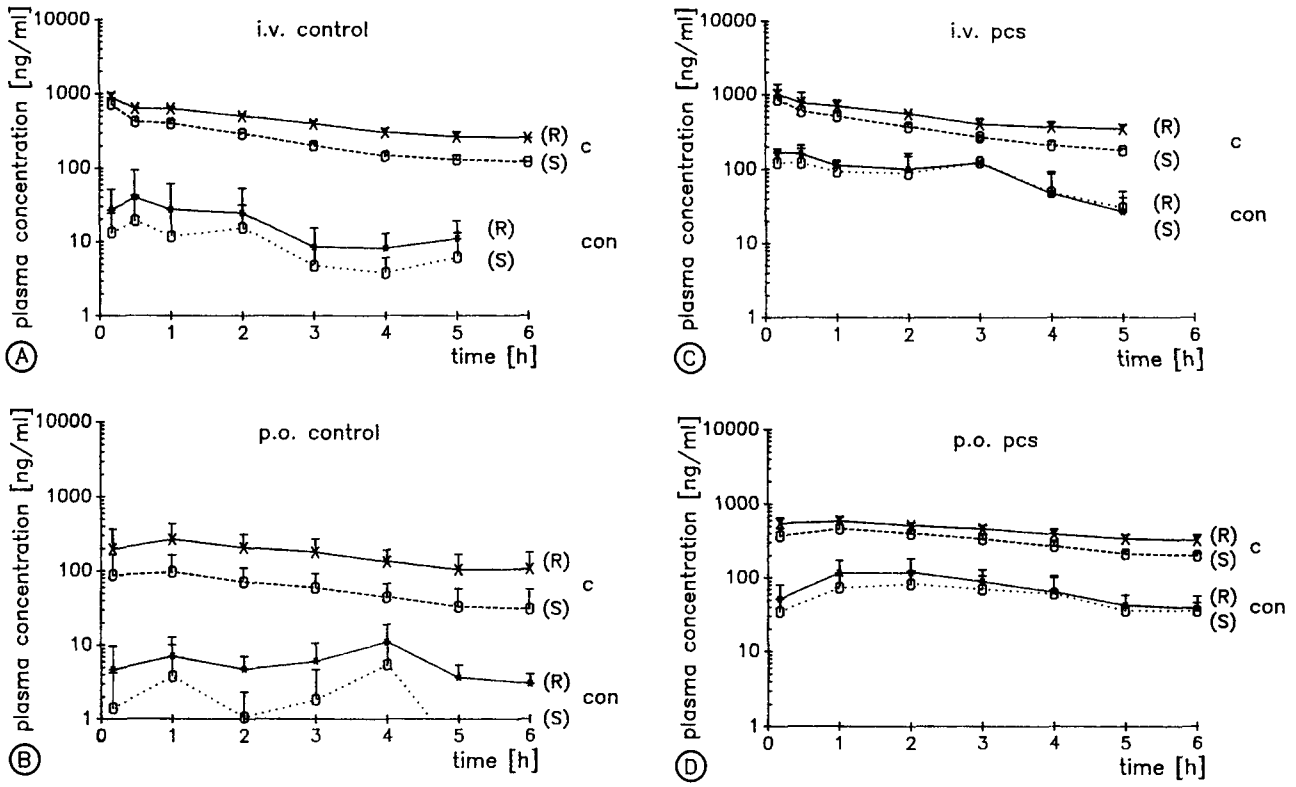

Fig. 2. Mean plasma-concentration-time curves ($\bar{x} \pm$ SD) of (S)- and (R)-carvedilol (c) and their conjugates (con) after i.v. (**A, C**) and p.o. (**B, D**) administration of 10 mg/kg rac-carvedilol in healthy rats (**A, B**) and rats with surgical portacaval anastomosis (**C, D**).

97.1% for (S) and 96.3% for (R), i.e., lower than for plasma ($P < 0.05$; S/R ratio for unbound carvedilol: albumin 0.79, plasma 1.26), which indicates that other binding proteins (α_1-acid glycoprotein) also play a role, as is known for propranolol for which reverse stereoselectivity has been observed in the binding to albumin and α_1-acid glycoprotein (or plasma).[29] Liver disease is frequently associated with reduced total plasma protein, since the liver represents the major site for protein, especially albumin, synthesis. Furthermore, the reduction of α_1-acid glycoprotein is proportional to the degree of cirrhosis.[30,31] Protein concentrations were reduced by 24% in pcs rats (control vs. pcs 54 vs 41 mg/ml; $P < 0.05$) and albumin concentration showed a 29% reduction (control vs. pcs 29 vs 21 mg/ml; $P < 0.05$) in pcs rats.

Alterations in Pharmacokinetic Parameters of Parent Carvedilol

Parent carvedilol in plasma and tissues. After p.o. as well as after i.v. administration, the plasma concentrations of the (R)-enantiomer exceeded those of the (S)-enantiomer in each group (Fig. 2). The plasma concentrations of both enantiomers increased in rats with portacaval shunt, while the differences between the stereoisomers decreased, especially after p.o. dosage (see Table 1; e.g., AUC, S/R ratio, 0.36 control vs. 0.63 pcs; $P < 0.01$). As would be expected from the morphologic changes, the estimated absolute bioavailability (F) was markedly enhanced from 0.29 to 1.08 for (S)- and from 0.37 to 0.95 for (R)-carvedilol. The pharmacokinetic parameters obtained in controls and pcs rats and the associated S/R ratios are listed in Table 1.

Compared to the control group, systemic clearance (CL) was decreased by the portosystemic shunt by approximately 30% of control (n.s.), with the S/R ratio being rather similar. Normalized to the corresponding liver weights, the systemic clearance did not differ between these groups. The oral clearance (CL_0) was significantly reduced to 10% of the baseline value ($P < 0.01$) as was its stereoselectivity with a reduction of 43% (S/R ratio of CL_0, 2.9 for control vs. 1.6 for pcs; $P < 0.01$). The average mean residence time, MRT, of the (R)-enantiomer increased from 4.5 (controls) to 6.0 h (pcs rats) (n.s.), to a higher extent than that of the (S)-enantiomer which increased from 3.5 to 4.1 h. The average steady-state volume of distribution (V_{SS}) was unchanged for (R)-carvedilol, but was approximately 25% lower for (S)-carvedilol (n.s.). Therefore an altered distribution into tissues, with changed S/R ratios, may also be of importance.

Tissue concentrations for the two enantiomers in control and pcs rats (liver, kidney, and heart) are listed in Table 2. Very high tissue concentrations were detectable in all livers. The stereoselectivities in the tissues were the inverse of those of the plasma.

Urinary and biliary excretion of parent drug. Excretion of unchanged carvedilol represents just a minor elimination pathway [< 1.3% for (S), < 0.5% for (R) in the control

TABLE 1. Pharmacokinetic parameters (mean ± SD) of carvedilol enantiomers and their conjugates (con) in healthy rats (control) and rats with portacaval shunt (pcs) after i.v. (A) or p.o. (B) administration of 10 mg/kg racemic carvedilol (values obtained for controls and pcs rats were compared applying the U-test: $*P \leq 0.01$)

		(−)-(S)	(+)-(R)	S/R
		A. i.v.		
Control				
$C_{max.con}$	[ng]	20.4 ± 22.0	42.4 ± 52.0	0.575
$t_{½}$	[h]	2.3 ± 0.4	3.0 ± 0.6	0.785
$AUC_{0\ \infty}$	[ng ml^{-1} · h]	1841 ± 212	3499 ± 605	0.530
MRT	[h]	3.5 ± 0.6	4.5 ± 0.9	0.779
CL_{tot}	[ml/min]	13.7 ± 4.3	7.4 ± 2.9	1.893
$CL_{tot/liver}$	[ml/min/g]	1.55 ± 0.60	0.84 ± 0.39	1.890
CL_R	[ml/min]	0.010 ± 0.005	0.004 ± 0.002	2.473
$CL_{R.con}$	[ml/min]	0.03 ± 0.03	0.05 ± 0.06	0.876
CL_B	[ml/min]	0.075 ± 0.043	0.016 ± 0.004	4.504
$CL_{c\ con}$	[ml/min]	0.11 ± 0.03	0.05 ± 0.01	2.008
$CL_{c\ others}$	[ml/min]	11.0 ± 0.7	5.6 ± 0.3	1.958
V_{ss}	[liter/kg]	0.52 ± 0.96	6.30 ± 0.29	1.515
pcs				
$C_{max.con}$	[ng]	169.9 ± 116.4	195.3 ± 61.9	0.802
$t_{½}$	[h]	2.9 ± 0.6	4.1 ± 0.5	0.703
$AUC_{0\ \infty}$	[ng ml^{-1} · h]	2524 ± 349	4606 ± 901	0.554
MRT	[h]	4.1 ± 0.5	6.0 ± 0.5	0.675
CL_{tot}	[ml/min]	8.9 ± 3.8	5.0 ± 2.6	1.818
$CL_{tot/liver}$	[ml/min/g]	1.51 ± 0.38	0.84 ± 0.27	1.818
CL_R	[ml/min]	0.018 ± 0.013	0.007 ± 0.003	2.554
$CL_{R.con}$	[ml/min]	0.04 ± 0.06	0.04 ± 0.06	1.226
CL_B	[ml/min]	0.209 ± 0.029	0.022 ± 0.005	10.01
$CL_{c\ con}$	[ml/min]	0.45 ± 0.37	0.15 ± 0.07	2.634
$CL_{c\ others}$	[ml/min]	8.8 ± 4.8	5.4 ± 3.3	1.665
V_{ss}	[liter/kg]	8.05 ± 0.28	6.62 ± 0.90	1.227
		B. p.o.		
Control				
C_{max}	[ng/ml]	99 ± 72	267 ± 167	0.355
$C_{max.con}$	[ng/ml]	3.8 ± 6.3	7.5 ± 5.6	0.303
t	[h]	3.1 ± 1.3	3.0 ± 1.1	1.060
$AUC_{0\ \infty}$	[ng ml^{-1} · h]	460 ± 235	1268 ± 612	0.358
MAT	[h]	1.22	0.25	4.880
CL_o	[ml/min]	69.2 ± 49.9	22.6 ± 11.4	2.894
CL_R	[ml/min]	0.004 ± 0.002	0.002 ± 0.001	2.444
$CL_{R.con}$	[ml/min]	0.02 ± 0.01	0.01 ± 0.01	5.200
CL_B	[ml/min]	0.587 ± 0.267	0.086 ± 0.050	7.832
$CL_{c\ con}$	[ml/min]	0.66 ± 0.31	0.23 ± 0.10	2.891
F		0.290	0.368	0.787
pcs				
C_{max}	[ng/ml]	472 ± 109*	602 ± 72*	0.779*
$C_{max.con}$	[ng/ml]	86.3 ± 40.3*	129.0 ± 61.3*	0.695*
$t_{½}$	[h]	3.1 ± 0.4	4.3 ± 0.6	0.717
$AUC_{0\ \infty}$	[ng ml^{-1} · h]	2855 ± 365*	4617 ± 546*	0.626*
MAT	[h]	0.98	0.75	1.307
CL_o	[ml/min]	8.1 ± 1.0*	5.0 ± 0.8*	1.638*
CL_R	[ml/min]	0.010 ± 0.008	0.005 ± 0.003	2.116
$CL_{R.con}$	[ml/min]	0.05 ± 0.07	0.02 ± 0.02	2.126
CL_B	[ml/min]	0.109 ± 0.008	0.012 ± 0.001	9.087
$CL_{c\ con}$	[ml/min]	0.21 ± 0.05	0.10 ± 0.02	1.985
F		1.076	0.954	1.129

TABLE 2. Tissue concentrations of carvedilol enantiomers in control and pcs rats 6 h after p.o. or 5 h after i.v. administration of 10 mg/kg rac-carvedilol, given in carvedilol equivalents per tissue (ng/g)

	Control			Pcs		
	(S)	(R)	S/R	(S)	(R)	S/R
i.v.						
Plasma	148	303	0.49	174	355	0.49
Heart	1020	718	1.42	1324	939	1.41
Liver	171	174	0.98	337	229	1.47
Kidney	1031	628	1.64	2536	1393	1.82
p.o.						
Plasma	12	56	0.22	194	347	0.57
Heart	62	84	0.73	2514	1911	1.32
Liver	179	131	1.36	530	367	1.45
Kidney	65	72	0.90	3267	2145	1.55

rats] as reflected by the small value obtained for renal and biliary clearance (Table 1).

After shunt surgery the total amount of the parent drug, calculated as percent of dose, excreted into urine ($Ae_{0\ \infty.Rc}$) increased 3-fold after i.v. (n.s.) and more than 10-fold after p.o. administration for both enantiomers ($P < 0.01$), while the renal clearance was similar in both groups (i.e., only up to 2-fold higher in pcs rats). These findings are in accordance with our previously published results with, for example, diuretics in reduced liver function, where we found that decreased hepatic clearance leads to decreased total clearance and an increase in the amount of drug excreted into urine, while the renal clearance remains almost unaffected.[32,33]

The highest biliary excretion rate for the parent compound was found for the p.o. controls, apparently because of the high first-pass extraction. The fact that the biliary clearance is higher in pcs rats is explained by a reduced biotransformation rate in the hepatocyte.

Systemic Blood Clearances and Maximum Bioavailabilities

Maximal bioavailabilities were calculated on the basis of blood concentrations or blood/plasma ratios and blood clearance. The blood to plasma ratio (C_b/C_p) in healthy rats showed a slight stereoselectivity with an S/R ratio of approximately 1.3 [(S) 0.62 and (R) 0.48]. In pcs rats this ratio was similar, 1.2 [(S) 0.68 and (R) 0.57]. According to Fujimaki et al.[12] the blood to plasma ratio in humans was higher with a comparable stereoselectivity [C_b/C_p) (S) 0.74, (R) 0.67; S/R 1.1].

The calculated systemic blood clearance ($CL_{sys.b}$) for the control and pcs groups was for (S)-carvedilol 22.0 vs. 14.4 ml/min and for (R)-carvedilol 15.3 vs. 10.5 ml/min, while the S/R ratio remained almost unchanged (1.43 vs. 1.37). The apparent oral blood clearance after pcs was similar to the systemic blood clearance, which is to be expected since there is no first-pass extraction and no reduction of the systemically available fraction of the dose.

When calculating F_{max} from the parameters obtained for healthy rats, values of 0.18 for (S) and 0.43 for (R) resulted,

which approximate the experimentally obtained F values sufficiently [(S) 0.29, (R) 0.37]. The expected F_{max} values for cirrhotic rats are 0.46 for (S) and 0.64 for (R); these values were predicted from the degree of reduction of hepatic function (40% decrease in liver weight). With a portacaval shunt (and no first-pass extraction) the measured bioavailabilities were close to 100% [(S) 1.08 and (R) 0.95], indicating complete absorption of the drug.

Carvedilol Conjugates and Metabolic Clearances

Similar to the parent compound, the (R)-carvedilol conjugates always exceeded the (S)-carvedilol conjugates in plasma. In the presence of pcs, the conjugates of both enantiomers increased significantly, while their stereoselectivity was reduced (e.g., after p.o. dosage, AUC, S/R ratio, 0.20 control vs. 0.93 pcs; $P < 0.01$) (Fig. 2).

Small amounts of conjugates were detected in the tissues with different stereoselectivities (Table 2). After i.v. dosage no relevant differences were found between the conjugate tissue concentrations of controls vs. pcs rats, while the concentrations in all investigated tissues after oral dosage were higher in pcs rats.

The fraction of the dose found in conjugated form in urine was smaller than of parent carvedilol. After i.v. administration, an average of 0.92% was found as a conjugate of the (R)- and 0.95% as a conjugate of the (S)-enantiomer in urine and bile of controls. The respective values for the pcs rats are 2.93% (R) and 4.34% (S) (n.s.). Just as for parent carvedilol, the fraction of conjugates excreted into urine was significantly higher in rats with pcs than in the controls (for p.o.-administration, $P < 0.01$), indicating a reduced biliary excretion of conjugates in pcs rats.

The calculated clearance of carvedilol enantiomers to their conjugates, $CL_{c\rightarrow con}$, after i.v. administration was up to 4-fold higher in pcs rats, with a 31% increase of the S/R ratio. This indicates that conjugation is not as much affected in pcs rats as is phase-I metabolism and that due to the reduced drug clearance by phase-I pathways greater amounts of the drug are available for phase-II metabolism. It is known that the perivenous hepatocytes are supplied with larger amounts of compounds and this acinar zone is mainly responsible for phase-II metabolism. It is also known that liver disease initially (e.g., hepatic fibrosis) affects oxidative metabolism,[34] and in an advanced state (e.g., cirrhosis) the conjugation is affected as well.[35] Interestingly, the apparent clearance of the unconjugated drug to other metabolites ($CL_{c\rightarrow others}$) which represents phase-I metabolism, was predominantly reduced for (S)-carvedilol by pcs. Therefore, the data obtained are in good accordance with observed changes in liver disease. With respect to phase-II metabolism, it is known in cirrhosis that the hepatic UDP-glucuronosyltransferase (UDP-GT) activity is correlated with the total serum bilirubin concentration[15], because increased bilirubin (which is increased in cirrhosis) competitively inhibits glucuronide formation from bilirubin and possibly also from xenobiotics. The observed decreased zinc concentrations may also affect the UDP-GT activity.[35] In rats with surgical portacaval anastomosis, fibrotic changes occurred without destruction of liver architecture. Due to intrahepatic shunts oxygen supply in certain liver areas may be reduced. Consequently, it may be concluded that, in comparison to healthy rats, lower amounts of oxidative phase-I metabolites are formed, resulting in higher concentrations of the parent compound and increased formation of phase-II metabolites.

CONCLUSIONS

In our experimental set-up with exising portacaval shunt, the first-pass extraction of carvedilol is completely eliminated. Because of the complete absorption F equals 100% and apparent oral clearance thus equals total plasma clearance in pcs rats. In reduced liver function in patients, depending upon its degree and the morphologic changes,[36] first-pass extraction may still play a role (namely as long as the intrinsic hepatic clearance represents a relevant contribution to total clearance).

The applicability of the pcs rat as an experimental pharmacokinetic model for liver cirrhosis, however, is also dependent upon the type of drug as well as the route of administration. The pcs rat is an appropriate model of cirrhosis to study the kinetics of low clearance drugs (where a first-pass effect does not occur) as well as the i.v. kinetics of high clearance drugs and kinetics in liver cirrhosis with portacaval anastomosis. It may be used as well for the kinetics after p.o. doses of high clearance drugs including influence of liver function on first-pass effect,[16] however, only when the portacaval shunt is removed and the portal blood supply is reconstituted prior to the pharmacokinetic study.

NOTE ADDED IN PROOF

While the present manuscript was in the revision process, a clinical study was published (Proceedings of a Satellite Symposium to the 5th ESH Meeting, Milan, June 1991), in which similar changes in carvedilol stereopharmacokinetics were described for cirrhotic patients (Neugebauer, G., Gabor, M., Reiff, K. Disposition of carvedilol enantiomers in patients with liver cirrhosis: Evidence for disappearance of stereoselective first-pass extraction. J. Cardiovasc. Pharmacol. 19 (Suppl. 1):S 142–146, 1992).

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