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Research Article

Evidence for Intestinal Secretion as an Additional Clearance Pathway of Talinolol Enantiomers: Concentration- and Dose-dependent Absorption in Vitro and in Vivo*

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Purpose. To evaluate carrier-mediated intestinal secretion of talinolol enantiomers in vivo and in vitro.
Methods. In clinical studies with i.v. and p.o. dosage of rac-talinolol (30 mg and 100 mg, resp.) performed in a small number of cholecystectomized patients total and partial clearances were determined on the basis of plasma, bile and urine concentrations. The dose-dependence of AUC was investigated in 12 healthy volunteers (25, 50, 100, and 400 mg rac-talinolol as single p.o. doses). Concentration-dependence of the permeability across Caco-2 cell monolayers included concentrations from 0.1 to 2.0 mM, inhibition by verapamil was tested at 0.5 mM.
Results. The total clearance as well as the apparent oral clearance (CL/F) were slightly higher for S-(−)- than for R-(+)-talinolol. Calculation of the partial clearances showed that also the residual clearance was higher for the S- than for the R-enantiomer. In the healthy volunteers, CL/F increased with increasing doses, while the S/R ratio decreased approaching unity for the highest dose. Also the results from Caco-2 cell permeation studies yielded a clear concentration-dependence with decreasing stereoselectivity for the higher concentration range. Permeability of both enantiomers was considerably higher for b→a than a→b transport, however, this difference disappeared when verapamil was added.
Conclusions. Although not very expressed, the detected stereoselectivities indicate a preferential absorption of R-(+)-talinolol in a lower concentration and dose range, which is most probably due to a moderate stereoselectivity at the carrier system involved in intestinal secretion.

KEY WORDS: intestinal secretion; intestinal absorption; bioavailability; non-linear pharmacokinetics; stereoselectivity; enantiomers; beta-adrenoceptor antagonists; talinolol.

INTRODUCTION

Several recent research articles on drug absorption have focussed on the impact of gastrointestinal (GI) physiology on the absorption process and the overall kinetic profile. This mainly includes gastric emptying (1–3) as well as site-dependence of absorption (4), complexation within the gut (5) and secretion processes into the gut (6). The studies recently performed with the β-adrenoceptor antagonist celiprolol in dogs demonstrated unambiguously that with p.o. dosage the occurrence of double peaks and shoulders in concentration-time profiles is correlated with the phase of high GI-motility (3). A compound that is structurally related to celiprolol is the also racemically administered talinolol, S(−)/R(+)-1-(4-cyclohexyl-ureido-phenoxy)-2-hydroxy-3-tert.butyl-aminopropane, the S-enantiomer of which is a highly selective β_1-adrenoceptor antagonist (8) without intrinsic sympathomimetic and membrane stabilizing activity. Like celiprolol the drug is a weak base with a pK_a of 9.4 and the solubility of the base in water is very poor (0.2 mg/ml). Talinolol has intermediate lipophilic properties (p.c. = 0.74, n-octanol/water at pH 7 and 37°C) when compared with other β-adrenoceptor antagonists.

Like with celiprolol, in preliminary bioanalytic/pharmacokinetic studies (9) expressed double peaks were detected in

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several volunteers following p.o. talinolol. Another aspect, which also turned out to be of relevance with respect to talinolol is the phenomenon of a dose-dependence of absorption, with an increasing extent for higher doses. This has also been observed with celiprolol in previous clinical studies. For the β-adrenoceptor antagonist pafenolol the dose-dependent bioavailability was found to be due to nonlinear intestinal uptake (10). One reason may be a saturable binding between the drug and bile acids in the gastrointestinal lumen that can form nonabsorbable complexes at low concentrations (5, 10). Formation of such a nonabsorbable complex has earlier been demonstrated to be the underlying mechanism for the low extent of intestinal absorption of nadolol, another beta-blocking agent (11, 12). Furthermore, for pafenolol it was found that intestinal secretion (exsorption) accounts for approximately 25% of an i.v. dose (13). Other drugs—e.g. celiprolol (14, 15) monoquaternary ammonium bases (16), cyclosporine A (17) and theophylline (18)—exhibit dose-dependent absorption properties. In addition, besides intestinal secretion processes (exsorption of drug into the intestinal lumen), also gut metabolism—in addition to metabolism in the hepatocytes—is discussed to contribute to the overall kinetic profile (18–20).

Although the mechanism of intestinal secretion has not been fully elucidated more evidence is evolving which suggests that the secretory process is maintained by P-glycoprotein (P-gp), a 170 kD plasma membrane protein in apical membranes of enterocytes (21). In tumor cells P-gp functions as an ATP-dependent efflux pump for many anticancer drugs and other compounds that are hydrophobic organic cations with molecular weights of 400–1000 (22). Immunocytochemistry techniques with monoclonal antibodies to human P-gp have revealed its localization in the plasma membranes of several normal human and animal tissues as well such as the liver, small and large intestine and brain capillaries (23). One reason for its existence in the intestine may be the protection against chemical carcinogens, which are abundant in plant and animal components of our diet. The interference of P-gp with the absorption of several drugs may therefore seem preprogrammed and further knowledge on this interference may help in explaining some of the phenomena and peculiarities seen after oral drug administration.

In the present study, the stereopharmacokinetics of a single i.v.- vs. p.o.-dose as well as different p.o. doses of rac-talinolol, a compound exhibiting a very low metabolic clearance (<1%), were studied in cholecystectomized patients or healthy volunteers, respectively, in order to investigate the evidence and potential relevance for carrier-mediated intestinal secretion of talinolol enantiomers. The 3 different aims of the project comprising in vivo studies in humans and in vitro experiments are as follows:

1. The quantification of accessible total and partial clearances (renal, biliary, residual), in order to find out, to which extent intestinal secretion may contribute to the overall kinetic profiles of the two talinolol enantiomers.
2. The evaluation of a potential in-vivo saturability (= concentration/dose dependence) of the hypothesized intestinal secretion process, which should result in a nonlinearity in the relationship between dose normalized AUC as well as apparent oral clearance and the administered dose.
3. The proof that in a cell culture model with Caco-2 cells in-vitro permeability is also concentration-dependent and saturable, and furthermore basolateral-to-apical directed and inhibitable by verapamil, a P-glycoprotein inhibitor. The cell line is originating from a human colorectal carcinoma and forms confluent monolayers of well differentiated enterocyte-like cells with many of the functional properties of the small intestinal epithelium (24). In addition to carrier systems involved in nutrient uptake as e.g. glucose-, amino acids- or peptide-transporters, Caco-2 cells also express P-gp (25), thus making this model a particularly useful part in investigations of intestinal drug secretion and also of its potential stereoselectivity.

METHODS

Drugs and Reagents

Talinolol and its enantiomers (S)- and (R)-talinolol were obtained from the Arzneimittelwerk Dresden GmbH, Germany. In the clinical studies rac-talinolol was administered as commercially available preparations (either intravenously as a 30 mg dose in physiological saline at a total volume of 250 mL or perorally as tablets with 100 mL tap water) or in gelatine capsules at doses of 25, 50, 100, and 400 mg, respectively. The i.v. and solid formulations were provided by the Arzneimittelwerk Dresden. Reagents for the preparation of the buffer solutions were purchased from Fluka (Buchs, Switzerland) and were of the highest purity available. Dulbecco's modified Eagle's medium (DMEM), MEM non essential amino acids (NEAA) (100 ×) solution, 0.05% trypsin/0.025% ethylene diamine tetraacetic acid (EDTA) solution, Hank's balanced salt solution (HBSS), Dulbecco's modified phosphate-buffered saline with and without Ca^{2+} and Mg^{2+} (PBS) were obtained from Gibco BRL-Life Technologies, Paisley, UK. Fetal calf serum was obtained from PAA Labor-und ForschungsGmbH, Linz, Austria.

Equipment

Cell culture flasks, cryotubes and 6-well multidishes were purchased from Nunc, Roskilde, DK, Snapwell cell culture chamber inserts from Costar, Badhoevedorp, NL, and side-by-side diffusion chambers, gas-manifold and block heater from Precision Instrument Design, Los Altos, USA.

Analytical Methods

To quantify talinolol enantiomers in plasma, a newly developed enantiospecific HPLC method was employed (9). The detection limit was 2.5 ng enantiomer per ml plasma. Both, intra- and interday coefficients of variation were below 5% except for concentrations at the detection limit, where it increased to 11%. Slight modifications were made for urine and bile samples, where 100 µl sample aliquots were used and similar volumes of 1 M sodium hydroxide were added. Also for the solutions obtained from the in-vitro cell culture studies, sample clean-up and chromatographic conditions were nearly identical with the method described for the clinical study with 0.5 ml sample and sodium hydroxide aliquots. However, it was necessary to introduce a second extraction step to reduce the buffer salt concentration in the organic layer by washing it with 2 ml 0.1 M NaOH. Under these conditions talinolol remained in the organic layer, while the buffer salts were reduced.

Clinical Studies

Estimation of Systemic and Partial Clearances Following a Single Intravenous or Peroral Racemate Dose

Six cholecystectomized patients with a T-drain were included into the study between days 5 and 8 post surgery, in order to assure normalization of bile flow. Half of the patients received a 30 mg i.v. dose as solution in physiological saline at a total volume of 250 ml as a 30-min short-infusion and the other three patients a 100 mg p.o. dose of rac-talinolol (tablet together with 100 ml tap water) after an overnight fast of at least 12 h and after having obtained blank blood, urine and bile samples. Breakfast was allowed at 2 h post-dose. Blood sampling was every 10 min in the first hour after i.v.-administration and every 20 min after p.o. dosage, then every 20 min up to 2 h, every 30 min up to 5 h, and after 6, 7, 8, 10, 12, 24, and 36 h. Urine was collected in intervals up to 72 h post-dose. Biliary excretion rates were determined via a 10-min collection of bile at a total of 8 time points (30 min, 1, 3, 4, 6, 12, and 24 h post dose).

Heparinized venous blood samples (10 ml) were centrifuged, in order to obtain plasma. All biological samples were stored frozen at −20°C and analyzed within 2 months.

Clinical Studies with Increasing P.O. Doses

Plasma samples had been obtained from a clinical study, which included an investigation of the kinetics-dynamics relationship of talinolol (de Mey et al., submitted) from each of twelve healthy male volunteers (ranging in age from 24 to 30 years) who had received four different doses—25, 50, 100, and 400 mg talinolol—in at least one-week intervals and in a randomized double blind cross-over design following a drug-free period of at least one month. The capsules were administered perorally with 100 ml tap water in the morning after an overnight fast for at least ten hours. The subjects had remained in fasted state up to 7 hours after administration and had not been allowed either to smoke or drink any alcohol on study days as well as 10 hours before.

Venous blood (5 ml) was collected in heparinized tubes before dosing, hourly for the first four hours and then after approximately 7.5, 10, and 24 h. Blood samples had immediately been centrifuged at 2500 g and plasma stored at −20°C until analysed.

Ethical Considerations

With respect to the clinical studies in cholecystectomized patients and healthy volunteers, respectively, written informed consent and ethics committee approval had been obtained.

Data Analysis

Numerical results were summarized as arithmetic means and standard deviations (SD_{n-1}). The pharmacokinetic parameters (AUC, $t_{1/2}$, C_{max}, t_{max}) were determined and calculated using standard procedures. The AUC values were calculated using the linear trapezoidal rule for the ascending part of the concentration-time profile and with the logarithmic trapezoidal rule for the descending part of the concentration-time-profile. Extrapolation to infinity was made on the basis of the terminal elimination rate constant (including at least 3 data points of the terminal disposition phase). Clearance terms were determined from the administered dose and the area under the plasma concentration-time profile (yielding the systemic clearance for i.v.- and the apparent oral clearance (CL/F) for p.o-administration). Partial clearances (renal clearance, CL_R, biliary clearance, CL_B, metabolic clearance, CL_M, (assumed to be negligible (<1%)); residual clearance, CL_{other}, as difference of the systemic clearance and the sum of unchanged renal and biliary clearances) were estimated for both enantiomers on the basis of plasma concentrations and urinary or biliary exretion rates, where the urine volume was measured and the average bile flow taken from the literature (30).

The total amounts of talinolol enantiomers excreted into urine and with bile (Ae) during the observation period (0-t) were extrapolated to infinity on the basis of the respective terminal rate constant (λ_z) according to the following equation:

$$Ae_{0\to\infty} = \frac{Ae_{0\to t}}{(1 - e^{-\lambda_z t})}$$

In addition, a compartmental analysis was performed employing the TOPFIT program (26) with a linear disposition model, because terminal half-lives were not significantly different between doses. For all but one of the concentration-time profiles appropriate fits and better fitting criteria (Akaike, Imbimbo, Schwarz) were obtained when 3- vs. 2-compartmental models as well as models with multi-segmental (2–3 segments) input were used, even when no expressed double peaks, but only shoulders were observed. When all available data were simultaneously fitted for one patient, different weighting factors were used for the different biological matrices: plasma, 1.0; urine, 0.2; and bile, 0.1.

With respect to p.o. dosage, the total dose/F was split into fractions that enter the systemic circulation at different times after dosing. Each segment was defined by three parameters, the relative dose fraction, the input rate constant and the lag-time (2). Plasma concentration-time profiles of one volunteer and one enantiomer were fitted simultaneously for all p.o. doses and for each enantiomer (weighting factors, unity for all profiles).

The total mean input time (MIT) was calculated as integral input parameter and equals the sum of the single MITs for the various input phases:

$$MIT = \sum_{i=1}^{n} f_{a,i}\left(\frac{1}{k_{in,i}} + t_{lag,i}\right)$$

where $f_{a,i}$ is the relative dose fraction, $k_{in,i}$ the input rate constant and $t_{lag,i}$ the lag time.

Statistical analyses with respect to differences between the two enantiomers as well as between the dose-normalized AUC and CL/F values were calculated using parametric procedures (ANOVA, $p > 0.05$ = n.s.)

Intestinal Cell Culture Studies

Cell Line

Caco-2 cells at passage 65 were a gift from Dr. Jürg Biber (Institute of Physiology, University of Zurich, Switzerland) and were used between passage 70 and 75. The cells were cultivated

according to previously published procedures (24). For the transport studies, Caco-2 cells were seeded at a density of 100'000 cells/cm^2 into polycarbonate filter inserts (Costar Snapwell cell culture inserts having a filter diameter of 1.13 cm^2 and a mean pore diameter of 0.4 μm). The cells were grown in Dulbecco's modified Eagle's medium containing 20% fetal calf serum, 1% L-glutamin, 1% non essential amino acids, 4.5 g/l L-glucose and 0.11 g/l sodium pyruvate in a humidified atmosphere of 10% CO_2:90% air at 37°C. The medium was changed every second day. The cells were routinely tested for mycoplasma infection applying DNA staining with 4',6-diamino-2-phenylindole-dihydrochloride (DAPI). No infections were observed. The cells were also characterized by light microscopy, scanning and transmission electron microscopy. Caco-2 cells were measured for transepithelial electrical resistance before and after undertaking the transport studies to check the integrity of the monolayer with a Millicell-ERS (Millipore Corporation, Bedford, USA). The cells were also tested positive for the presence of P-glycoprotein with FITC-labelled C219 monoclonal antibody (Centocor Diagnostics Division, Malvern, PA, USA) specific for an internal epitope of P-gp, which is highly conserved between species, and with anti-P-gp monoclonal antibody MRK 16 (Kamiya Biomedical, Thousand Oaks, CA, USA) using FACS analysis.

Permeation Studies

Transport experiments were performed at 37°C in Hanks' Balanced Salt Solution (HBSS) containing 10 mM MES buffered to pH 6.5. Five milliliters of the prewarmed 0.25–5.0 mM rac-talinolol solution in buffered HBSS were added to either the apical (a) or the basolateral (b) side of the cell monolayer and drug-free buffered HBSS was added to the opposite side. Each chamber was bubbled with carbogen (95% O_2, 5% CO_2) for homogeneous mixing of each cell's contents. The permeation experiment lasted for 2 hours. Samples (600 μl) were taken every 20 minutes (for apical-to-basal transports from the basal side and vice versa) and were replaced with equal volumes of fresh buffered HBSS immediately. The samples were stored at −20°C until analysis. Experiments with the P-gp inhibitor verapamil were performed at 500 μM verapamil concentrations on the apical and basolateral side; verapamil concentrations of 100 μM have already been reported to reduce the efflux of drugs (25)).

Effective permeability coefficients (P_{eff}) were calculated according to the following equation:

$$P_{eff} = \frac{dC/dt \cdot V}{AC_0} \; [cm\ s^{-1}]$$

where (dC/dt) is the flux across the monolayer (μg ml^{-1} s^{-1}) at steady state, A is the diffusion area (cm^2), V is the volume of the acceptor compartment (ml) and C_0 the initial concentration in the donor compartment (μg/ml). Data are presented as means ± standard deviation of n Caco-2 cell monolayers.

RESULTS

Clinical Studies

Cholecystectomized Patients

Following the 30 mg i.v.-dose of rac-talinolol the plasma concentrations of the S-enantiomer of talinolol were almost always slightly exceeded by those of the respective R-enantiomer in the three patients included into this part of the study (Fig. 1) (Table 1A). The total fraction of the respective enantiomer dose excreted into urine amounted to 50% for the S- and to 64% for the R-enantiomer when talinolol was given intravenously, while the respective values for biliary excretion were 9.1 (S) and 8.9% (R). The average total clearance was calculated to be 321 for S- and 269 ml/min for R-talinolol. The respective apparent oral clearance values (CL/F) obtained with p.o.-dosage of 100 mg racemate were in the range of 473–914 ml/min for S- and 448–807 ml/min for R-talinolol. CL/F was always slightly higher for S-talinolol. In this part of the clinical studies the half-lives were usually longer (1.5 times) than in the below mentioned study with different p.o. doses. When urinary as well as bile excretion data were included and the data simultaneously fitted, even longer terminal half-lives resulted, since small amounts of talinolol were still detectable in urine after 72 h.

The renal clearances were in the range of 139–166 for R- and of 147–169 ml/min for S-talinolol [i.v.: 153 (S), 163 ml/min (R); p.o.: 160 (S), 154 ml/min (R)]. Significant differences were neither detected between enantiomers nor between administration routes. These clearance values exceeded those of the glomerular filtration rate (GFR), which was calculated to be in the range of 70–80 ml/min for a patient population of this age (average age, 55 ± 6 yrs).

Biliary clearance appeared to be higher following p.o.- than after i.v.-dosage with a slight preference for the S-enantiomer [i.v.: 28 (S), 23 ml/min (R); p.o.: 55 (S), 36 ml/min (R)]. For 5 of the 6 i.v.- and p.o.-treated patients the residual clearance

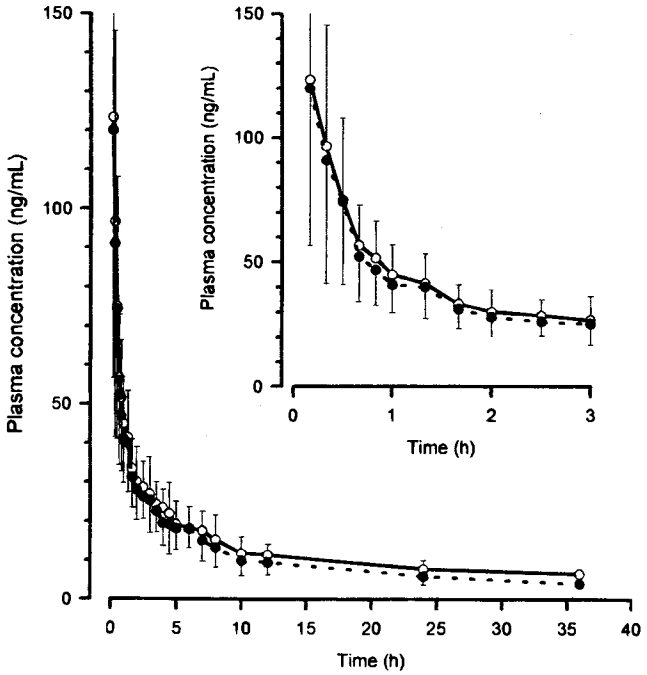

Fig. 1. Average plasma concentration-time profiles for R-talinolol (open circles) and S-talinolol (closed circles, broken line) following an i.v. short infusion of 30 mg rac-talinolol to cholecystectomized patients (n = 3, arithmetical means ± SD). Average plasma concentrations of the R-enantiomer are always exceeding those of the S-enantiomer (eutomer).

Table 1A. Pharmacokinetic Parameters for Talinolol Enantiomers Obtained After I.V. and P.O. Dosage of 30 mg or 100 mg Rac-Talinolol (15 and 50 mg of Each Enantiomer), Respectively, in Cholecystectomized Patients (n = 3 for Each Group; Arithmetical Means ± SD)

	30 mg i.v. dose (= 15 mg enantiomer)		100 mg p.o. dose (= 50 mg enantiomer)	
	S-(−)	R-(+)	S-(−)	R-(+)
C_O (ng/ml)	1428 ± 697	1484 ± 673	—	—
C_{max} (ng/ml)	—	—	208 ± 38.5*	217 ± 39.4
t_{max} (h)	—	—	1.44 ± 0.51	1.44 ± 0.51
$t_{1/2,z}$ (h)	36.1 ± 12.0+	47.2 ± 18.7+	20.7 ± 2.0	23.0 ± 11.1
$AUC_{0\to\infty}$ (ng $ml^{-1}h$)	803 ± 181	936 ± 107	1043 ± 460*	1433 ± 415*
MRT_{sys}, MRT_{tot} (h)	19.4 ± 6.8	33.4 ± 22.0	16.5 ± 3.0	15.5 ± 2.5
V_c (l)	12.2 ± 5.4	11.6 ± 5.1	—	—
V_{ss} (l)	646 ± 148	779 ± 372	—	—
Ae_{urine} (% of dose)	49.7 ± 16.5	64.3 ± 6.5	25.3 ± 9.2	27.0 ± 10.0
Ae_{bile} (% of dose)	9.1 ± 4.8	8.9 ± 5.1	7.7 ± 3.3	5.6 ± 1.9
CL, CL/F (ml/min)	321 ± 65	269 ± 31	685 ± 221	616 ± 181
CL_R (ml/min)	153 ± 15	163 ± 5	160 ± 11	154 ± 14
CL_B, $Cl_{B,app}$ (ml/min)	28 ± 12	23 ± 10	55 ± 30	36 ± 16
$CL_{residual}$ (ml/min)	140 ± 84	84 ± 43	471 ± 207	426 ± 182

Abbreviations: C_O, initial concentration (as if the i.v. dose was a bolus); C_{max}, maximum concentration at time t_{max}; $t_{1/2,z}$, terminal half-lives (as obtained from simultaneous curve fitting of plasma, urine and bile data); MRT_{tot}, total mean residence time (AUMC/AUC); MRT_{sys}, systemic mean residence time for i.v. talinolol (difference between MRT_{tot} and the mean input time, which equals the mean time during the infusion period); V_c, initial volume of distribution (D/C_O); V_{ss}, volume of distribution at steady-state (CL·MRT); $AUC_{0\to\infty}$, area under the concentration-time curve (extrapolated to infinity); Ae, amount excreted (into urine (U) or bile (B), respectively); CL, systemic (plasma) clearance; CL/F, apparent oral clearance; CL_R, renal clearance; CL_B, biliary clearance; $CL_{residual}$, fraction of total (systemic or apparent oral) clearance, which is not explained by excretion into urine or bile.

*Statistically significant between R- and S-talinolol ($p < 0.05$, ANOVA).

+Includes one very high value.

[i.v.: S, 44%, R, 31% of CL] was higher for S-than for R-talinolol. In one of the i.v.-treated patients only very little difference between the systemic clearance of the two enantiomers was detected. For this patient the residual enantiomer clearances were low and tended to be inverse when compared with the other patients' data.

Dose-dependence in Healthy Volunteers

Irrespective of the dose, the shapes of the plasma concentration-time profiles of S- and R-talinolol were quite similar in the study in healthy volunteers. In all subjects concentrations of R-(+)-were higher than those of S-(−)-talinolol. Only in very few samples higher S-(−)-concentrations were detected. In this study as well, the AUC and C_{max} values were found to be constantly slightly higher for R-(+)-than for S-(−)-talinolol. The corresponding average pharmacokinetic parameters are listed in Table 1B. For the two major bioavailability measures, $AUC_{0\to\infty}$ and C_{max}, the average S/R ratios were calculated (for the 25, 50, 100 and 400 mg dose, respectively) yielding the following values: 0.83, 0.90, 0.88, 0.92 for $AUC_{0\to\infty}$ and 0.91, 0.96, 0.94, 0.96 for C_{max}. For both parameters, the decrease of the ratios reached statistical significance when comparing the highest and the lowest dose ($p < 0.05$). For both enantiomers the dose-normalized AUC increased with increasing doses, while absorption rates as well as CL/F decreased. Average CL/F values amounted to 1062 (S) and 830 ml/min (R) for the lowest and 498 (S) and 452 ml/min (R) for the highest dose ($p < 0.05$), the respective average S/R ratios to 1.3 and 1.1. The dose-normalized values of AUC as well as CL/F for both enantiomers indicate dose-proportionality only for the two highest doses, whereas for the lowest dose (25 mg) an average of only 50% of the AUCs of the 400 mg dose was obtained (Fig. 2A).

When increasing p.o.-doses of rac-talinolol were administered to the 12 healthy volunteers, an expressed biphasic drug input was observed in almost 40% of all observed plasma concentration-time profiles with a first maximum at approximately 1 hour postdose and a second—usually higher—maximum at about 3–4 hours postdose. The occurrence of concentration-time profiles with two distinct concentration maxima was neither specific for any individual nor related to the administered dose. The t_{max} values were always shorter with higher doses, and this observation reached statistical significance when comparing the highest and the lowest dose ($p < 0.05$). Accordingly, the mean input times decreased with increasing doses, while the terminal half-lives remained unchanged, i.e., they were independent of dose. Therefore, total clearance was assumed to be largely the same for high and low oral talinolol doses.

Permeation Studies with Caco-2 Cell Monolayers

The apical to basolateral transport of both talinolol enantiomers across Caco-2 monolayers clearly showed a concentration dependence and the percentage of drug transported to the basolateral side increased with rising apical drug concentrations. This is shown in Fig. 2B and expressed as an increase in the effective permeability of talinolol, e.g. from $3.59 \cdot 10^{-7}$ ($\pm 9.51 \cdot 10^{-8}$) cm s^{-1} to $8.44 \cdot 10^{-7}$ ($\pm 5.21 \cdot 10^{-8}$) cm s^{-1} (SD, n = 3–4) for apical concentrations from 0.11 to 1.94 mmoles/l of R-talinolol, respectively. For the S-enantiomer and the same apical concentrations, the permeabilities increased from 3.18 ·

Table 1B. Pharmacokinetic Parameters Obtained for S-(−)- and R-(+)-Talinolol Following Increasing P.O. Doses of Rac-Talinolol (25, 50, 100, 400 mg or 12.5, 25, 50, 200 mg of Each Enantiomer) in Healthy Volunteers (n = 12, Arithmetical Means ± SD)

Enantiomer dose (mg)	S-(−) 12.5	R-(+) 12.5	S-(−) 25	R-(+) 25	S-(−) 50	R-(+) 50	S-(−) 200	R-(+) 200
C_{max} (ng/ml)	22 ± 11*	24 ± 10*	72 ± 32	72 ± 33	159 ± 64	164 ± 61	791 ± 271*	824 ± 269*
1st t_{max} (h) [S:[a]; R:[a]]	3.6 ± 1.5	3.5 ± 1.6	3.0 ± 1.0	3.0 ± 1.0	2.8 ± 1.2	2.8 ± 1.2	2.0 ± 1.1	2.0 ± 1.0
$t_{1/2}$ (h)	12.9 ± 1.5	14.0 ± 4.4	12.9 ± 1.5	14.0 ± 4.4	12.9 ± 1.5	14.0 ± 4.4	12.9 ± 1.5	14.0 ± 4.4
λ_z (1/h)	0.054 ± 0.006	0.053 ± 0.012	0.054 ± 0.006	0.053 ± 0.012	0.054 ± 0.006	0.053 ± 0.012	0.054 ± 0.006	0.053 ± 0.012
$t_{1/2\ n.c.}$ (h)	14 ± 3	15 ± 5	12 ± 5	12 ± 2	12 ± 2	18 ± 4	11 ± 1	11 ± 1
AUC (ng·h/ml) [S:[a,b,c,d,e,f]; R:[a,b,c,d,e]]	226 ± 81*	274 ± 77*	582 ± 222*	656 ± 218*	1536 ± 344*	1746 ± 443*	6985 ± 1408*	7701 ± 1688*
MRT_{tot} (h) [S:[a]; R:[a]]	18.4 ± 2.6	20.0 ± 5.4	17.7 ± 1.9	19.4 ± 4.8	17.8 ± 1.9	19.5 ± 5.3	16.6 ± 1.8	18.1 ± 4.8
CL/F (ml/min) [S:[b,e,f]; R:[a,b,d,e,f]]	1062 ± 438*	830 ± 285*	861 ± 480	711 ± 270	578 ± 177*	508 ± 139*	498 ± 112*	452 ± 99*
t_{lag1} (h)	0.44 ± 0.87	0.67 ± 1.09	0.23 ± 0.26	0.18 ± 0.17	0.40 ± 0.81	0.40 ± 0.81	0.08 ± 0.09	0.09 ± 0.10
t_{lag2} (h) [S:[e,f]; R:[a,f]]	2.37 ± 1.15	2.35 ± 1.16	2.36 ± 0.94	2.49 ± 0.61	2.26 ± 0.82	2.26 ± 0.81	1.68 ± 0.87	1.71 ± 0.92
MIT (h) [S:[a,e,f]; R:[a,e,f]]	3.56 ± 1.12	3.81 ± 1.64	2.89 ± 1.04	3.27 ± 1.40	2.93 ± 0.91	3.37 ± 1.72	1.82 ± 1.03	1.94 ± 1.32
$f_{a,1}$	53.9 ± 33.0	49.5 ± 29.8	45.0 ± 31.3	42.7 ± 30.3	45.2 ± 25.3	46.5 ± 24.7	64.0 ± 31.3	63.5 ± 31.8

*R vs. S significant at $p < 0.05$; According to ANOVA the following differences were statistically significant at $p < 0.05$.
[a]200 vs. 12.5 mg.
[b]25 vs. 12.5 mg.
[c]50 vs. 12.5 mg.
[d]50 vs 25 mg.
[e]200 vs 25 mg.
[f]200 vs. 50 mg (The dose-normalized data were included for C_{max} and AUC) for S-(−)-talinolol (S) and R-(+)-talinolol (R), respectively.
Definitions are as follows: C_{max}, maximum measured concentration at time t_{max}; AUC, area under the concentration-time curve extrapolated to infinity (lin/log trapezoidal rule); $t_{1/2}$, terminal half-lives as obtained from simultaneous curve fitting with λ_z as the respective terminal elimination rate constants; $t_{1/2\ n.c.}$, terminal half-lives as calculated via regression analysis from the terminal log-linear phase (n.c. = non compartmental); CL/F, apparent oral clearance (calculated as D/AUC); MRT_{tot}, total mean residence time (AUMC/AUC); $t_{lag,1}$ and $t_{lag,2}$, lag-times for the two consecutive input processes; MIT, mean input time.

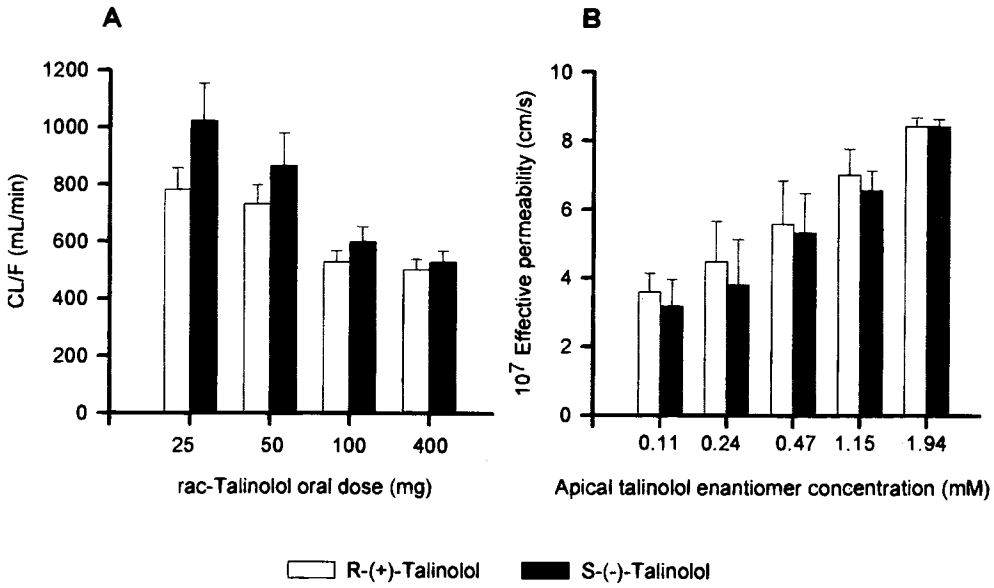

Fig. 2. A: Dose-dependence of the apparent oral clearance of both talinolol enantiomers: Average CL/F of S-(−)-talinolol is always exceeding that of R(+)-talinolol. CL/F values as well as the difference between enantiomers are higher at the subtherapeutic dose level **B:** Effective permeabilities of talinolol enantiomers in Caco-2 cell monolayers and the effect of increasing apical rac-talinolol concentrations. The permeation direction was from apical to basolateral (arithmetical means ± SD, n = 3–4).

10^{-7} ($\pm 1.33 \cdot 10^{-7}$) cm s^{-1} to $8.44 \cdot 10^{-7}$ ($\pm 4.20 \cdot 10^{-8}$) cm^{-1}. The differences between enantiomer permeabilities were statistically significant for the low concentrations ($p < 0.05$, Dixon and Mood (31)).

The transport rates of talinolol enantiomers were significantly higher in the basolateral to apical as compared to the reverse direction. At enantiomer concentrations of 0.2 mmoles/l the a→b drug fluxes were 180.9 (±117.8) ng cm^{-2} h^{-1} and 199.9 (±108.8) ng $cm^{-2}h^{-1}$ for S- and R-talinolol, respectively, whereas the b→a drug fluxes were 1736.4 (±313.6) ng cm^{-2} h^{-1} and 1737.0 (±352.3) ng cm^{-2} h^{-1} for S- and R-talinolol, respectively (Fig. 3). In the presence of 0.5 mmoles/l of racemic verapamil, the a→b transport rates increased to 457.4 (±0.5) ng cm^{-2} h^{-1} whereas the b→a transport rates decreased to 644.9 (±17.6) ng $cm^{-2}h^{-1}$ for S-talinolol. Similarly for R-talinolol, verapamil increased the a→b transport rates increased to 459.0 (±14.9) ng $cm^{-2}h^{-1}$ whereas the b→a transport rates decreased to 639.4 (±58.8) ng $cm^{-2}h^{-1}$. These findings can be attributed to a carrier-mediated intestinal secretion mechanism involving P-glycoprotein. In the presence of the P-gp inhibitor verapamil the secretory pathway is inhibited leading to very similar transport rates of the compound in both directions, presumably mainly by a passive diffusion mechanism.

DISCUSSION

Provided that intestinal secretion plays a role in the kinetics of talinolol enantiomers, particular phenomena should be observed: contribution of the process to total clearance following i.v. dosage, dose- and concentration-dependence as well as saturability and transport inhibition.

The results of the study performed in a limited number of cholecystectomized patients indicates that the systemic clearance of the compound cannot be fully explained by renal and biliary excretion. Since in the studies of Oertel et al. (26) metabolic clearance was found to be smaller than 1% with no relevant stereoselectivity, it may be assumed that an alternative route—intestinal secretion (exsorption)—may be involved. While the total clearance values calculated from i.v. data were higher for S- than for R-talinolol, no difference between enantiomers was detected for renal clearance, which reached values half as high as CL. Presumably, CL_R is smaller in the included middle-aged patients than it would have been in young healthy volunteers. The difference in renal function and a decreased CL_R may explain the differences of the terminal half-lives observed between the patients and volunteers. Active tubular secretion is very likely to occur, since CL_R values considerably exceeded the GFR values to be expected for this population. Approximately another 10% of the total clearance can be explained by biliary secretion (see also 28). Here, the fractional clearance is slightly higher for S- than for R-talinolol. The residual clearance—potentially via intestinal secretion—reaches approximately 1/3 of CL with some preference for S-talinolol. The p.o. data from cholecystectomized patients support this hypothesis, since the difference between the apparent oral clearance values of the two enantiomers was more expressed than with i.v. dosage, again with preference for S-talinolol. While CL_R values were almost identical to those after i.v. application, CL_B of both enantiomers was higher (1.5–2-fold) and the above mentioned stereoselectivity was more expressed, an observation that may be explained as a first-pass phenomenon. In these patients the average apparent oral clearance after application of 100 mg rac-talinolol was higher than with the same dose in healthy volunteers indicating a smaller bioavailability in the patients.

Observations from the p.o. study on dose-dependence of particular parameters are supplementary and appear to be confirming the hypothesis that exsorption plays a significant role

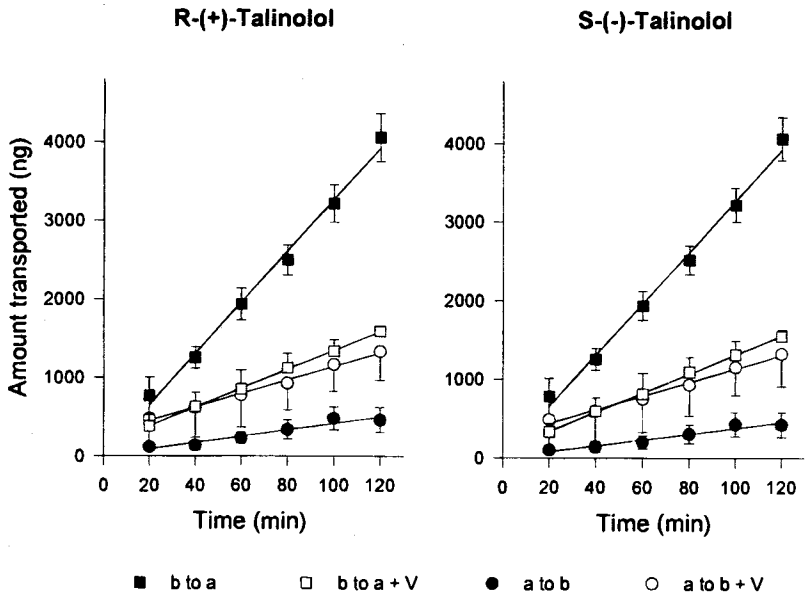

Fig. 3. Amount transported versus time of R-(+)- (left) and S-(−)-talinolol (right) across Caco-2 cell monolayers as a function of transport direction (a→b, apical to basolateral transport; b→a, basolateral to apical transport) and the effect of the P-gp inhibitor verapamil (V) at 0.5 mM on the permation rates in both directions. The donor concentrations were 0.4 mM rac-talinolol (arithmetical means ± SD, n = 3–4).

in the kinetic profile of talinolol. When an active secretion process is involved this process should be saturable at particular concentrations. However, in the performed kinetic studies ***concentrations*** at the absorption site are unknown and assumed to be highly variable. Yet also when trying to correlate kinetic parameters with ***dose***, some obvious relationships were found. For both enantiomers the dose-normalized AUC and C_{max} values increased with dose, while the apparent oral clearance decreased with minor dose-dependent changes and significantly smaller interindividual variabilities in the therapeutic dose range. The dose dependence of AUC/D and CL/F may largely be attributed to input phenomena, because no significant differences between doses were found for the terminal half-lives of both talinolol enantiomers, which indicates that the systemic clearance is not dependent upon dose to a relevant extent. Also the data on systemic availability are in accordance with the hypothesis of saturable intestinal secretion too, because under such circumstances the transporter should be saturated and the amount that reaches the systemic circulation should be higher at higher dose levels. Interestingly, the apparent oral clearance of the S-enantiomer always exceeded that of the R-enantiomer with the difference being more expressed in the subtherapeutic (=low) dose range. Another observation that may be regarded as supportive with respect to the intestinal secretion hypothesis is that t_{max} and MRT are decreasing at higher doses, reaching statistical significance when comparing the lowest and the highest p.o. dose ($p < 0.05$).

The occurrence of two "absorption windows" appear to be the most plausible explanation for the observed phenomenon of discontinuous drug input with a minor fraction of the dose being absorbed in the more proximal part of the gastrointestinal tract (stomach or upper region of the small intestine), followed by cessation or substantial reduction of the amount absorbed per time unit until the drug reaches the ileocaecal region where most probably the major amount is absorbed, as it has been shown experimentally for the beta-blocking agent pafenolol (7). This may as well be explained by a varying extent of intestinal secretion due to varying amounts or activities of the respective carrier system in different regions. However, although discontinuous input models were applicable to most of the profiles the extent of discontinuity was highly variable indicating that the involved carrier system shows considerably higher variability than observed for other "secretion" processes.

With the help of the Caco-2 cell studies it was possible to show that the presence of a carrier-mediated secretory system for talinolol in the intestine may be the basis for the reduction in bioavailability of talinolol at low doses. The intestinal secretion of quaternary ammonium compounds has been studied in guinea-pigs, and the role of saturable secretion on the non-linear absorption outlined (16). A similar mechanism may also be present for talinolol. At low doses, secretion of talinolol in the intestinal tract serves to limit the absorption of talinolol. As the secretion system begins to saturate overall absorption increases, resulting in non-linear increases in bioavailability. Once secretion is totally saturated, passive diffusion dominates and a new equilibrium is reached with linear absorption now evident. It appears highly probable that talinolol is transported by P-gp as in the presence of verapamil, a competitive P-glycoprotein inhibitor and substrate (29), basal-to-apical transport of talinolol was clearly inhibited.

Finally, the data obtained with talinolol in clinical and cell culture studies are indicative of a higher affinity of S-talinolol to the respective carrier with a consequently lower concentrations of S-talinolol at the basolateral side. However, for talinolol the stereoselectivity in pharmacokinetics is of minor relevance in the therapeutic dose range. For other compounds, this inverse transport mechanism might (dependent upon the dose and the

affinity to the carrier) turn out to be stereoselective *and* therapeutically relevant.

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