Human Pharmacokinetics of Betaxolol Enantiomers

GRAZIA STAGNI*, PATRICK J. DAVIS,* AND THOMAS M. LUDDEN*‡x

Received January 22, 1990, from the *College of Pharmacy, The University of Texas at Austin, Austin, TX 78703, and the ‡Department of Pharmacology, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284. Accepted for publication May 21, 1990.

Abstract □ Betaxolol is a cardioselective β-adrenergic antagonist effective in the treatment of hypertension. The pharmacokinetic behavior of betaxolol enantiomers in healthy male subjects is reported. Betaxolol enantiomer concentrations were determined in samples collected up to 48 h after iv administration of a 10-mg dose over a 30-min period by constant-rate infusion in 12 subjects and after oral administration of 40-mg capsules to eight of the same subjects. Betaxolol extracted from whole blood was reacted with (+) or (−)-1-naphthylethyl isocyanate. The resulting diastereoisomeric derivatives were analyzed by reversed-phase HPLC with fluorimetric detection. Following the iv dose, there were no differences in clearance or volume of distribution for the two enantiomers (15.6 ± 4.4 versus 16.4 ± 4.1 L/h and 342 ± 62 versus 340 ± 65 L, respectively). Likewise, after the oral dose, there were no differences in the maximum concentration, time of maximum concentration, bioavailability, or apparent absorption rate constant (41.0 ± 8.6 versus 42.0 ± 7.0 ng/mL, 214 ± 59 versus 215 ± 56 min, 0.89 ± 0.26 versus 0.94 ± 0.23, and 1.0 ± 0.6 versus 1.2 ± 0.6 h^{-1}, respectively). Thus, the pharmacokinetic behavior of racemic betaxolol accurately reflects the behavior of betaxolol enantiomers in this subject group.

Betaxolol hydrochloride [(±)-1-[*P*-[2-(cyclopropylmethoxy) ethyl]phenoxy]-3-(isopropyl-amino)-2-propanol hydrochloride] is a β_1-adrenoceptor blocking agent chemically related to a group of cardioselective β-blockers such as metoprolol, atenolol, and practolol.[1] Studies based on measurement of racemic betaxolol indicate that it has a high bioavailability (*F* = 70–90%) and its disposition is independent of the amount of drug administered.[2,3] Such properties distinguish betaxolol from related *para*-alkyl phenoxypropanolamines and make this agent particularly attractive for once-a-day therapy. Betaxolol has a chiral center and it is synthesized and administered as a racemate. Enantiomers often have different pharmacokinetic and pharmacodynamic properties.[4] Therefore, it is desirable to measure each enantiomer individually in biological fluids after administration of the racemate to better correlate the pharmacological effect with the pharmacokinetic profiles.[5] In the specific case of β-blockers, the therapeutic activity is usually associated with the *S* enantiomer,[6] and significant differences have been found in the pharmacokinetic behavior of *R*- and *S*-β-blocker enantiomers (i.e., for propranolol,[7] metoprolol,[8] and pindolol[9]).

The pharmacokinetics of betaxolol enantiomers has been studied in only three subjects receiving 20-mg oral doses.[10] This preliminary study did not show kinetic differences between the two enantiomers.

The purpose of the present study was to investigate the potential differences in the absorption and disposition of betaxolol enantiomers on a larger number of subjects after both iv infusion and oral administration of a single dose of betaxolol racemate. Blood collected from a previously published study[2] of racemic betaxolol kinetics was available for analysis of the enantiomers.

Experimental Section

Subjects—Blood samples were the same as those obtained for a study of the absolute bioavailability and dose proportionality of betaxolol in normal healthy subjects, performed at The University of Texas Health Science Center, San Antonio, TX.[2] Twelve healthy nonobese, nonsmoking, adult male subjects (24.8 ± 2.6 years; 73.6 ± 7.5 kg) were enrolled in the study after providing written, informed consent. The protocol and consent form were approved by The University of Texas Health Science Center San Antonio Institutional Review Board. On separate occasions, each subject received 10 mg of betaxolol · HCl, diluted in 40 mL of sterile normal saline and administered by constant-rate iv infusion over a period of 30 min, and oral capsules containing 10, 20, or 40 mg of betaxolol · HCl in a randomized crossover design. Subjects fasted for 8 h prior to drug administration and for 4 h after drug administration. Oral doses were administered with 100 mL of water. Subjects remained recumbent for the first 3 h, except for periodic measurement of standing heart rate and blood pressure. The concentrations of the individual enantiomers were determined only in the samples collected after iv infusion for all 12 subjects and after administration of the 40-mg capsules. For the oral dose, there were a sufficient number of samples with adequate residual volume for only eight subjects.

Sampling Procedure—For the iv doses, blood samples were obtained just prior to the beginning of the infusion, at 15 min into the infusion, at the end of the infusion, and then at 5, 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 4, 6, 12, 24, 36, and 48 h after the end of the infusion. For the oral doses, the blood sampling was similar except that samples at 5 min after the dose were not obtained and there were no samples corresponding to those collected during the infusion.

Sample Analysis—The blood samples were divided per collection time and assayed in singlet. Each day, 14–15 samples were extracted with 3–5 standards of comparable concentrations, and a blank. The peak height ratios (PHR) from the chromatogram were weighted by the reciprocal of the respective known concentrations expressed as betaxolol free base for the preparation of the calibration curve. The concentration of each enantiomer in the unknown samples was calculated from the slope and the intercept of the regression equation. At the end of the analysis, inspection of the concentration–time profile for each betaxolol enantiomer showed some outlier points. When possible, the relevant samples were reassayed.

Analytical Method—One milliliter of whole blood was mixed with 1.4 mL of deionized water, 100 μL of internal standard solution [(±)-2-propanol 1-[4-[2-(cyclobutylmethoxy)ethyl] phenoxy]-3-[(1-methylethyl)amino] hydrochloride; Lorex Pharmaceuticals, Skokie, IL; 0.69 ng/μL in distilled water], and 200 μL of 2 M NaOH in a screw-capped culture tube. Betaxolol was then extracted from blood using a published procedure.[2] After extraction, the ether phase was transferred to a clean 16 × 125-mm screw-capped culture tube and evaporated to dryness under dry nitrogen at 50 °C. The derivatization and quantitation of betaxolol enantiomers were performed using a modification of the method proposed by Darmon and Thenot.[10] To each tube were added 10 μL of 0.01% (+) or (−)-1-naphthylethyl isocyanate in dichloromethane and 200 μL of dichloromethane, dispensed automatically by a SYVA diluter dispenser (Palo Alto, CA). The tube was capped, mixed on a vortex for 30 s, and allowed to react at room temperature for 1.5 h. Excess solvent was then removed by evaporation under a stream of nitrogen at room temperature. Each sample was reconstituted with 150 μL of methyl alcohol: 0.4% (v/v) TEMED (pH 3; 1:1), and 100 μL was injected onto the chromatographic column by filling a 100-μL loop injector.

High-performance liquid chromatography was conducted using a

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Beckman model 110A pump and a model 210 sample injector valve (Beckman Instruments, Fullerton, CA). A Schoeffel model FS 970 L.C. Fluorometer was used as the detector. The excitation wavelength was 222 nm and the emission cut-off filter was set at 345 nm. Chromatograms were recorded with a Hewlett-Packard model 3390A integrator. The post-integration report was set in peak-height mode. The column was a Beckman Ultrasphere C18, 5μ 150 × 4.6 mm (Alltech Associates, Deerfield, IL) and was maintained at 37 °C with a Rainin column jacket model CJB (Rainin Instrument Company, Woburn, MA) connected to a heating bath (Thermomix 1420, B.Braun, Melsungen FRG or Chicago Surgical and Electrical Company, Melrose Park, IL). An absorbosphere CN guard column (Alltech Associates, Deerfield, IL) was installed between the injector and the column. The mobile phase consisted of methanol:tetrahydrofuran: 0.4% (v/v) TEMED (pH 3; 52:14:34). The TEMED solution was filtered on membrane filter cellulose nitrate (pore size 0.45 μm; Micro Filtration System, Dublin, CA). After mixing, the mobile phase was sonicated for 2–3 min. Peaks were identified by elution order based on the work by Darmon and Thenot.[10]

Racemic betaxolol (Lorex Pharmaceuticals, Skokie, IL) was used in the preparation of standard curve samples over the range 0.7–70 ng/mL. Freshly prepared standard curves were analyzed simultaneously with each set of unknowns. Absolute recovery was 88.1 ± 17.0 (SD)% for the *R* enantiomer of betaxolol and 87.0 ± 12.6% for the *S* enantiomer. Intraday precision was 10.1 and 8.7% for *S* and *R* enantiomers, respectively, at a concentration of 1.5 ng/mL, and 5.6 and 4.7%, respectively, at a concentration of 17.5 ng/mL. Each standard curve yielded an r^2 value of 0.98 or higher. The standard curves used to assay the majority of samples are summarized in Table I. In some cases, a standard curve with a higher range was used for samples likely to contain high concentrations (i.e., samples collected shortly after iv administration). No sample was found with a concentration of <1.1 ng/mL. Chromatograms are shown in Figure 1.

Pharmacokinetic Data Analysis—Since all enantiomer concentrations were expressed as the free base, all doses of (±)betaxolol · HCl were corrected to reflect the content of the dose of (±)betaxolol free base (89.4%) and divided by two to represent the amount of each enantiomer.

Pharmacokinetic data were analyzed by the nonlinear regression program NONLIN84.[11] The iv data were fitted by a two-compartment model, and estimates of the exponential terms (λ_i) and of the intercepts at the time of the end of the infusion (R_i) were obtained. The intercepts that would have occurred if the dose had been administered as a bolus (C_i) were calculated as follows:

$$C_i = R_i \cdot \text{Dose} \cdot \lambda_i / (k_o (1 - \exp(-\lambda_i \cdot t_d))) \quad (1)$$

where k_o is the infusion rate and t_d is the duration of infusion. The following secondary parameters were then computed:

$$\text{AUC} = C_1/\lambda_1 + C_2/\lambda_2 \quad (2)$$

$$CL = \frac{\text{dose}}{\text{AUC}} \quad (3)$$

$$V_z = CL/\lambda_z \quad (4)$$

Table I—Summary of Standard Curves

Concentration Added, ng/mL	N	Concentration Found, ng/mL[a]	
		R	*S*
0.69	5	0.88 ± 0.14	0.84 ± 0.14
1.74	8	1.60 ± 0.12	1.65 ± 0.19
5.21	6	4.40 ± 0.58	4.40 ± 0.59
10.4	5	9.83 ± 0.59	9.98 ± 0.55
15.6	4	15.3 ± 0.50	15.5 ± 0.38
20.9	4	19.9 ± 1.00	20.5 ± 0.80
27.8	4	27.7 ± 2.16	26.1 ± 2.20
41.2	2	43.7 ± 4.02	44.0 ± 3.91

[a] Mean ± SD.

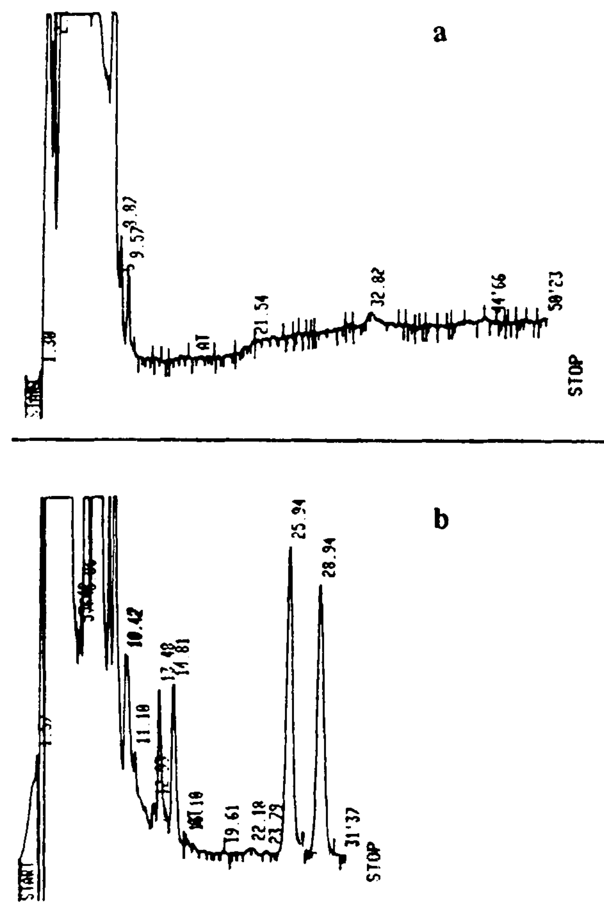

Figure 1—The HPLC separation of whole blood extracts derivatized with *R*(−)-NEI: (**a**) Blood blank; (**b**) blood supplemented with 50.5 ng of internal standard (IS) and with 24.8 ng of racemic betoxolol. Retention times were as follows: 13.48 min for *S*(−)-betaxolol derivative; 14.81 min for *R*(+)-betaxolol derivative; 25.94 min for *S*(−)-IS derivative; and 28.94 min for *R*(+)-IS derivative.

where $\lambda_z = \lambda_2$, AUC is the area under the concentration–time curve, *CL* is clearance, and V_z is volume.

The oral data were fitted by a one-compartment absorption–disposition model, yielding estimates of t_{lag}, λ_z, the apparent first-order absorption rate constant (K_a), C_{max}, t_{max}, and V_z/F. The secondary parameters computed in this case were: AUC, *CL/F*, and *F* corrected for the difference in doses.

The potential differences in pharmacokinetic behavior between the *R*- and the *S*-enantiomers were evaluated by the paired *t* test at the 5% level.

Results

The mean blood concentration versus time profiles of the *R*- and the *S*-betaxolol enantiomer, of the sum of the concentrations of the two isomers, and of the racemate are shown in Figure 2. These last two are displayed to show that the total concentrations found during the present analysis and those found using the racemic assay[2] are quite similar. Visual inspection of the concentration versus time curves reveals that the iv infusion pharmacokinetics has a multicompartmental character; a biexponential equation provided a good fit of the iv data in all the subjects. Since the distribution phase was masked by the absorption phase, a first-order input, first-order output equation fitted the data following oral administration of the (±)-betaxolol capsules.

Table II lists the means and the standard deviations of the pharmacokinetic parameters estimated for the *R* and the *S* enantiomer. It is apparent from these data that there are not

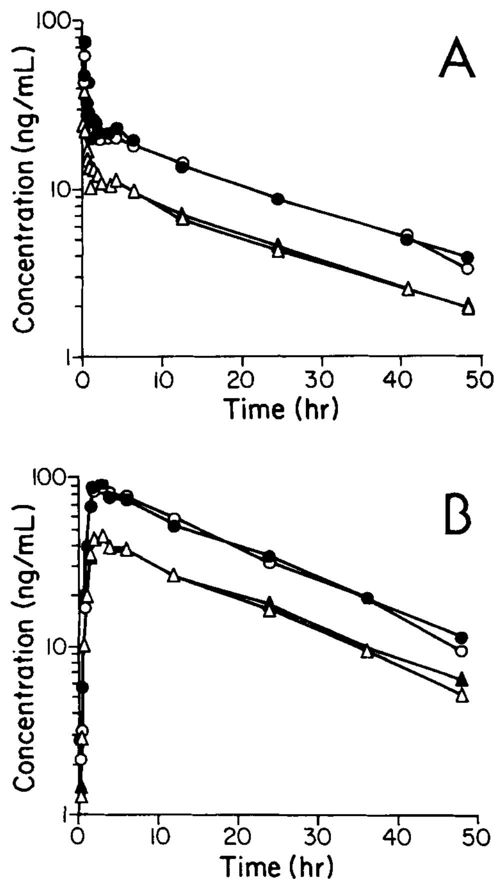

Figure 2—Mean betaxolol enantiomer concentration–time profiles: (**A**) 10-mg iv dose; (**B**) 40-mg oral dose. Key: (▲) *R* enantiomer; (△) *S* enantiomer; (●) sum of the two enantiomers; (○) racemate.

pronounced differences between the pharmacokinetics of the two isomers. The means for each type of parameter for the *R* and the *S* enantiomer are very similar to each other. However, the standard deviations of the *CL* and *CL/F* for the *R* enantiomer are generally larger than those of the *S* enantiomer. This behavior is evident from the box plot of the normalized values of pharmacokinetic parameters presented in Figure 3.

Statistical analyses did not reveal any significant difference between the pharmacokinetics of betaxolol enantiomers.

Discussion

The analyses reported here permitted the description of the pharmacokinetic behavior of the *R* and *S* enantiomers of betaxolol following iv and oral administration of the racemate. Calculation of pharmacokinetic parameters assumed that there was no metabolic inversion of the parent drug during its residence time in the body; no evidence for such inversion has been found for β-adrenergic antagonists. Thus, the pharmacokinetic analysis was based on the assumption that the concentration measured depended only on the fate of the administered dose of each isomer.

The results of this study failed to reveal any important difference between the pharmacokinetics of the *R* and *S* enantiomer of betaxolol (Table II) and are in agreement with the finding of Darmon and Thènot.[10] In their study, three subjects received a single oral administration of 20 mg of betaxolol racemate. It was concluded that the blood concentrations of betaxolol enantiomers were virtually equal by visual inspection of the blood concentration versus time curves.

The pharmacokinetics of the *R*- and the *S*-betaxolol enantiomers differ from the kinetics observed for other β-blockers such as propranolol, alprenolol, and metoprolol. It is particularly interesting to compare betaxolol with metoprolol since they are quite similar in molecular structure and pharmacological activity. Their pharmacokinetic profiles, however, are very different, mainly due to differences in metabolic rates. Metoprolol is rapidly metabolized in the liver; it undergoes significant first-pass elimination, resulting in an oral bioavailability of only 40–50% of the administered dose.[12] Moreover, its terminal half-life is 3–6 h. On the other hand, betaxolol has a high bioavailability (80–90%) and a longer terminal half-life of 16–22 h.[2,3] The difference between metabolic clearances of metoprolol and betaxolol can be explained by the combined effects of the higher plasma protein binding (55% versus 20%) of betaxolol and the difference in their molecular structure; that is, the cyclopropyl side chain of betaxolol, which is believed to decrease intrinsic hepatic clearance via steric hindrance. In fact, *O*-dealkylation followed by oxidation of the resulting primary alcohol accounts for the 24% of betaxolol metabolism[13] and for 65% of metoprolol metabolism.[14] In vitro studies show that with metoprolol this reaction is stereoselective: *R*(+)-metoprolol is more rapidly *O*-demethylated than *S*(−)-metoprolol.[15] A minor metabolic pathway for metoprolol (10%) is α-hydroxylation on the benzylic carbon. This reaction is selective for the *S*-enantiomer.[16] Moreover, α-hydroxylation is under polymorphic control and, along with *O*-demethylation, it contributes to the large interphenotype differences in plasma metoprolol concentrations,[8] whereas α-hydroxylated betaxolol metabolites account for <2% of the dose.[13]

A metabolic pathway that is more relevant for betaxolol than for metoprolol is the oxidation of the carbon α to the isopropylamino group, with removal of the latter.[13] The

Table II—Pharmacokinetic Parameters for Betaxolol Enantiomers[a]

Parameter	Intravenous Dose (10 mg)			Oral Dose (40 mg)		
	Enantiomer		Racemate[b]	Enantiomer		Racemate[b]
	R	*S*		*R*	*S*	
C_{max}, ng/mL	—	—	—	41.0 ± 8.61	42.0 ± 7.01	89.8 ± 16.0
t_{max}, min	—	—	—	214 ± 59.1	215 ± 55.6	170 ± 50.3
V_z, L/kg[c]	4.63 ± 0.55	4.61 ± 0.60	5.07 ± 0.74	5.37 ± 0.70	5.15 ± 0.56	5.34 ± 0.62
CL, L/h[d]	15.6 ± 4.35	16.4 ± 4.13	15.1 ± 2.9	18.5 ± 6.51	17.8 ± 3.04	17.7 ± 5.0
AUC, μg · h/L	314 ± 118	290 ± 75.5	610 ± 122	1077 ± 362	1030 ± 162	2096 ± 246
λ_z, h^{-1}	0.0466 ± 0.0146	0.0498 ± 0.0165	0.0415 ± 0.0050	0.0467 ± 0.0104	0.0479 ± 0.0072	.0455 ± 0.0057
K_a,h^{-1}	—	—	—	1.02 ± 0.56	1.24 ± 0.64	0.510 ± 0.156[e]
F, %	—	—	—	89 ± 26	94 ± 23	84 ± 6

[a] The results are expressed as mean ± SD of 12 subjects for the iv infusion and of eight subjects for the oral administration. [b] From ref 2. [c] V_z/F for the oral administration. [d] *CL*/F for the oral administration. [e] K_a for racemate obtained by simultaneous analysis of iv and oral data.

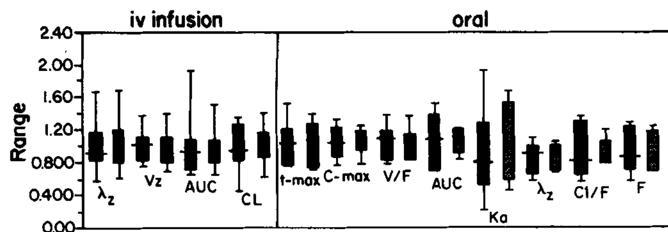

Figure 3—Box plot of pharmacokinetic parameters normalized by their respective means. Each box encloses 50% of the values for the parameter (± 25%), with the median of that parameter marked with a bold line. The maximum and the minimum of the parameters are marked with a tie above and below the box. [■ *R* enantiomer (left), ▩ *S* enantiomer (right)].

corresponding acid (SL77 009) accounts for 35% of the administered dose of betaxolol and for 10% of the metoprolol dose.[14] No information on the stereoselectivity of this metabolic route is available.

The similar kinetic characteristics of betaxolol enantiomers could be the result of different stereoselective metabolic pathways proceeding at similar rates. Certainly, this hypothesis cannot be ruled out by the present study.

The present study involved only single-dose administration of betaxolol to healthy male subjects. Inspection of the blood concentration–time curves and analysis of pharmacokinetic parameters revealed no important differences in profiles of *R* and *S* enantiomers. Generally speaking, β-blocking activity has been associated only with the *S* configuration of similar agents.[6] The implications of this study are that the racemic measurements may correlate with pharmacologic activity, either directly or indirectly, but that any concentration-related pharmacodynamic parameters such as an EC_{50} (concentration producing 50% of a maximum effect) obtained from racemic measurements would be relative to the total concentration.

Additional studies of the enantiomers following chronic administration to different patient groups should be performed before these results are extrapolated to circumstances where betaxolol disposition may be altered by pathophysiologic changes.

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