Nonlinear accumulation of propranolol enantiomers

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The accumulation of (+)- and (–)-propranolol was investigated in nine subjects who received 160 mg of racemic propranolol as a single dose and then once daily for 7 days. The serum concentrations of propranolol enantiomers were measured by h.p.l.c. using a novel chiral stationary phase allowing direct resolution of underivatized propranolol. The (+)-propranolol AUC increased from 412 ± 223 ng ml⁻¹ h after single doses (0–∞) to 584 ± 279 ng ml⁻¹ h at steady-state (0–24 h) ($P < 0.05$). Similarly, (–)-propranolol AUC increased from 609 ± 304 to 777 ± 370 ng ml⁻¹ h ($P < 0.05$). The AUC ratio (–)/(+) was 1.52 ± 0.36 and 1.32 ± 0.17 after single doses and steady-state, respectively ($P > 0.05$). Therefore, nonlinear accumulation occurs with both enantiomers although there is a trend for the (–)/(+) ratio to decrease at steady-state.

Keywords propranolol enantiomers pharmacokinetics

Introduction

Propranolol is an enantiomeric molecule commercially available as a racemate. Major differences are evident in the β -adrenoceptor-blocking activity (Barrett & Cullum, 1968) and pharmacokinetics of the two enantiomers (Silber *et al.*, 1982). Nevertheless, the overwhelming majority of propranolol pharmacokinetic studies are done by measuring the concentration of racemic drug and, therefore, information is usually lacking on the disposition of the active S (–)-enantiomer (Ariens, 1984).

After chronic dosing, racemic propranolol is known to accumulate to an extent greater than expected based on single dose pharmacokinetics (Straka *et al.*, 1987; Wood *et al.*, 1978). This is due to an apparent decrease in the hepatic intrinsic clearance of unbound drug leading to a decrease in first-pass metabolism, at steady-state. It is unknown if this change in hepatic drug metabolism is also stereoselective and, therefore, may preferentially affect the (+)- or (–)-enantiomer.

A study with dogs by Bai *et al.* (1983) showed that there was stereoselective accumulation of (–)-propranolol after chronic dosing. However, appreciable differences in enantiomer disposition exist between dog and man (Von Bahr *et al.*, 1982). Therefore, the purpose of this study was to investigate the accumulation of propranolol enantiomers after multiple doses in humans. We report on the use of a novel analytical method that resolves underivatized propranolol enantiomers in serum.

Methods

Nine healthy male volunteers ranging in age from 23 to 39 years (mean age 27 years) and ranging in weight from 66 to 82 kg (mean weight 75 kg), participated in the study. The subjects had no evidence of previous or current cardiac, respiratory, renal or hepatic dysfunction. All were non-

smokers and abstained from taking caffeine containing products, other drugs and alcohol for the duration of the study.

The study was a two-phase comparison. During Phase I, each subject received a single 160 mg oral dose of propranolol (Inderal tablets, Ayerst Laboratories Inc., New York). After a washout of at least 1 week, each subject entered Phase II (multiple dose phase) and received oral propranolol 160 mg once daily for 7 days. The doses were administered at approximately 08.00 h and, on study days, the subjects fasted for 8 h before and 4 h after drug administration. After the single dose in Phase I and the last dose in Phase II, blood was collected by direct venepuncture at 0, 1, 2, 4, 6, 8, 12 and 24 h. No heparin was used in the sample collection. The serum was separated and frozen at -20°C until analysis.

The serum concentrations of underivatized propranolol enantiomers were measured by h.p.l.c. using a chiral stationary phase (Straka *et al.*, 1988). Briefly, 300 ng of verapamil (internal standard) and 0.5 ml of carbonate buffer (pH 10.0) were added to 1 ml of serum and then extracted with 7 ml of diethylether. The ether layer was collected, evaporated to dryness under nitrogen, then each sample was reconstituted with mobile phase prior to injection. The mobile phase consisted of hexane:2-propanol:*N,N*-dimethyloctylamine (92:8:0.01 vol/vol/vol). The chiral stationary phase was a cellulose-tris(3,5-dimethylphenylcarbamate) polymer coated on macroporous silica (Okamoto *et al.*, 1986) and packed in a stainless-steel column (Daicel Chemical Industries, New York). The mobile phase flow rate was 1 ml min^{-1} and the fluorescence of the compounds was detected using an excitation wavelength of 290 nm and a 340 nm emission cut-off filter. At serum concentrations of 30 ng ml^{-1} and 80 ng ml^{-1} of each propranolol enantiomer, the within-day coefficients of variation were less than 5% and the between-day coefficients of variation were less than 10%.

Standard noncompartmental equations were used to calculate the area under the serum concentration-time curve (AUC), the terminal elimination rate constant (k) and the elimination half-life for each enantiomer. The AUC was calculated using the logarithmic trapezoidal rule. At steady-state, the AUC was calculated over 24 h, whereas after single doses the calculated AUC was extrapolated to infinity.

Pharmacokinetic parameters between enantiomers and study phases were compared by *a priori* orthogonal contrasts using an F test and a two-way analysis of variance model (Kirk, 1982). The two major contrasts involved the comparison of each enantiomer between single doses *vs*

Figure 1 Mean serum concentration-time curves of (+)- (○) and (-)-propranolol (Δ) after a single 160 mg dose of racemic propranolol.

steady-state. The third possible orthogonal contrast allowed the comparison of the combined (+)-propranolol data (single doses plus steady-state) *vs* (-)-propranolol. AUC ratios were compared by a two-tailed paired *t*-test. The 5% level of significance was used.

Results

Mean serum concentration-time curves are shown in Figure 1 and the individual pharmacokinetic parameters are summarized in Table 1. The AUC of (-)-propranolol increased from 609 ± 304 after single doses to $777 \pm 370\text{ ng ml}^{-1}\text{ h}$ at steady-state ($P < 0.05$). Similarly, the AUC of (+)-propranolol increased from 412 ± 223 to $584 \pm 279\text{ ng ml}^{-1}\text{ h}$ at steady-state ($P < 0.05$). The combined (+)-propranolol AUCs were smaller than those of (-)-propranolol ($P < 0.05$), indicating that there was stereoselective disposition. The terminal half-lives were not significantly different between enantiomers or study phases ($P > 0.05$). The ratio of (-)/(+) AUC was not statistically different between single doses and steady-state, although there was a trend for it to decrease ($P = 0.18$). This trend was not statistically significant, in part because the study did not have sufficient power. A 28% difference in (-)/(+) ratio would have been necessary to detect with a power of 80%.

Discussion

The observed increase in the AUC of each propranolol enantiomer at steady-state is consistent with previous studies measuring racemic propranolol concentrations (Straka *et al.*, 1987; Wood *et al.*, 1978). Interestingly, the (-)/(+)

Table 1 Pharmacokinetic parameters of propranolol enantiomers after single doses and at steady-state

Subject	AUC (ng ml ⁻¹ h)				Half-life (h)				AUC ratio ((-)/(+))	
	Single dose		Steady-state		Single dose		Steady-state		Single dose	Steady-state
	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)		
1	345	815	509	603	4.15	5.33	4.17	3.94	2.36	1.24
2	501	716	826	1300	4.65	4.41	4.68	5.97	1.43	1.57
3	842	1156	1170	1354	4.39	5.41	4.33	4.53	1.37	1.16
4	449	607	339	468	4.08	4.30	3.92	4.25	1.35	1.38
5	89	130	266	312	3.59	3.35	5.82	5.06	1.46	1.17
6	327	448	620	821	2.65	2.67	2.34	2.17	1.37	1.32
7	622	805	379	411	3.48	3.01	6.93	5.59	1.29	1.08
8	275	338	501	771	3.41	3.35	3.16	3.27	1.23	1.54
9	255	464	647	922	2.26	2.45	3.83	3.75	1.82	1.43
Mean	412a	609b	584a	777b	3.63	3.81	4.35	4.28	1.52	1.32
± s.d.	(223)	(304)	(279)	(370)	(0.791)	(1.102)	(1.363)	(1.180)	(0.36)	(0.17)

a $P < 0.05$ between (+)-propranolol AUCb $P < 0.05$ between (-)-propranolol AUC

AUC ratio tended to decrease slightly from single doses to steady-state, reflecting the fact that the relative increase in (+)-propranolol AUC was greater than that of (-)-propranolol. Therefore, the enantiomer with the lower AUC (higher oral clearance), (+)-propranolol, showed the larger increase in AUC at steady-state. In the study of Bai *et al.* (1983), the enantiomer with the lower AUC in dogs was also the one showing the greater increase in AUC at steady-state. However, it is (-)-propranolol which has the lower AUC in dogs. Nevertheless, the consequences of these changes in AUC (or oral clearance) are similar in both man and dog,

in that the enantiomeric differences in AUC are relatively less at steady-state than after single doses. Further studies are necessary to confirm these trends at other doses and dosing intervals of propranolol. Obviously, since (-)-propranolol is largely responsible for the β -adrenoceptor-blocking effect, it is the change (or lack thereof) in pharmacokinetics of this enantiomer that will have clinical importance. If (+)-propranolol concentrations increase proportionately more than those of (-)-propranolol, the expected change in β -adrenoceptor blockade may not be as large as expected based on the increase in total (+) + (-) concentrations.

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