International Journal of Pharmaceutics, 43 (1988) 261–265
Elsevier

IJP 01466

Reduction of first-pass metabolism of propranolol after oral administration of ester prodrugs

B.D. Anderson, W.W. Chu and R.E. Galinsky

Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, UT 84112 (U.S.A.)

(Received 11 September 1987)
(Modified version received and accepted 6 November 1987)

Key words: Prodrug; Propranolol; First-pass metabolism; Bioavailability; Ester; Hydrolysis

For orally administered drugs, first-pass metabolism by the liver is a formidable barrier to efficient delivery. Extensive first-pass metabolism, in addition to decreasing the percentage of dose reaching its intended site of action, often leads to significant variability in bioavailability, necessitating careful monitoring of patient blood levels. While first-pass metabolism can be avoided by selecting alternate routes of administration (i.v., transdermal, rectal, etc.), the oral route is generally preferred. Rational approaches for minimizing first-pass metabolism would therefore be quite valuable.

Propranolol, used extensively in the treatment of angina pectoris, hypertension, and cardiac arrhythmia, has been shown to undergo extensive presystemic metabolism after oral administration leading to reduced bioavailability (Paterson et al., 1970; Shand et al., 1970; Lo et al., 1982) and significant inter-subject variability in blood levels (Kornhauser et al., 1978). Garceau et al. (1978) have shown that the hemisuccinate ester of propranolol, when administered orally to beagle dogs, yields propranolol levels 8 times higher than after an equivalent dose of propranolol hydrochloride. The mechanism suggested was that the succinate ester bypassed the gut-wall glucuronide formation during absorption. Others have shown, however, that there is complete absorption and no metabolism of propranolol by the gastrointestinal wall (Lo et al., 1982; Iwamoto et al., 1985). The improved bioavailability of propranolol succinate must therefore be the result of its bypassing first-pass metabolism by the liver.

The rational application of bioreversible chemical modification (prodrug) strategies to minimize first-pass metabolism of drugs requires an understanding of the sequence of events leading to clearance of drugs by the liver and the influence of substrate chemical structure on each step in this sequence. Prodrugs of propranolol appear to be appropriate model compounds for such mechanistic studies. In this study, the relative oral bioavailabilities in rats of two prodrugs of propranolol, the succinate and acetate esters, have been compared with propranolol in order to establish their utility as model compounds for further mechanistic studies in rats.

O-Acetylpropranolol, HCl salt, was prepared by a previously published procedure (Crowther and Smith, 1968). *O*-Hemisuccinylpropranolol, HCl salt, was used as received (99.5% purity, Ayrest Laboratories, New York, NY) or synthesized by reacting propranolol HCl (500 mg) with succinic anhydride (500 mg) in dimethylfor-

Correspondence: B.D. Anderson, Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, UT 84112, U.S.A.

mamide (1 ml) at 85–90 °C for approximately 4 h. Following extractive purification and recrystallization, the identity and purity of the synthesized materials were established by IR, TLC, UV and HPLC.

To determine oral bioavailabilities of the prodrugs in comparison to propranolol, 9 male Sprague–Dawley rats weighing 250–400 g were randomly divided into 3 groups. On the first day of the study, a two-piece silastic–polyethylene catheter was implanted into the right jugular vein of each animal under ether anesthesia (Weeks and Davis, 1964; Galinsky, 1986). Only the silastic catheter portion of the catheter was inserted into the vein. For sampling, the polyethylene extension of the catheter was passed subcutaneously to emerge at the dorsal base of the neck. To avoid the use of heparin, the catheter was flushed daily with a small volume of saline (0.5 ml) to maintain patency. Food was withheld overnight before the experiment. On the second day each group received 10 mg/kg (propranolol equivalents) of either propranolol HCl (PRO-HCl), propranolol acetate HCl (AC-HCl), or propranolol succinate (HS-HCl) orally by gavage of solutions in normal saline adjusted to pH 4 (phosphoric acid). Two-hundred μl of blood was withdrawn from the jugular vein through the indwelling catheter at timed intervals for 4 h. Blood was immediately

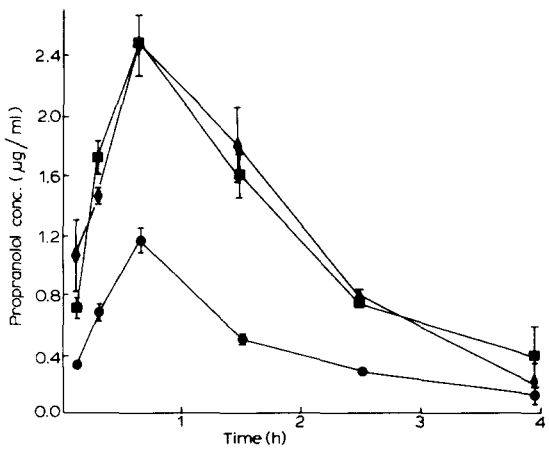

Fig. 1. Mean (±S.E.) blood concentration of propranolol following oral administration (10 mg/kg propranolol equivalents) of PRO-HCl (●), HS-HCl (■), and AC-HCl (◆) to Sprague–Dawley rats ($n = 3$ per group).

added to 2 ml of methanol to quench plasma esterase activity.

Quenched samples were centrifuged at 3000 rpm for 2 min and the supernatant was collected. The precipitate was rinsed twice with methanol, recentrifuged, and the rinse solutions were collected and added to the first supernatant. The combined solution was evaporated under nitrogen and reconstituted with 1 ml of 20% MeOH/H_2O adjusted to pH 3 with phosphoric acid. This solution was passed through a previously prepared C_{18} Sep-Pak cartridge (Waters Chromatography Div., Millipore, Milford, MA) followed by an additional 1 ml of the same solvent to remove serum proteins. Propranolol and the intact prodrugs were then eluted with 5 ml of a solution containing 50% CH_3CN, 20% MeOH, and 0.6% triethylamine adjusted to pH 3.5. The 5 ml samples were evaporated under nitrogen and reconstituted with mobile phase for HPLC analysis.

HPLC analyses were conducted using a reverse-phase column (μBondapak C_{18}, Waters Chromatography Div., Millipore, Milford, MA) and a mobile phase consisting of 25% CH_3CN and 10% MeOH in water with 0.05% *N,N*-dimethyloctylamine added as a polar modifier and pH adjusted to 3.5 with phosphoric acid. Detection was by UV at 219 nm.

The recovery of PRO-HCl, HS-HCl, and AC-HCl from spiked plasma samples was shown to be nearly quantitative over concentrations ranging from 10^{-7} to 10^{-5} M, the range of concentrations analyzed in vivo, with percent recovery averaging 99.1%, 97.1%, and 94.3% for PRO-HCl, HS-HCl, and AC-HCl, respectively.

The blood concentrations of propranolol versus time after oral doses of PRO-HCl, HS-HCl, and AC-HCl (10 mg/kg propranolol equivalents) are shown in Fig. 1. Clearly apparent from Fig. 1 is that the oral bioavailability, in terms of propranolol concentration in the blood, is significantly higher from either prodrug. Estimates of mean area under the concentration–time curves (AUC) obtained from trapezoidal integration of the data between 0 and 4 h are listed in Table 1. Student's t-test applied to the 0–4 h data indicated that the oral bioavailabilities of both prodrugs are significantly higher than propranolol ($P < 0.001$ and

TABLE 1

Pharmacokinetic parameters obtained from least-squares regression of the propranolol blood concentrations resulting from oral administration of propranolol and its acetate and succinate esters according to Eqn. 1

Rat	Compound	K (h^{-1})	k_a (h^{-1})	AUC (h·μg/ml)	
				0–4 h [a]	0–∞ [b]
1	PRO-HCl	0.6	2.9	1.9	1.9
2	PRO-HCl	0.5	4.5	2.0	2.1
3	PRO-HCl	0.7	3.1	1.7	1.6
Average ±S.D.		0.6±0.1	3.5±0.9	1.8±0.2	1.9±0.3
4	HS-HCl	0.5	2.4	5.2	7.0
5	HS-HCl	1.1	1.3	4.0	4.1
6	HS-HCl	1.3	1.4	4.9	4.7
Average ±S.D.		1.0±0.4	1.7±0.6	4.7±0.7	5.2±1.5
7	AC-HCl	1.3	1.3	5.4	5.1
8	AC-HCl	1.4	1.4	3.9	3.4
9	AC-HCl	0.6	2.8	5.2	5.6
Average ±S.D.		1.1±0.4	1.9±0.8	4.8±0.8	4.7±1.2

[a] From trapezoidal integration.
[b] From best fit according to Eqn. 1.

$P < 0.0025$ for the hemisuccinate and acetate, respectively). According to these AUC estimates, bioavailability increased by a factor of approximately 2.5 when propranolol was administered in prodrug form. In male Wistar rats the systemic availability (F) of propranolol at an oral dose of 10 mg/kg was shown to be 0.253 (Iwamato and Watanabe, 1985). The present finding of a 2.5-fold increase in availability for prodrugs of propranolol in Sprague–Dawley rats is consistent with this reported value and may suggest that the acetate and succinate esters are themselves not fully bioavailable.

Blood concentration (C)–time curves for each animal were analyzed by least-squares regression (RSTRIP, Micromath, Salt Lake City, UT) using a biexponential equation

$$C = A(e^{-Kt} - e^{-k_a t}) \quad (1)$$

to reflect simple first-order absorption and elimination processes. The pharmacokinetic parameters obtained are shown in Table 1.

The mean plasma elimination half-life of 69 min obtained in this study after oral administration of propranolol is similar to the value of 63 min reported in Sprague–Dawley rats after i.v. administration (Bianchetti et al., 1980). However, the $t_{1/2}$ for disappearance of propranolol after prodrug administration appears to be decreased ($t_{1/2} = 42$ min and 38 min, respectively, for HS-HCl and AC-HCl). Thus, propranolol may undergo elimination more rapidly when first-pass metabolism is bypassed. A plausible explanation for this is that drug concentrations in the liver are sufficiently high after oral propranolol to saturate metabolic enzymes, leading to reduced hepatic extraction from the systemic circulation. Consistent with this interpretation, the mean hepatic extraction ratio of [^{14}C]propranolol given intravenously has been reported to be reduced after pretreatment with unlabelled propranolol or during portal venous administration of unlabelled propranolol (Suzuki et al., 1980).

The apparent absorption rate constants, k_a (Table 1), based on propranolol appearance in the blood are decreased after prodrug administration. Either slower absorption of the prodrugs compared to propranolol or relatively slow (rate-limiting) bioconversion after absorption could account for this. Information on the concentrations of intact prodrugs (Figs. 2 and 3) are useful in addressing this question. Again the data were analyzed by least-squares regression using a simple biexponential equation. The rate constants obtained are listed in Table 2. The relatively high concentrations of the prodrugs in the blood and higher values of their absorption rate constants compared to their elimination rate constants coupled with the comparable magnitudes of the prodrug elimination rate constants and the propranolol formation constants suggest that prodrug bioconversion, rather than absorption, governs the rate of propranolol appearance in the blood.

The mechanism by which prodrugs of propranolol produce significantly higher bioavailabilities of propranolol is unknown. The simplest rationalization is that the prodrugs themselves are not metabolized in the first-pass through the liver and subsequently undergo hydrolysis in the systemic circulation to propranolol. In vitro studies

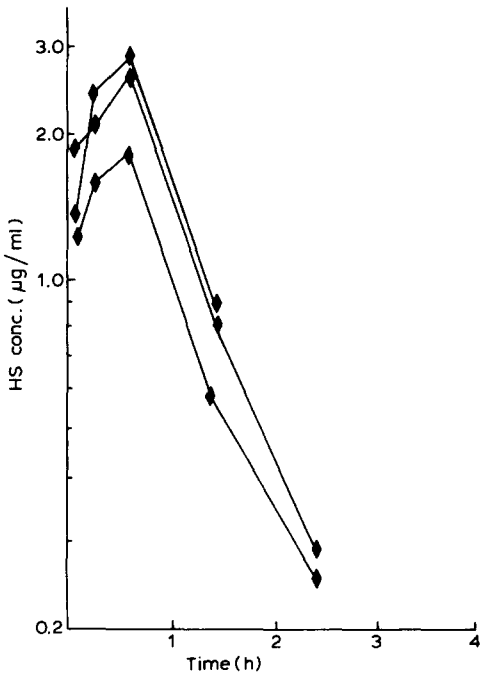

Fig. 2. Blood concentration of intact prodrug following oral administration of HS-HCl (10 mg/kg propranolol equivalents) to each of 3 rats.

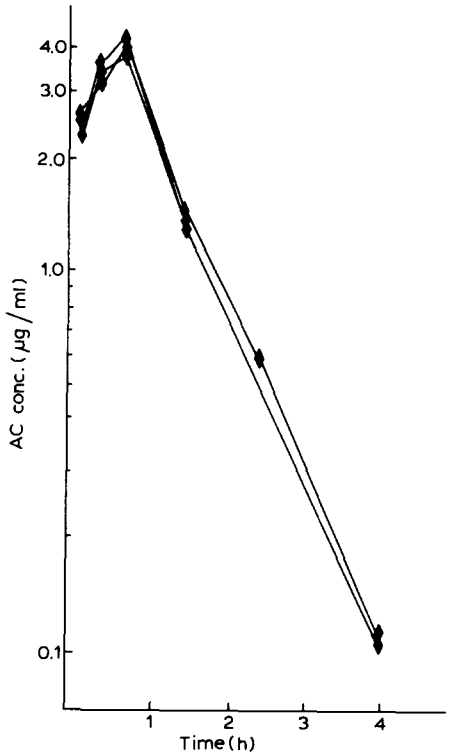

Fig. 3. Blood concentration of intact prodrug following oral administration of AC-HCl (10 mg/kg propranolol equivalents) to each of 3 rats.

in these laboratories of the hydrolysis of HS-HCl and AC-HCl in buffer and in rat plasma (pH 7.4, 37 °C) establish that propranolol ester hydrolysis is catalyzed in plasma. The $t_{1/2}$ values were 281 min and 35 min for HS-HCl and 84 min and 9 min for AC-HCl in buffer and in plasma, respectively. Thus, hydrolysis rates in plasma were sufficiently rapid for hydrolysis in the systemic circulation to contribute to the overall rates of ester hydrolysis in vivo.

Further experiments to determine the mechanism by which prodrugs avoid metabolism in their first-pass though the liver and the extent to which this favorable property can be optimized are planned.

TABLE 2

Apparent absorption and elimination rate constants obtained from least-squares regression of the propranolol acetate and succinate blood concentrations after oral administration according to Eqn. 1

Rat	Compound	K (h^{-1})	k_a (h^{-1})
4	HS-HCl	1.9	2.4
5	HS-HCl	1.0	6.3
6	HS-HCl	1.2	5.4
Average ± S.D.		1.4 ± 0.5	4.7 ± 2.0
7	AC-HCl	1.1	4.8
8	AC-HCl	1.1	5.7
9	AC-HCl	2.2	2.3
Average ± S.D.		1.5 ± 0.4	4.3 ± 1.8

Acknowledgements

The authors are grateful to Dr. I. Lai for his assistance in the syntheses and to Ayerst Laboratories, New York, NY for providing a sample of the hemisuccinate ester of propranolol. This work was supported by a grant from the University of Utah Research Committee and a University of Utah Biomedical Research Support Grant.

References

Crowther, A.F. and Smith, L.H., β-Adrenergic blocking agents. II. Propranolol and related 3-amino-1-naphthoxy-2-propanols. *J. Med. Chem.*, 11 (1968) 1009–1013.

Galinsky, R.E., Role of glutathione turnover in drug sulfation: Differential effects of diethylmaleate and buthionine sulfoximine on the pharmacokinetics of acetaminophen in the rat. *J. Pharmacol. Exp. Ther.*, 236 (1986) 133–139.

Garceau, Y., Davis, I. and Hasegawa, J., Plasma propranolol levels in beagle dogs after administration of propranolol hemisuccinate ester. *J. Pharm. Sci.*, 67 (1978) 1360–1363.

Iwamoto, K. and Watanabe, J., Dose-dependent presystemic elimination of propranolol due to hepatic first-pass metabolism in rats. *J. Pharm. Pharmacol.*., 37 (1985) 826–828.

Kornhauser, D.M., Wood, A.J.J., Vestal, R.E., Wilkinson, G.R., Branch, R.A. and Shand, D.G., Biological determinants of propranolol disposition in man. *Clin. Pharmacol. Ther.*, 23 (1978) 165–174.

Lo, M.-W., Effeney, D.J., Pond, S.M., Silber, B.B. and Riegelman, S., Lack of gastrointestinal metabolism of propranolol in dogs after portacaval transposition. *J. Pharmacol. Exp. Ther.*, 221 (1982) 512–515.

Paterson, J.W., Conolly, M.E., Dollery, C.T., Hayes, A. and Cooper, R.G., The pharmacodynamics and metabolism of propranolol in man. *Pharmacol. Clin.*, 2 (1970) 127–133.

Shand, D.G. and Rangno, R.E., The disposition of propranolol. I. Elimination during oral absorption in man. *Pharmacology*, 7 (1972) 157–168.

Suzuki, T., Isozaki, S., Ohkuma, T. and Rikihisa, T., Influence of route of administration on the mean hepatic extraction ratio of propranolol in the rat. *J. Pharmacol. Dyn.*, 3 (1980) 603–611.

Weeks, J.R. and Davis, J.D., Chronic intravenous cannulas for rats. *J. Appl. Physiol.*, 19 (1964) 540–541.