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The influence of bile salts on the absorption *in vitro* and *in vivo* of propranolol

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Abstract: The lipophilicity of propranolol is increased by some bile salts which form ion-pairs. In the presence of taurodeoxycholate, the logarithm of the apparent partition coefficient (log *P*) of propranolol is increased. Moreover, the apparent diffusion constants *in vitro* of propranolol as ion-pairs at pH 3.0–6.0 are about 5–6 times higher than those of propranolol alone.

The area under the curve values of plasma concentration–time profiles of propranolol, following its oral administration to rabbits together with taurodeoxycholate, are about 1.4 times higher than those after administration of propranolol alone. Moreover, after the administration of propranolol with taurodeoxycholate the plasma concentration rises more rapidly, with a point of inflection between 0.5 and 1.5 h, than after administration of propranolol alone.

Taurodeoxycholate does not modify the first-pass effect of propranolol in rabbits following intravenous and intraportal administration. The absorption of an oral dose of propranolol in the presence of taurodeoxycholate increases from 70% to 100%, due to the higher lipophilicity of the ion-pair. The plasma concentration–time curves suggest the hypothesis that greater absorption of the ion-pair occurs mainly in the upper region of the gastrointestinal tract.

Keywords: *Propranolol–taurodeoxycholate ion-pair; propranolol absorption; bile salts; diffusion rate* in vitro*; bioavailability; first-pass effect.*

Introduction

Propranolol, the first beta-adrenergic receptor-blocking drug introduced in clinical practice, shows a wide variability in inter-individual disposition after oral administration in man [1]. As much as a 20-fold variation of the steady-state plasma concentration of propranolol has been observed in patients receiving identical multiple-dose therapy [2].

The low bioavailability of oral doses of propranolol, as well as the variations in the blood levels, were attributed to the extensive first-pass effect on metabolism of the drug [3, 4]. Many authors have shown that propranolol is primarily eliminated through metabolism after massive hepatic uptake [5–7].

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Tse *et al.* [8] examined the pharmacokinetics of propranolol in a fasting dog after oral, intraportal and intravenous single doses. The absorption efficiency, as unchanged drug, of orally administered propranolol was 71%, while first-pass metabolism accounted for a further loss of 62% before the drug reached the systemic circulation; the overall bioavailability was 27%. Taylor and Grundy [9] compared the absorption *in situ* of propranolol in different regions of the rat gastrointestinal tract; whereas no absorption of propranolol was detected in the stomach, the drug was rapidly absorbed from the small and large intestine, according to first-order kinetics.

In previous reports [10, 11] the changes in lipophilicity induced by ion-pair formation of some drugs were reported, together with changes in the drug permeability constants [12] induced by the counter-ions.

The present study reports ion-pair formation between propranolol and some bile salts, the kinetics of the diffusion through a lipophilic membrane and the pharmacokinetic behaviour in rabbit.

Experimental

Materials

Propranolol hydrochloride (Icpharma, Italy) and the bile salts taurodeoxycholate (Sigma), deoxycholate, cholate, glycocholate, glycodeoxycholate and taurocholate (Merck) were used as received. The organic phase used in the partition studies was 1-octanol (Merck). Gastric barrier M 1, intestinal barrier D 1 and RS-40 membranes were supplied by Sartorius.

The instruments employed were: pH meter, Orion 701-A; UV-visible spectrophotometer, Perkin–Elmer model EPS-3T; absorption simulator, Sartorius No. 16750; vortex G mixer; spectrofluorimeter, Aminco–Bowman; Varian model 5010 high-performance liquid chromatograph, equipped with a Rheodyne injector, model 7125; fluorimeter, Varian Fluorichrom; variable wavelength spectrophotometer, Varian UV 10. For high-performance liquid chromatography (HPLC) 250 × 4 mm i.d. stainless-steel columns packed with 5-μm LiChrosorb RP-18 (Merck) were used.

Determination of in vitro *absorption rate constants*

Various artificial lipid barriers were used, supported by RS 40 membranes. The artificial gastric barrier M 1 was used at pH 1.1 and 3.0 and the intestinal barrier D 1 was used at pH 6.0, 7.0 and 8.0. 1-Dodecanol was then used as a lipid barrier at pH 1.1 and 3.0 and at pH 9.0.

A 100 ml portion of each solution of the drug, alone or in the presence of the bile salt (Phase 1), maintained at 37°C for 30 min, was transferred to the absorption simulator. Phase I was buffered at pH 1.1 and at pH 3.0 by hydrochloric acid–glycine buffer and at pH 6.0, 7.0, 8.0 and 9.0 with phosphate buffer [13]. In all experiments the simulated plasma solution (Phase II) consisted of 100 ml of a pH 7.5 phosphate buffer solution as proposed by Stricker [13].

Propranolol solutions ($1.25 \times 10^{-4} - 1 \times 10^{-3}$M), buffered at the chosen pH, were used as controls for the simulated absorption. The bile salt solutions were prepared using a $1.25 \times 10^{-4} - 1 \times 10^{-3}$M drug solution in the presence of the bile salt at fixed molarity. Diffusion cells of 40 cm^2 and of 4.6 cm^2 were used. Samples were removed from both sides of the lipid barriers at 0, 30, 60, 90 and 120 min and propranolol was assayed spectrophotometrically at 292 nm.

The diffusion rate constants, K_d, and the simulated absorption rate constants, K_i, were calculated from the formulae [13, 14]:

$$K_d = \frac{C_2^{II} - C_1^{II}}{t_2 - t_1} \cdot \frac{1}{C_0^I} \cdot \frac{100}{B} \text{ cm min}^{-1},$$

where t_1 and t_2 = time (min); C_2^{II} and C_1^{II} = concentration in phase II at t_2 and t_1; C_0^I = initial concentration in phase I; B = barrier area (cm^2);

$$K_i = G(K_d - K_{d_o}) \text{ min}^{-1},$$

where $G = 4.3$ for the artificial gastric barrier, $G = 10.0$ for the artificial intestinal barier, $K_{d_o} = 0.7 \times 10^{-4}$ for the artificial gastric barrier and 1.8×10^{-4} for the artificial intestinal barrier.

Partition studies

Octanol saturated with the buffer selected for the diffusion experiment and the same buffer saturated with octanol were used for partition studies. The bile salt and propranolol were dissolved in the chosen buffer at concentrations identical to those used in the diffusion experiments.

Samples (10 ml) of aqueous buffer solution containing propranolol, alone or in the presence of bile salt, and 2–10 ml of octanol were placed in a screw-scapped tube and shaken vigorously for 2 min with a Vortex G mixer [15].

The aqueous layer was withdrawn by a syringe and centrifuged at 1000 rpm for 20 min. The concentration of propranolol in the aqueous phase, before and after partitioning, was measured spectrophotometrically at 292 nm, and the log of the apparent partition coefficient (log P) for propranolol was then calculated.

Drug administration and blood sampling

Six New Zealand rabbits each weighing 3 kg were used, maintained under constant humidity and temperature conditions. Food was withheld for 12 h prior to each experiment. Each rabbit was treated with single doses of propranolol hydrochloride (PL) and of propranolol-taurodeoxycholate (PLT) (molar ratio 1:2), administered orally (o.s.), intravenously (i.v.) or intraportally (i.p.) according to the following two-stage protocol.

Stage 1: single doses of PL and PLT were given orally (10 mg/kg propranolol) and intravenously (1 mg/kg propranolol) in a randomized crossover protocol.

Stage 2: the rabbits were then cannulated for intraportal administration. The rabbits were anaesthetized with Althesin (0.1 ml/kg in repeated doses i.v. at 2-min intervals). With sterile technique, a midline incision was made in the abdomen. A polyethylene tube (PE 50) was inserted into a tributary of the superior mesenteric vein near its junction with the portal vein, and was guided upward into the portal vein. It was then secured by ligation with a nylon suture. The other end of the catheter was passed subcutaneously and led to the exterior through a stab incision at the nape of the neck, before closing the abdomen with sutures. Clot formation was minimized with routine heparin injection. Three days after surgical recovery, single doses of PL and PLT (1 mg/kg propranolol) were given intraportally according to a randomized cross-over scheme.

In both stages each single dose was given at ten-day intervals to ensure complete drug elimination, thereby avoiding changes in pharmacokinetic parameters due to prior exposure to propranolol.

The rabbits were placed in restraining apparatus so that they could stand normally, but could not disturb the dosing and blood sampling processes. For intravenous dosing, a catheter was positioned in the marginal vein of the ear. PL and PLT were administered over a period of 30 s. The catheter was flushed with 5 ml of saline solution and was then removed.

The intraportal dose of PL or PLT was injected directly into the cannula leading to the portal vein over a 30 s period. Each oral dose of PL or PLT was administered in a total volume of 5 ml of 0.5% m/v carboxymethylcellulose through a gastric cannula.

Blood samples (3 ml) were collected via a catheter positioned in the ear central artery before and up to 8 h after drug administration. Blood samples were placed in heparinized tubes and plasma was separated by centrifugation and frozen immediately (−80°C).

Assay of plasma samples

Plasma concentrations of propranolol were determined by a sensitive and specific HPLC method published by Drummer *et al.* [16].

Pharmacokinetic analysis

The apparent first-order elimination rate constants (β) (and the relative half-lives) of PL and PLT were computed by means of the least squares method on the terminal tail of each plasma concentration–time curve.

The area under the curve (AUC) was estimated by the trapezoidal rule:

$$AUC_0^{\infty} = AUC_0^{8\ h} + \frac{C_{8\ h}}{\beta}$$

where $C_{8\ h}$ = plasma concentration of propranolol at the eighth hour; and β = apparent first-order elimination rate constant.

The bioavailability of propranolol following administration of both PL and PLT was assessed by comparing AUC values for each of the different routes of administration. The overall bioavailability of an oral dose is the product of two factors, $F' \times f$, where F' represents the fraction of dose absorbed unchanged, and f is the fraction of absorbed propranolol which escapes first-pass hepatic elimination. Thus:

$$F' = \frac{AUC_{o.s.}}{AUC_{i.p.}} \cdot \frac{Dose_{i.p.}}{Dose_{o.s.}} \tag{1}$$

$$f = \frac{AUC_{i.p.}}{AUC_{i.v.}} . \tag{2}$$

The relative bioavailability index (F_{rel}) for propranolol as the ion-pair was estimated for each rabbit, by the AUC ratio:

$$F_{rel} = \frac{AUC_0^{\infty}\ (PLT,\ o.s.)}{AUC_0^{\infty}\ (PL,\ o.s.)}$$

Statistical comparison between the data groups was based on one-way analysis of variance.

Results and Discussion

Propranolol is normally administered orally in man, although variable absorption has been reported [17]. Propranolol is a weak base ($pK_a = 9.45$) [9] which is mainly absorbed in the intestine; studies on absorption *in situ* of the drug from different regions of the rat gastrointestinal tract [9] showed that absorption is rapid through the small intestine, particularly the duodenum, and the large intestine whereas it is minimal through the stomach.

In a study of the buccal absorption and physicochemical properties of propranolol [18], the absorption data were interpreted qualitatively, in terms of the pH-partition theory, as passive diffusion of non-ionized drug species. Ion-pair formation between drug and anions is another hypothesis proposed by Higuchi and Kato [19] to explain drug absorption.

In the present work, the effects of bile anions on the lipophilicity of propranolol and therefore on the absorption of the drug were examined *in vitro* using an absorption simulator. The diffusion of propranolol alone and in the presence of a bile salt through an artificial lipid membrane was studied over a wide pH range using simulated gastric and intestinal juices.

Effect of taurodeoxycholate

Sartorius barriers M 1 and D 1 were used at first. The composition of the two lipid coatings on the membranes was different but unknown. The diffusion rate constants observed are reported in Table 1; the diffusion constants of propranolol alone, in the pH range 6.0–8.0, are rather close and do not increase progressively as was observed by Schurmann and Turner [18]. In addition the apparent log P of propranolol, determined in the same simulated fluid (Table 1), cannot be related to the K_d values obtained. The same behaviour was observed for propranolol in the presence of taurodeoxycholate (Table 1).

These results, which do not agree with other biological and chemical data [18], can probably be related to interactions between propranolol and the lipids of the coatings. To overcome this problem 1-dodecanol was used as a lipid coating on the RS-40 membrane (Table 1). A progressive increase of the diffusion constant occurs in the pH range 6.0–9.0. In Fig. 1, log P of propranolol alone is plotted as a function of log K_d. An approximately linear relationship is observed.

Moreover, another interesting relationship is observed between the apparent rate constant obtained by Schurmann and Turner [18] and the diffusion constants obtained in the absorption simulator, using 1-dodecanol as a lipid barrier. The plot of the apparent rate constants determined *in vivo* for buccal absorption [18] in the pH range 5.08–8.94 against the extrapolated absorption rate *in vitro* shows an almost linear relationship (Fig. 2).

At pH 1.1 the diffusion constant of propranolol hydrochloride alone (Fig. 3, lower curve; Table 1) is greater than at pH 3.0. This may be due to the hydrochloric acid concentration used to simulate the gastric juice; probably the chloride common-ion suppresses the ionization of propranolol hydrochloride, thus increasing the lipophilicity of the drug molecule and increasing the diffusion rate.

Table 1
Diffusion rate constants K_d, absorption rate constants K_i, and log P of propranolol alone and in the presence of taurodeoxycholate (molar ratio 1:2)

In absence of taurodeoxycholate						In presence of taurodeoxycholate				
	Lipid barrier					Lipid barrier				
pH	M_1, D_1		1-Dodecanol		log P	M_1, D_1		1-Dodecanol		log P*
	$K_d \times 10^3$ (cm·min^{-1})	$K_i \times 10^3$ (min^{-1})	$K_d \times 10^3$ (cm·min^{-1})	$K_i \times 10^3$ (min^{-1})		$K_d \times 10^3$ (cm·min^{-1})	$K_i \times 10^3$ (min^{-1})	$K_d \times 10^3$ (cm·min^{-1})	$K_i \times 10^3$ (min^{-1})	
1.1	0.70	2.77	0.76	2.96	0.25	1.01	4.06	0.98	3.91	0.91
3.0	0.51	1.89	0.46	1.66	0.18	4.04	17.10	2.50	10.45	1.50
6.0	2.04	18.60	0.50	3.21	−0.04	4.56	43.9	2.57	2.89	1.59
7.0	3.72	35.40	1.66	14.80	0.65	4.37	41.9	3.89	37.1	1.69
8.0	4.17	39.90	7.24	70.60	1.52	4.79	46.1	8.51	89.3	1.80
9.0	—	—	36.3	361.0	2.20	—	—	35.5	353.0	2.30

* Log P was obtained in the same media used to determine K_d and K_i.

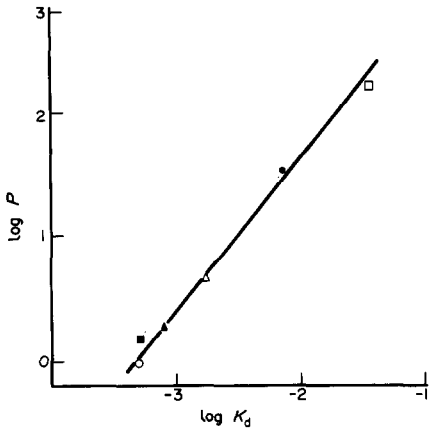

Figure 1
Log P (apparent partition coefficient) of propranolol against log K_d (diffusion rate constant, cm min^{-1}, obtained using 1-dodecanol as lipid barrier) at different pH values. pH 1.1 (▲); pH 3.0 (■); pH 6.0 (○); pH 7.0 (△); pH 8.0 (●); pH 9.0 (□).

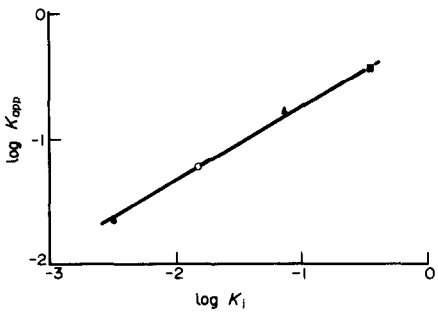

Figure 2
Log apparent absorption rate constant K_{app} (min^{-1}) of propranolol determined by buccal administration (Schürmann and Turner [18]) against log absorption rate constant K_i (min^{-1}), determined using the absorption simulator.

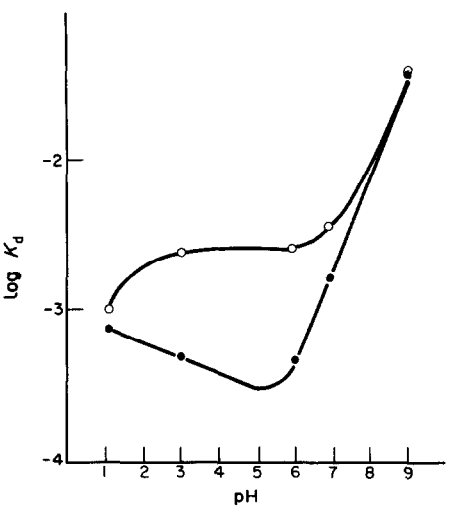

Figure 3
Log diffusion constant K_d (cm min^{-1}) of propranolol alone (●) and in the presence of taurodeoxycholate (○) (molar ratio 1:2) obtained using 1-dodecanol as lipid barrier, against pH of the medium.

At pH 3.0 and pH 6.0 the propranolol salt is more highly dissociated and log K_d is consequently lower; at higher pH the non-ionized base, which is more lipophilic than the protonated form, diffuses quickly. Analogous behaviour can be observed for log P with a minimum at about pH 6.0 (Fig. 4, lower curve; Table 2).

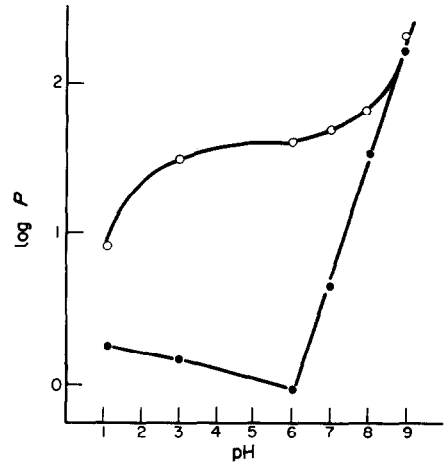

Figure 4
Log P of propranolol alone (●) and in the presence of taurodeoxycholate (○) (molar ratio 1:2) against pH of the medium.

Table 2
Diffusion rate constants K_d, absorption rate constants K_i and log P of propranolol (1.25×10^{-4}M) in the presence of increasing concentration of taurodeoxycholate.

Taurodeoxycholate $\times 10^{-4}$M	Molar ratio	$K_d \times 10^3$ (cm.min^{-1})	$K_i \times 10^3$ (min^{-1})	log P*
0	—	0.46	1.66	0.18
1.25	1:1	1.66	6.84	1.05
2.50	1:2	2.50	10.45	1.52
6.25	1:5	3.02	12.70	1.65
10.00	1:8	3.31	13.90	1.70

* Log P of propranolol was obtained in the same medium used to determine K_d and K_i.

Of the bile salts, taurodeoxycholate can be studied over a wide pH range since it is sufficiently soluble in an acid medium. The presence of taurodeoxycholate, at a concentration twice that of propranolol, greatly increases the diffusion constant of propranolol (Fig. 3, upper curve). At pH 1.1 a sharp increase is observed; the greatest rise appears in the pH range 3.0–6.0.

The greater lipophilicity of propranolol as an ion-pair is confirmed by the increase of log P over a wide pH range (Fig. 4). At pH 9.0 no difference between K_d and log P of propranolol alone or in the presence of bile salt can be observed.

A number of absorption simulations were performed at pH 3.0 in which the concentration of propranolol was kept constant (1.25×10^{-4}M), while the concentration of the bile salt was varied. The results are illustrated in Table 2 and Fig. 5. An increase is noted both in diffusion constants and in log P as the bile salt concentration rises. The increment is highest for a 1:2 molar ratio; for this reason a 1:2 ratio was used in the experiments *in vitro* and *in vivo*. The study was performed below the critical micelle

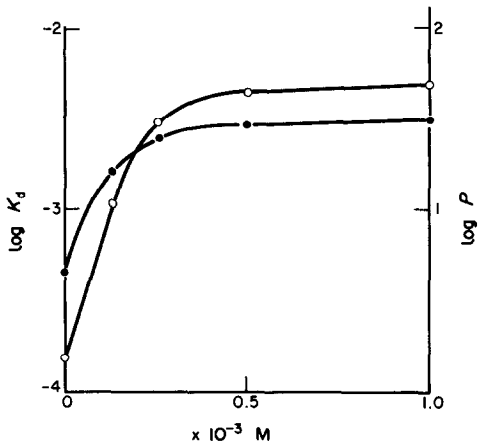

Figure 5
Log K_d (cm min^{-1}) (●) and log P (○) of propranolol ($1.25 \times 10^{-4}M$) against increasing concentrations of taurodeoxycholate.

concentration of taurodeoxycholate (1.1×10^{-3}M) [20] at pH 6.0, because propranolol can be entrapped in micelles with a decrease in diffusion and in absorption, as was noted *in situ* by other workers [21].

In Fig. 5 log P is plotted against taurodeoxycholate concentration. Behaviour similar to that of the diffusion constant is evident, showing that an increase in the bile salt concentration results in an increase in the lipophilicity of the drug.

Effect of other bile salts

The effect of the other bile salts on diffusion rates of propranolol was studied at pH 6.0. At this pH these bile salts are sufficiently soluble, except for deoxycholate, whose diffusion rate constant cannot be determined with certainty owing to its low solubility. The diffusion constants and log P values of propranolol, both with and without bile salts at pH 6.0, are reported using lipid barrier D 1 of 1-dodecanol (Table 3). The range of K_d values for propranolol in the presence of the different bile salts is wider for 1-dodecanol than for the lipid barrier D 1. The diffusion constants of propranolol in the presence of the bile deoxyacids are higher than those obtained with the respective hydroxylated

Table 3
Diffusion rate constants K_d, absorption rate constants K_i and log P of propranolol in the presence of bile salts (molar ratio 1:2) by using as lipid barriers (a) D_1 and (b) 1-Dodecanol (pH 6.0)

	Lipid barrier					
	D_1		1-Dodecanol			
Bile salt	$K_d \times 10^3$ (cm·min^{-1})	K_i (min^{-1})	$K_d \times 10^3$ (cm·min^{-1})	K_i (min^{-1})	Log P*	pK_a†
—	2.04	18.6	0.50	3.21	−0.04	—
Glycocholic	4.27	40.9	1.55	13.70	0.74	3.95
Glycodeoxycholic	4.57	43.9	2.11	19.30	1.04	4.69
Taurocholic	3.72	35.4	0.57	3.95	1.02	1.85
Taurodeoxycholic	4.57	43.9	2.57	23.90	1.59	1.93
Cholic	5.01	48.3	0.50	3.71	0.45	4.98

* Log P of propranolol was obtained in the media used to determine K_d and K_i.
† pK_a of bile acids [20].

acids. These results are probably related to the greater lipophilicity of the deoxyacids in comparison with the corresponding hydroxylated acids. The behaviour of the different bile salts at pH 6.0 cannot be related to their pK_a as shown in Table 3.

Investigations in vivo

The results obtained *in vitro* prompted the question as to whether they could be correlated with the *in vivo* results. Figure 6 describes mean plasma concentration–time curves obtained in rabbit by administering single oral doses (10 mg/kg) of propranolol, both with and without taurodeoxycholate. In rabbit the absorption of propranolol alone is low in the first two hours and shows a lag-time of about 30 min. The maximum peak concentration (C_{max}) of 82.3 ± 8.8 ng/ml is observed at 2.67 ± 0.52; after this the concentration falls to 18.5 ± 6.7 ng/ml after 8 h. The decrease follows the first-order rate law (Table 6) with a rate constant of 0.2929 ± 0.0678 h^{-1} corresponding to a half-life, $t_{1/2}$, of 2.47 ± 0.54 h.

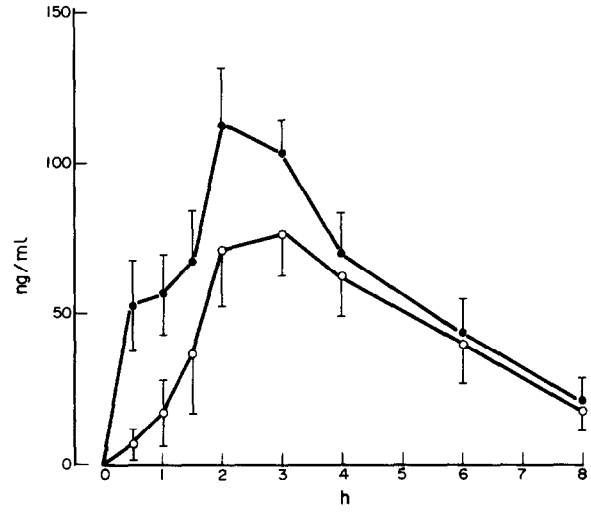

Figure 6
Plot of mean plasma concentration–time curves (n = 6) of propranolol (ng/ml) in rabbit, following single oral administration of propranolol (10 mg/kg) along (○) or with taurodeoxycholate (●) in a molar ratio of 1:2.

The simultaneous administration of taurodeoxycholate markedly affects the absorption phase of propranolol. The plasma concentration–time curves exhibit a C_{max} of 114.2 ± 18.1 ng/ml after 2.17 ± 0.41 h. No lag-time is apparent, but 30 min after administration plasma concentrations of about 50 ng/ml were observed (Fig. 6).

After administration of PLT, plasma concentrations of propranolol rise faster than after PL alone and show a point of inflection between 0.5 and 1.5 h. The decay curve, after the peak maximum, follows first-order kinetics, with a rate constant of 0.2928 ± 0.053 h^{-1} corresponding to a $t_{1/2}$ of 2.44 ± 0.48 h. The rate constants and the relative half-life of propranolol after oral administration of both PL and PLT are not significantly different from those observed after i.v. administration.

The area under the curve obtained for propranolol administered with taurodeoxycholate is considerably higher than that obtained for propranolol alone.

In Table 4, the index of the relative bioavailability of propranolol–taurodeoxycholate

Table 4
Pharmacokinetic analysis and relative bioavailability of propranolol administered orally (10 mg/kg) to rabbits as propranolol–taurodeoxycholate ion-pair (PLT) or as propranolol alone (PL)

Animal No.	β* (h^{-1})		$t_{1/2}$† (h)		C_{max}† (ng/ml)		t_{max}§ (h)		AUC_o^{∞}‖ ($ng \cdot h \cdot ml^{-1}$)		
	PLT	PL	PLT	PL	PLT	PL	PLT	PL	PLT	PL	$F_{(rel)}$¶
1	0.3102	0.3784	2.23	1.83	126	90	2	3	510.70	345.75	1.48
2	0.3002	0.3726	2.31	1.86	98	79	2	2	399.13	287.40	1.39
3	0.2146	0.2370	3.23	2.92	122	95	2	3	633.93	487.90	1.30
4	0.2476	0.2634	2.80	2.63	135	83	2	3	560.77	374.23	1.50
5	0.3215	0.2859	2.16	2.42	87	75	3	3	361.02	271.46	1.33
6	0.3625	0.2203	1.91	3.14	117	72	2	2	451.87	333.86	1.35
$\bar{x}$	0.2928	0.2929	2.44	2.47	114.2	82.3	2.17	2.67	486.24	350.1	1.39
σ	0.0533	0.0678	0.48	0.54	18.1	8.8	0.41	0.52	102.39	77.4	0.08
P (one-way AOV)**	N.S.††		N.S.		<0.01		N.S.		<0.05		

* β = first order apparent elimination rate constant.
† $t_{1/2}$ = elimination half-life.
‡ C_{max} = maximum drug concentration in plasma, following administration of single dose.
§ t_{max} = time at which C_{max} occurs.
‖ AUC_o^{∞} = area under the curve.
¶ $F_{(rel)}$ = relative bioavailability index.
** P (one-way AOV) = probability calculated by one-way analysis of variance. †† NS = not significant.

to that of propranolol is reported. The table shows that the bioavailability of propranolol in the presence of taurodeoxycholate is about 1.39 times higher than that of propranolol alone, although valves for β and $t_{1/2}$ do not vary significantly in the two series of data.

Since kinetic analysis shows that, after oral administration, the propranolol elimination phase is not influenced by taurodeoxycholate, the change in the PLT plasma concentration–time curve and the increase of the PLT bioavailability could be related to the absorption phase.

To exclude the influence of taurodeoxycholate on the first-pass hepatic effect of propranolol, the plasma concentration–time curves were measured, following intravenous and intraportal administration of a single dose of the drug (1 mg/kg) administered over 30 s as PL or as PLT.

Plasma levels of propranolol following intravenous and intraportal doses of PLT and PL are shown in Fig. 7. The values of β and $t_{1/2}$ are summarized in Tables 5 and 6 for intravenous and for intraportal administration, respectively.

After intraportal administration of PLT or PL, the plasma concentration of propranolol, the apparent elimination rate constant and the AUC are significantly lower than those observed after intravenous administration. These results agree with data previously obtained for intravenous administration of propranolol and also with rapid hepatic uptake of the drug [5].

However, after both intraportal and intravenous administration, no statistically significant differences between PLT and PL were observed in the values of β, $t_{1/2}$ and AUC. Therefore it can be concluded that taurodeoxycholate does not interfere with the first-pass hepatic effect of propranolol in rabbits, at the dose tested.

Further evidence is reported in Table 7, which lists the fraction of unchanged dose absorbed (F'), the fraction of absorbed propranolol which escapes first-pass hepatic

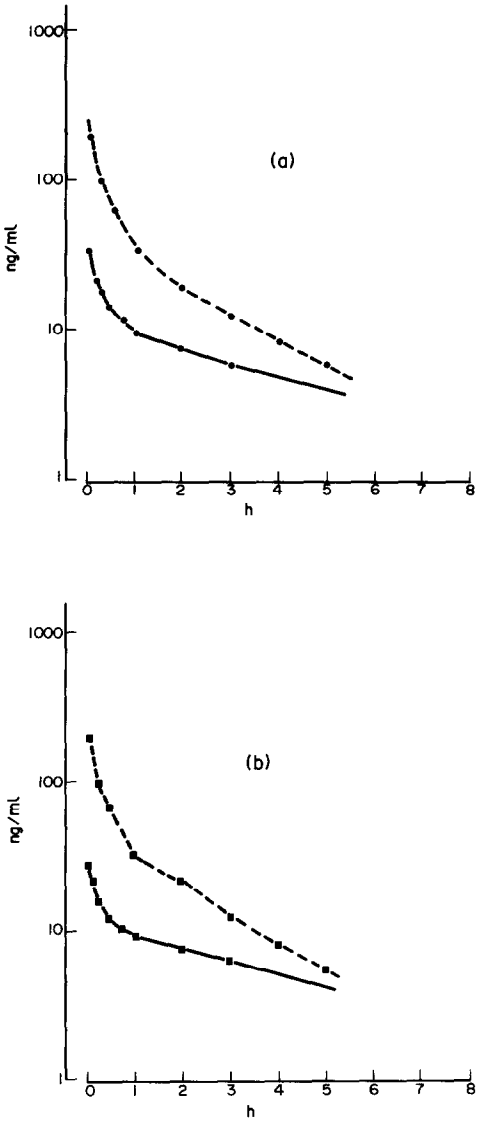

Figure 7
Semilog-plot of mean plasma concentration–time curves (n = 6) of propranolol (ng/ml) in rabbit, following a single dose of propranolol (1 mg/kg over 30) administered intravenously (– – –) or intraportally (———) as propranolol, either alone (a) or with taurodeoxycholate (b) in a molar ratio of 1:2.

elimination (f) and the overall bioavailability of an oral dose of propranolol administered to rabbits as ion-pairs (PLT) or alone (PL).

The fraction of absorbed propranolol which escapes first-pass hepatic elimination is not statistically different after PLT or PL administration. In contrast, the fraction of the dose absorbed (F') is statistically considerably different after oral administration of PLT or PL, rising to almost 100% when propranolol is administered as an ion-pair with taurodeoxycholate, and to about 70% when administered alone. Based on the AUC

Table 5
Pharmacokinetic parameters of propranolol administered intravenously (1 mg/kg over 30 s) to rabbits as propranolol–taurodeoxycholate ion-pair (PLT) or as propranolol alone (PL)

Animal No.	β (hr^{-1})		$t_{1/2}$† (h)		AUC_0^∞‡ ($ng \cdot h \cdot ml^{-1}$)	
	PLT	PL	PLT	PL	PLT	PL
1	0.3458	0.4832	2.00	1.43	152.72	148.40
2	0.6222	0.5992	1.11	1.16	139.49	134.00
3	0.4585	0.4199	1.51	1.65	130.74	141.81
4	0.4149	0.4196	1.67	1.65	155.69	171.77
5	0.5043	0.5533	1.37	1.25	174.52	124.66
6	0.5829	0.3701	1.19	1.87	124.13	147.70
$\bar{x}$	0.4881	0.4742	1.47	1.50	146.21	144.72
σ	0.1036	0.0880	0.33	0.27	18.47	15.99
P (one-way AOV)§	N.S.‖		N.S.		N.S.	

* β = first order elimination rate constant.
† $t_{1/2}$ = elimination half-life.
‡ AUC_0^∞ = area under the curve.
§ *P* (one-way AOV) = probability calculated by one-way analysis of variance.
‖ NS = not significant.

Table 6
Pharmacokinetic parameters of propranolol administered intraportally (1 mg/kg over 30 s) to rabbits as propranolol–taurodeoxycholate ion-pair (PLT) or as propranolol alone (PL)

Animal No.	β* (h^{-1})		$t_{1/2}$† (h)		AUC_0^∞‡ ($ng \cdot h \cdot ml^{-1}$)	
	PLT	PL	PLT	PL	PLT	PL
1	0.2157	0.1737	3.21	3.99	50.05	59.10
2	0.2425	0.3273	2.86	2.12	42.01	38.87
3	0.1579	0.1689	4.39	4.10	58.88	69.10
4	0.2177	0.2037	3.18	3.40	60.79	59.05
5	0.2736	0.2862	2.53	2.42	37.87	36.85
6	0.2267	0.2196	3.06	3.16	48.61	46.02
$\bar{x}$	0.2223	0.2299	3.20	3.19	49.70	51.50
σ	0.0381	0.0638	0.63	0.81	9.03	12.87
P (one-way AOV)§	N.S.‖		N.S.		N.S.	

* β = first order elimination rate constant.
† $t_{1/2}$ = half-life of elimination.
‡ AUC = area under the curve.
§ *P* (one-way AOV) = probability calculated by one-way analysis of variance.
‖ NS = not significant.

ratio an increase of bioavailability of 1.39 times is estimated. This value is the maximum relative to the reference point. The results are reasonably consistent with the data *in vitro*.

It can be concluded, therefore, that the statistically significant differences observed for the overall bioavailability of PLT, in comparison with PL, must be related to the absorption phase. Nevertheless the first part of the plasma concentration–time curves *in vivo* obtained after the administration of the propranolol–taurodeoxycholate ion-pair

Table 7
Fraction of unchanged dose absorbed (F')*, fraction of absorbed propranolol which escapes first-pass hepatic elimination (f)†, and the overall bioavailability (F) of an oral dose of propranolol administered to rabbits as PLT or as PL

Animal No.	F'		f		$F = F' \cdot f$	
	PLT	PL	PLT	PL	PLT	PL
1	1.0204	0.5850	0.3277	0.3982	0.3344	0.2329
2	0.9501	0.7394	0.3012	0.2900	0.2862	0.2144
3	1.0766	0.7061	0.4504	0.4873	0.4849	0.3441
4	0.9225	0.6338	0.3905	0.3438	0.3602	0.2179
5	0.9533	0.7367	0.2170	0.2956	0.2069	0.2178
6	0.9296	0.7255	0.3916	0.3116	0.3640	0.2261
$\bar{x}$	0.9754	0.6877	0.3464	0.3544	0.3394	0.2422
σ	0.0605	0.0637	0.0824	0.0763	0.0923	0.0504
P (one-way AOV)‡	<0.001		N.S.		<0.05	

* F' is defined in terms of the ratio of AUC values for oral:intraportal administration, normalized for dose (cf. equation 1).
† f is defined in terms of the ratio of AUC values for intraportal:intravenous administration (cf. equation 2).
‡ P (one-way AOV) = probability calculated by one-way analysis of variance.

(Fig. 6) appears to be very steep. The maximum peak concentrations are significantly higher and the t_{max} is shorter than those obtained with propranolol alone. These results are in agreement with an increased apparent absorption rate of propranolol administered as an ion-pair.

Taylor and Grundy [9] showed that propranolol *in vivo* is not absorbed in the stomach, but is rapidly absorbed in the duodenum of the rat. The absorption of propranolol in the rabbit after oral administration shows a lag-time of about 30 min. Subsequently the concentration progressively rises, probably due to the passage of the gastric contents into the duodenum. When the same criteria are applied to the curve corresponding to the administration of propranolol–taurodeoxycholate, the main difference observed is the lack of a lag-time. These data may indicate that absorption begins at the gastric level and is completed at the intestinal level.

The pharmacokinetic data are in agreement with the results obtained *in vitro* at the pH of the stomach and of the intestine. According to the present work the role of bile salts in gastrointestinal absorption could be related not only to the formation of micelles, but also to the possibility of forming ion-pairs with bioactive molecules containing basic functions. The stability of the ion-pair depends, of course, on several properties of the counter-ion such as lipophilicity, steric and electronic factors.

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