J. Pharm. Pharmacol. 1993, 45: 246–252
Received May 20, 1992

An In-vitro and In-vivo Correlative Approach to the Evaluation of Ester Prodrugs to Improve Oral Delivery of Propranolol

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Abstract—A series of ester prodrugs of propranolol was synthesized by incorporating substituents (straight alkyl, branched alkyl, acyloxyalkyl and cycloalkyl) into the β-hydroxy function of propranolol with the aim of protecting the drug against first-pass metabolism following oral administration. The in-vitro hydrolysis rates of the prodrugs were, in increasing order, liver homogenate ≫ plasma > buffers. The pH-rate profile of the prodrugs showed maximum stability around pH 4·0; the hydrolysis rates were drastically increased over pH 6·8. QSAR analysis revealed hydrophobic (π) and electronic (σ) effects of the substituents play the main roles for prodrug hydrolysis in buffers and plasma, while hydrolysis in liver homogenate could not be well explained by any of these parameters. Four prodrugs (*O*-acetyl-, *O*-butyryl-, *O*-isovaleryl- and *O*-cyclopropanoyl-propranolol) were selected for oral administration based on their hydrolysis in-vitro. Following oral administration of prodrugs to beagle dogs the absolute bioavailabilities (F) of propranolol were about 2–4-fold that after an equivalent dose of propranolol. The prodrugs were rapidly absorbed and regenerated propranolol to attain peak plasma levels at 0–0·5 h. Intact prodrug levels were also observed, which varied depending on their respective stabilities in in-vitro media. A linear relationship between F of propranolol and log P was obtained. F further appeared to be parabolically dependent on the observed hydrolysis rates of prodrugs in liver homogenate suggesting optimal design manipulation. The overall in-vitro and in-vivo results showed that lipophilic prodrugs having higher chemical and enzymatic stability in buffers and plasma, but susceptible to hydrolysis in the liver homogenate, to be the most promising prodrugs for improving oral bioavailability of propranolol.

Propranolol, an adrenergic β-receptor blocker, is a widely accepted and clinically effective cardiovascular agent. Very low and variable bioavailability of the drug results following oral administration due to extensive stereoselective hepatic first-pass metabolism (Paterson et al 1970; Shand & Rangno 1972; Silber et al 1982; Walle et al 1984), which is influenced by both environmental and genetic factors (Riddell et al 1987). As a model drug to explore different aspects of drug delivery systems, and to avoid the first-pass metabolism, propranolol has been of much research interest in the last two decades.

Alternative routes of administration including intravenous, rectal (De Boer et al 1981; Iwamoto & Watanabe 1985), transdermal (Ogiso & Shintani 1990) and nasal (Hussain et al 1980; Vyas et al 1991) routes have been attempted to improve the systemic bioavailability of propranolol, as these routes can allow the drug to reach the systemic circulation, bypassing the liver and escaping first-pass metabolism. However, complicated application through routes other than oral are still not so acceptable and some of them are still in the development stage. Acceptance of oral delivery has also encouraged prodrug development (Garceau et al 1978; Anderson et al 1988; Buur et al 1988; Irwin & Belaid 1988a, b) and lymphatic delivery formulations of propranolol (White et al 1991). The major metabolites after oral administration of propranolol are *O*-glucuronide, 4-hydroxypropranolol and its glucuronide, and naphthoxyl-acetic acid. The former two are generated in the presystemic metabolism of the drug, and prodrugs were designed to escape this presystemic glucuronidation. Ester prodrugs of the hemisuccinate and acetyl type were found to improve the oral bioavailability of propranolol in dogs (Garceau et al 1978) and rats (Anderson et al 1988) through partial avoidance of glucuronidation. However, the physicochemical and kinetic properties for optimal prodrug design are not clear.

In a further attempt to establish the oral delivery of propranolol, we have synthesized a series of ester prodrugs and after their in-vitro regeneration kinetics and bioavailability evaluation, focused on the optimal physicochemical characteristics and degradation kinetics to improve the oral delivery of propranolol.

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Materials and Methods

Apparatus

Melting points were determined by a micromelting point apparatus (Yanaco Ltd, Japan). Elemental microcombustion analysis was performed by CNH coder (MT-5, Yanaco Ltd, Japan). Infrared (IR) spectra were measured on a Jasco A-1000 IR spectrophotometer by the KBr disc method. Proton magnetic resonance (^{1}H NMR) was taken on a JEOL JNM-GX 400 NMR spectrometer in $CDCl_3$, using tetramethylsilane (TMS) as an internal standard. HPLC was performed with a system consisting of a Hitachi 655A-11 liquid chromatograph, a Hitachi 655A variable UV monitor, a Hitachi F 1000 fluorescence detector, a rheodyne injector with a 20 μL loop and a recorder. The column (250 × 4 mm, i.d.) was a Lichrosorb RP select B (7 μm) type.

Materials

(±)-Propranolol HCl was obtained from Sigma Chemical Company (USA). Saccharose (Nacalai Tesque, Japan), Water for Injection (Fuso Chemical Industry, Japan) and bovine serum albumin (Fraction V, Sigma, USA) were purchased. All other chemicals and reagents were of analytical grade. Deionized distilled water was used throughout.

Synthesis of prodrugs

The synthesis was adapted from the methods described by Crowther & Smith (1968) and Irwin & Belaid (1987). Propranolol HCl (1 g, 3·4 mmol) was dissolved in chloroform (20 mL) and heated under reflux for 2 h with the required acid chloride (6·8 mmol). Excess of acid chloride was removed under high vacuum and the residue obtained, repeatedly treated with benzene or toluene and evaporated to exclude completely traces of acid chloride. The solid products were recrystallized from a mixture of isopropyl alcohol and ether to obtain prodrugs as hydrochloride salts. Yields as well as physical and analytical data for the esters are shown in Table 1. New carbonyl bands in the IR spectrum appeared from 1724 to 1764 cm^{-1}. The ^1H NMR data was interpreted as shown in Table 1. The IR and ^1H NMR data of the esters were consistent with their structures. The purity of the esters was confirmed by elemental analysis (C, H and N) that was within ±0·4% of the theoretical values and by the HPLC analysis which revealed > 99% purity.

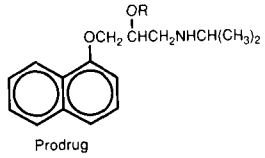
Hydrolysis study

In buffers. Phosphate buffers ($\mu = 0{\cdot}15$) of pH 1·2–8·0 were used as the chemical media for the hydrolysis study. Stock solutions (0·2 mL) of prodrug (0·05 M) in dimethylsulphoxide were added to 100 mL buffer at 37°C to initiate the reaction at 10^{-4} M. At appropriate intervals samples were taken and directly analysed by HPLC. For prodrugs VIII and IX samples quenched by 0·5 M HCl were subsequently analysed by HPLC.

In plasma. The blood was taken from beagle dogs using heparinized syringes and centrifuged at 3000 rev min^{-1} for 10 min. The average protein content of plasma was 87·67 mg mL^{-1} as determined by the method of Lowry et al (1951) with bovine serum albumin as standard. The reaction was initiated as described above using 4 mL plasma in each experiment. At appropriate intervals, samples of 200 μL were withdrawn and added to 200 μL acetonitrile kept in an ice–water bath to deproteinize plasma, and immediately 100 μL of 0·5 M HCl was added to stop further hydrolysis. After centrifugation for 10 min at 3000 rev min^{-1} at 0°C, the clear supernatant was filtered for direct analysis by HPLC.

In liver homogenate. The liver was removed from a beagle dog under ether anaesthesia and thoroughly washed with 0·15 M KCl. Sliced wet liver was added to 3 vol of 0·01 M phosphate buffer (pH 7·4) with 0·15 M KCl and homogenized at 4°C. The homogenate obtained (25% wet wt/v) was centrifuged at

Table 1. Physical and analytical data of ester prodrugs (hydrochloride salts) of propranolol.



Prodrug

Ester	R	Yield (%)	mp (°C)	PC	^1H NMR[a], δ (ppm), J in Hz
Propranolol	H	—	163–164	2·40	
Acetyl (I)	$COCH_3$	93	165–166	4·15	2·56 (3H, s, $COCH_3$)
Propionyl (II)	$COCH_2CH_3$	69	123–124	11·36	1·13 (3H, t, J = 7·3, CH_2CH_3), 2·43, 2·70 (each 1H, dq, J = 17·2, 7·3, CH_2CH_3)
Butyryl (III)	$COCH_2CH_2CH_3$	84	142–143	34·60	0·89 (3H, t, J = 7·5, $CH_2CH_2CH_3$), 1·65 (2H, m, $CH_2CH_2CH_3$), 2·44, 2·61 (each 1H, dt, J = 16·3, 7·5, $COCH_2CH_3$)
Valeryl (IV)	$COCH_2CH_2CH_2CH_3$	65	145–146	97·35	0·82 (3H, t, J = 7·3, CH_2CH_3), 1·31 (2H, h, J = about 7, $CH_2CH_2CH_3$) 1·60 (2H, m, $COCH_2CH_2CH_3$) 2·45, 2·63 (each 1H, dt, J = 6·5, 7·3, $COCH_2CH_2$)
Isobutyryl (V)	$COCH(CH_3)_2$	84	133–134	30·50	1·50, 1·19 (each 1H, d, J = 7·0, $CH(CH_3)_2$), 2·80 (1H, qq, J = 7·0, 7·0, $CH(CH_3)_2$)
Isovaleryl (VI)	$COCH_2CH(CH_2)_2$	85	145–147	88·90	0·90 (each 3H, d, J = 6·6, $CH(CH_3)_2$), 2·10, (1H, p, J = 6·96, 6·60, $CH(CH_3)_1$) 2·39, 2·46 (each 3H, dd, J = 15·7, 7·3, $COCH_2CH$)
Pivaloyl (VII)	$COC(CH_3)_3$	91	144–145	61·60	1·23 (9H, s, $C(CH_3)_2$)
Methylmalonyl (VIII)	$COCH_2COOCH_3$	67	202–205	3·90	3·21 (3H, s, $COOCH_3$), 4·70, 4·90 (each 1H, d, J = 16·1, $COCH_2COO$)
Ethylmalonyl (IX)	$COCH_2COOC_2H_5$	37	156–158	5·21	1·10 (3H, t, J = 7·3, OCH_2CH_3), 3·65, 3·71 (each 1H, d, J = 16·3, $COCH_2COO$) 4·05 (2H, m, two overlapped dq signals, $COOCH_2CH_3$)
Methylsuccinyl (X)	$CO(CH_2)_2COOCH_3$	88	151–152	4·47	2·69 (2H, m, CH_2CH_2COO), 3·55 (3H, s, $COOCH_3$)
Methylglutaryl (XI)	$CO(CH_2)_3COOCH_3$	87	101–103	7·48	1·96 (2H, p, J = 7·3, $CH_2CH_2CH_2$), 2·56, (2H, t, J = 7·3, CH_2CH_2CO) 2·50, 2·73 (each 1H, dq, J = 16·8, 7·3, $COCH_2CH_2$), 3·57 (3H, s, $COOCH_3$)
Cyclopropanoyl (XII)	$COcC_3H_5$	83	153–154	14·40	0·91, 1·10 (each 2H, m, $cCH(CH_2)_2$), 1·79 (1H, m, $cCH(CH_2)_2$)
Cyclobutanoyl (XIII)	$COcC_4H_7$	85	149–150	37·97	1·92, 2·21 (each 3H, m, $cCH(CH_2)_3$), 3·40 (1H, m, $cCH(CH_2)_3$)
Cyclopentanoyl (XIV)	$COcC_5H_9$	58	145–146	52·73	1·64 (8H, m, $cCH(CH_2)_4$), 3·0 (1H, m, $cCH(CH_2)$)
Cyclohexanoyl (XV)	$COcC_6H_{11}$	97	174–175	112·00	1·20, 1·43, 1·95 (10H, m, $cCH(CH_2)_5$), 2·54 (1H, m, $cCH(CH_2)_5$)

PC is the partition coefficient between 1-octanol and aqueous buffer pH 4·0 at 25°C.

Propranolol HCl, δ: 1·49, 1·52 (each 3H, d, J = 6·6, $NHCH(CH_3)_2$), 3·48 (2H, br s, $CH(OH)CH_2NH$), 3·54 (1H, m, $NHCH(CH_3)_2$), 4·41 (2H, d, J = 4·0, $COCH_2CH(OH)$) 5·78 (1H, m, $CH_2CH(OH)CH_2$), 6·81 (1H, d, J = 7·7, ArH), 7·35 (1H, t, J = 8·1, ArH), 7·48 (3H, m, ArH), 7·80 (1H, br d, J = 7·0, ArH), 8·18 (1H, br d, J = 7·2, ArH) 9·49, 10·01 (each 1H, br s, NH_2^+).

[a] Substituents (R) δ values only, the rest of the signals from the compounds were almost the same as obtained from propranolol HCl.

Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, h = hexet, m = multiplet(s), c = cyclic protons.

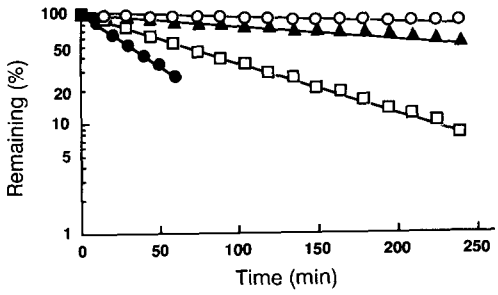


FIG. 1. First order plots for hydrolysis of prodrug I in buffers pH 6·0 (○), 6·8 (▲), 7·4 (□) and 8·0 (●) at 37°C.

10 000 rev min^{-1} for 20 min at 0°C, and the supernatant in divided fractions was stored at −25°C. The hydrolysis study was performed as described for plasma using stocks diluted to 0·1% (v/v) with 0·1 M phosphate buffer (pH 7·4). The average protein content of diluted liver homogenate supernatant (0·1% v/v) was 892·46 $\mu g\ mL^{-1}$. The hydrolysis of *O*-propionyl propranolol (II) was used as a control to ascertain the enzymatic validity of the homogenate preparation.

HPLC analysis. The mobile phase comprised 0·05 M KH_2PO_4 and acetonitrile varied from 50:50 to 30:70. The flow rate was at 1 mL min^{-1} with detection at 290 nm.

Measurement of partition coefficient

The partition coefficients (PC) of propranolol and the prodrugs were determined in 1-octanol/pH 4·0 phosphate buffer system as the prodrugs were stable at pH 4·0. The buffer solution and 1-octanol were mutually saturated at 25°C before use. The absorbance in pH 4·0 buffer was measured by UV spectrophotometry (UV-240, Shimadzu, Japan) at 290 nm before and after shaking with equal volumes of 1-octanol for 1 h. The partition coefficients were determined as the ratios between the absorbances in 1-octanol and pH 4·0 phosphate buffer.

Animals and administration

Four beagle dogs (10–12 kg, 2–4 years old) were used in cross-over bioavailability experiments with intervals of one week for washing out, and were maintained on a liquid diet (Besvion: Snow Brand Milk Product Co. Ltd, Japan) for two days followed by fasting for 24 h with free access to water

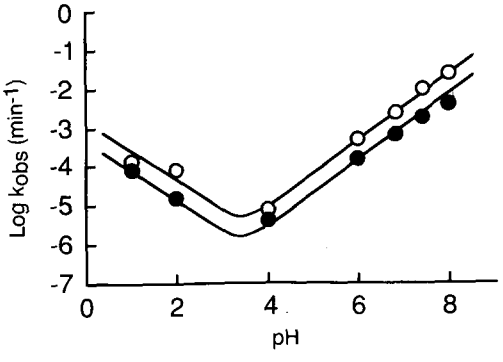


FIG. 2. The pH-rate profiles for the hydrolysis of prodrug I (○) and VI (●) in aqueous solution at 37°C.

before drug administration. Propranolol HCl (2 mg kg^{-1}) and equivalent doses of prodrugs I, III, VI and XII were administered orally after dissolution in 0·1 M phosphate buffer (pH 4·0) with 20% saccharose. The administration of samples (2–3 mL) was performed with 5 mL water. For intravenous bolus administration at the same dose, propranolol HCl was dissolved in isotonic phosphate buffer, pH 7·4, prepared using Water for Injection and the solution was filtered through a 0·45 μm membrane filter (Millipore, USA) before use.

Simultaneous determination of prodrug and propranolol in plasma

Blood samples of 3 mL were withdrawn at appropriate intervals and immediately centrifuged at 3500 rev min^{-1} for 5 min. Plasma, 0·5 mL, was mixed with 0·5 mL acetonitrile to precipitate protein and centrifuged at 3000 rev min^{-1} for 5 min. The clear solution of 0·5 mL was then filtered through a cotton plug and added to 2 mL 0·1 M phosphate buffer (pH 4·0) saturated with NaCl. In this procedure, prodrugs were found to degrade slightly in the plasma (< 2%). Propranolol and intact prodrug were simultaneously extracted with 6 mL ether after shaking for 20 min. Five millilitres of the organic phase was evaporated to dryness and dissolved in 100 μL of the mobile phase before injection of 20 μL into the HPLC. The mobile phase comprised 0·01 M KH_2PO_4–acetonitrile (2:3). The flow rate was at 1 mL min^{-1}. Fluorescence detection was performed with excitation at 285 nm and emission at 340 nm.

Determination of propranolol in plasma

Plasma samples (0·5 mL) obtained after oral or intravenous administration of propranolol, were added to 0·5 mL 1 M NaOH, extracted with 6 mL ether and analysed by HPLC as above.

Results and Discussion

Chemical hydrolysis

The hydrolysis of all prodrugs, I–XV was investigated in aqueous solution at 37°C over a wide pH range. At constant pH and temperature the disappearance of the prodrugs displayed strict first order kinetics for several half-lives as shown in Fig. 1. All prodrugs were mainly hydrolysed to propranolol in buffer (pH 1·2–8·0) although the propranolol prodrugs undergo hydrolysis and intramolecular aminolysis in alkaline solution (Buur et al 1988). The hydrolysis rate of prodrug was increased with increasing pH (Fig. 2). The shape of the pH-rate profile indicated proton and hydroxyl catalysed degradation of the protonated amine together with a hydroxyl catalysed degradation of the unprotonated form. The V-shaped pH-rate profile (slope close to unity) indicated that water-catalysed degradation does not significantly contribute to the overall reaction. The maximum stability of the prodrugs was at pH 4·0. These features of chemical kinetics are in agreement with those findings earlier reported for propranolol esters (Buur et al 1988) but are in contrast to those of timolol esters (Bundgaard et al 1988). The measured rate constants of hydrolysis of the prodrugs are listed in Table 2.

All the prodrugs were only slightly hydrolysed up to pH

Table 2. Rate data for the hydrolysis of propranolol prodrugs in buffers ($\mu = 0{\cdot}15$), plasma and liver homogenate (0·1%) at 37°C.

	Rate constant, k_{obs} ($\times 10^2$ min^{-1})				
Compound	pH 6·0	pH 6·8	pH 7·4	Plasma	Liver homogenate
I	0·047	0·232	1·00	3·97	3·70
II	0·044	0·229	0·95	2·20	20·50
III	0·038	0·137	0·53	1·82	26·10
IV	0·037	0·118	0·50	1·55	44·90
V	0·034	0·174	0·63	1·22	14·90
VI	0·015	0·065	0·18	0·94	6·20
VII	0·005	0·029	0·15	0·61	1·60
VIII	1·87	15·20	58·90	95·40	75·00
IX	0·512	3·465	10·40	17·00	32·40
X	0·079	0·479	1·49	5·60	16·90
XI	0·054	0·286	1·36	4·90	13·10
XII	0·045	0·039	0·12	0·67	2·00
XIII	0·081	0·425	1·79	7·71	61·60
XIV	0·029	0·168	0·66	1·82	27·60
XV	0·037	0·113	0·19	0·77	12·70

All values are average of two experiments.

6·0; above pH 6·8, hydrolysis was rapid. The branched chain alkyl prodrugs (V, VI) were comparatively more stable than the corresponding long-chain alkyl prodrugs (III, IV), while *tert*-butyryl propranolol (VII) was the most stable. Acyloxyalkyl prodrugs (VIII–XI) were the most susceptible to hydrolysis. The behaviour of the cycloalkyl esters was similar to that reported for the equivalent timolol esters (Bundgaard et al 1988). In both cases the cyclopropanoyl prodrug (XII) showed the highest stability, presumably because of the formation of a sterically hindered ester.

Enzymatic hydrolysis

The first-order degradation of the esters in plasma and liver homogenate is shown in Fig. 3. Absence of *N*-acyl propranolol in the chromatogram indicated the possibility of ester hydrolysis without rearrangement. Similar findings have been reported for some propranolol esters using rabbit serum (Irwin & Belaid 1988b). All prodrugs were more rapidly hydrolysed in the enzyme systems than in buffers, but followed the same order as that in buffers (Table 2).

The hydrolysis rate in liver homogenate was faster than in plasma, although the protein content in liver homogenate was only one-tenth that in plasma. Liver is assumed to be the most active site for prodrug degradation by nonspecific esterases and metabolic enzymes. With increasing alkyl chain (I, II, III and IV) the rate of hydrolysis increased, the reverse of that observed in plasma and buffers, suggesting that

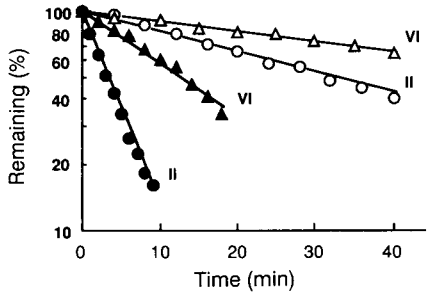


FIG. 3. First-order plots for hydrolysis of prodrugs II (circles) and VI (triangles) in plasma (open symbols) and liver homogenate (closed symbols).

hydrophobicity is important in the interaction of prodrug with enzymes and that there may be specific esterases or microsomal enzymes in liver. In the above kinetic studies, only liberated propranolol was monitored, although the presence of other degradation products through metabolism is possible.

These kinetic investigations suggested that most of the ester prodrugs are expected to be more or less stable in the gastrointestinal pH during absorption, but will be hydrolysed rapidly and spontaneously after absorption. In-vivo application of these prodrugs would therefore appear to be possible.

QSAR analysis

To explore the influence of different substituents on the hydrolysis of the prodrugs, the structure–activity relations were quantitatively interpreted after multiple regression analysis. Equations were constructed considering the observed rate constant in different media vs π, σ (Taft) and molar reflection (MR) for hydrophobic, electronic and steric effects, respectively. For pH 7·4 buffer one-variable equations with the different parameters were as follows:

$$\log k_{obs} = -0{\cdot}61\pi(\pm 0{\cdot}17) - 1{\cdot}30(\pm 0{\cdot}22) \quad (1)$$
$$n = 15 \quad s = 2{\cdot}94 \quad r = 0{\cdot}76 \quad F = 18{\cdot}21$$

$$\log k_{obs} = 1{\cdot}59\sigma(\pm 0{\cdot}13) - 2{\cdot}09(\pm 0{\cdot}09) \quad (2)$$
$$n = 15 \quad s = 1{\cdot}57 \quad r = 0{\cdot}88 \quad F = 45{\cdot}54$$

$$\log k_{obs} = 0{\cdot}003\ MR(\pm 0{\cdot}28) - 2{\cdot}11(\pm 0{\cdot}66) \quad (3)$$
$$n = 15 \quad s = 7{\cdot}06 \quad r = 0{\cdot}02 \quad F = 0{\cdot}08$$

These equations indicated that hydrophobic and electronic influences of the substituents are important for prodrug hydrolysis, while the steric parameter showed almost no influence. A two-variable equation with π and σ led to better correlation as shown below.

$$\log k_{obs} = -0{\cdot}09\pi(\pm 0{\cdot}23) + 1{\cdot}44\sigma(\pm 0{\cdot}23) - 1{\cdot}99(\pm 0{\cdot}25) \quad (4)$$
$$n = 15 \quad s = 1{\cdot}47 \quad r = 0{\cdot}89 \quad F = 22{\cdot}81$$

For hydrolysis in plasma the following equations were obtained:

$$\log k_{obs} = -0{\cdot}52\pi(\pm 0{\cdot}17) - 0{\cdot}90(\pm 0{\cdot}17) \quad (5)$$
$$n = 15 \quad s = 1{\cdot}92 \quad r = 0{\cdot}78 \quad F = 20{\cdot}57$$

$$\log k_{obs} = 1{\cdot}32\sigma(\pm 0{\cdot}14) - 1{\cdot}59(\pm 0{\cdot}08) \quad (6)$$
$$n = 15 \quad s = 1{\cdot}19 \quad r = 0{\cdot}87 \quad F = 41{\cdot}07$$

$$\log k_{obs} = 0{\cdot}003\ MR(\pm 0{\cdot}28) - 1{\cdot}61(\pm 0{\cdot}55) \quad (7)$$
$$n = 15 \quad s = 4{\cdot}95 \quad r = 0{\cdot}03 \quad F = 0{\cdot}013$$

$$\log k_{obs} = -0{\cdot}14\pi(\pm 0{\cdot}24) + 1{\cdot}06\sigma(\pm 0{\cdot}24) - 1{\cdot}41(\pm 0{\cdot}22) \quad (8)$$
$$n = 15 \quad s = 1{\cdot}12 \quad r = 0{\cdot}88 \quad F = 20{\cdot}53$$

These equations for various parameters were similar to those in buffer which indicated that the influences of hydrophobic and electronic effects were similar in enzyme-catalysed and uncatalysed hydrolysis of prodrugs. Construction of two-variable equations (eqns 4 and 8) were only significantly different when compared with equation 1 and equation 5 ($P < 0{\cdot}01$). Accordingly, the electronic parameter seems to be the main influence on the hydrolysis of prodrugs in buffer and plasma. Moreover, the positive regression coefficient with σ indicated electron withdrawal by the substituents has

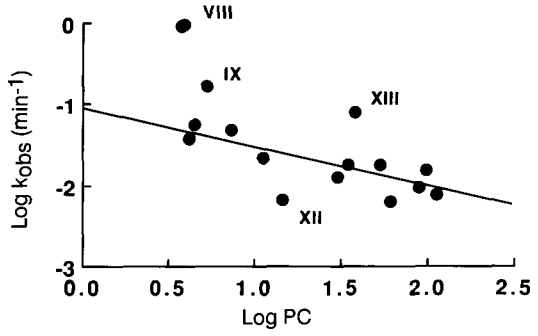


FIG. 4. Relation between the observed hydrolysis rate in plasma and partition coefficient of prodrugs.

facilitated hydrolysis. Although the prodrug hydrolysis was less influenced by the hydrophobic effect than by the electronic effect, the importance of hydrophobicity on interaction of drugs with proteins such as enzymes has been reported by many authors (Carotti et al 1984; Hansch & Klein 1986; Altomare et al 1988).

To establish the effect of hydrophobicity of the prodrug more precisely, partition coefficients were plotted against observed rate constants in plasma (Fig. 4). A relatively poor correlation was observed ($r = 0{\cdot}69$) when all the prodrugs were considered. However, a good correlation ($r = 0{\cdot}90$) was obtained by excluding only four prodrugs (VIII, IX, XII and XIII); VIII and IX are very unstable due to the low electric density around the carbonyl carbon, while the latter two prodrugs are very stable (XII) or unstable (XIII) due to steric factors.

A poor correlation was found between the parameters and the observed rate constant in liver homogenate (r for π, σ and MR were 0·30, 0·59 and 0·23, respectively). Including π and σ in the two-parameter equation slightly improved the correlation ($r = 0{\cdot}67$). The electronic effect was also assumed to be a major factor for hydrolysis in liver. Further, no correlation between rate constant in liver and partition coefficient was obtained in contrast to the finding in plasma. When particular ester types such as *n*-alkyl, branched alkyl, acyloxyalkyl or cycloalkyl were considered as a group and hydrolysis was compared with the different physicochemical parameters of the R substituent, good to moderate correlation was found. These limited data suggest that the degradation of the prodrugs in liver are complex and there may be specific esterases for different groups of prodrugs.

Bioavailability evaluation

The systemic bioavailability of propranolol was determined in beagle dogs following oral and intravenous administration. Fig. 5 represents the mean plasma concentration–time profile of propranolol after oral and intravenous administration of 2 mg kg^{-1} propranolol HCl solution. The systemic or absolute bioavailability (F) of propranolol was about 0·08. The value of F was earlier reported to vary from about 0·06 to 0·27 in beagle dogs in comparable studies (Garceau et al 1978; Tse et al 1980; Yoshimura et al 1985). The F value for propranolol after oral administration varied widely (0–0·28) depending on the dose (Pond & Tozer 1984).

The major objective of the present work was to identify an ester prodrug showing good bioavailability of the parent

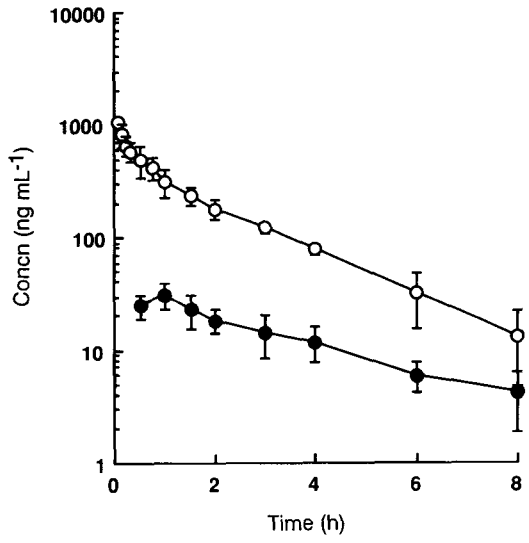


FIG. 5. Plasma levels of propranolol ($\pm$ s.d., $n = 4$) following oral (●) and intravenous (○) administration of 2 mg kg^{-1} propranolol HCl to beagle dogs.

drug following oral administration and also to determine physicochemical and kinetic parameters for optimization of oral delivery. We selected prodrugs I, III, VI and XII from those initially studied for in-vivo trials giving emphasis to different groups of prodrugs and in-vitro hydrolysis kinetics. The plasma concentration–time profiles are illustrated in Fig. 6 and the bioavailability parameters are listed in Table 3. A very rapid appearance of propranolol in the blood after prodrug administration indicated rapid absorption and hydrolysis of the prodrugs in-vivo, the peak plasma levels of propranolol being reached between 0–0·5 h. The overall time-courses from the prodrugs were similar in nature, providing higher propranolol levels and lower intact prodrug levels for a relatively short period. Prodrugs I and XII (Fig. 6a, d) showed similar plasma profiles with moderate increase of propranolol and intact prodrug. Comparatively high propranolol levels and low intact prodrug levels were recorded for less than 3 h from prodrugs III and VI (Fig. 6b, c). The larger AUC of propranolol and smaller AUC of intact prodrug as obtained from III and VI were probably due to lower stability in liver and higher stability in the gastrointestinal tract and portal system during absorption as indicated by their hydrolysis data (Table 2). The higher stability of prodrug XII in liver and other media caused less regeneration of propranolol and subsequently higher AUC of intact prodrug for XII (Table 3). The small propranolol AUC increase from prodrug I could be explained from the presystemic degradation as expected from its relative instability in buffers and plasma, releasing some propranolol before absorption.

The absorbed fraction (F) values for propranolol from prodrugs were calculated as follows from the appearance of propranolol in blood:

$$F = AUC_{p.o.(prodrug)}/AUC_{i.v.(propranolol)}$$

The F values indicated that a 2- to 4-fold increase in bioavailability was achieved through the use of these prodrugs. The mechanism of these improvements may be due to

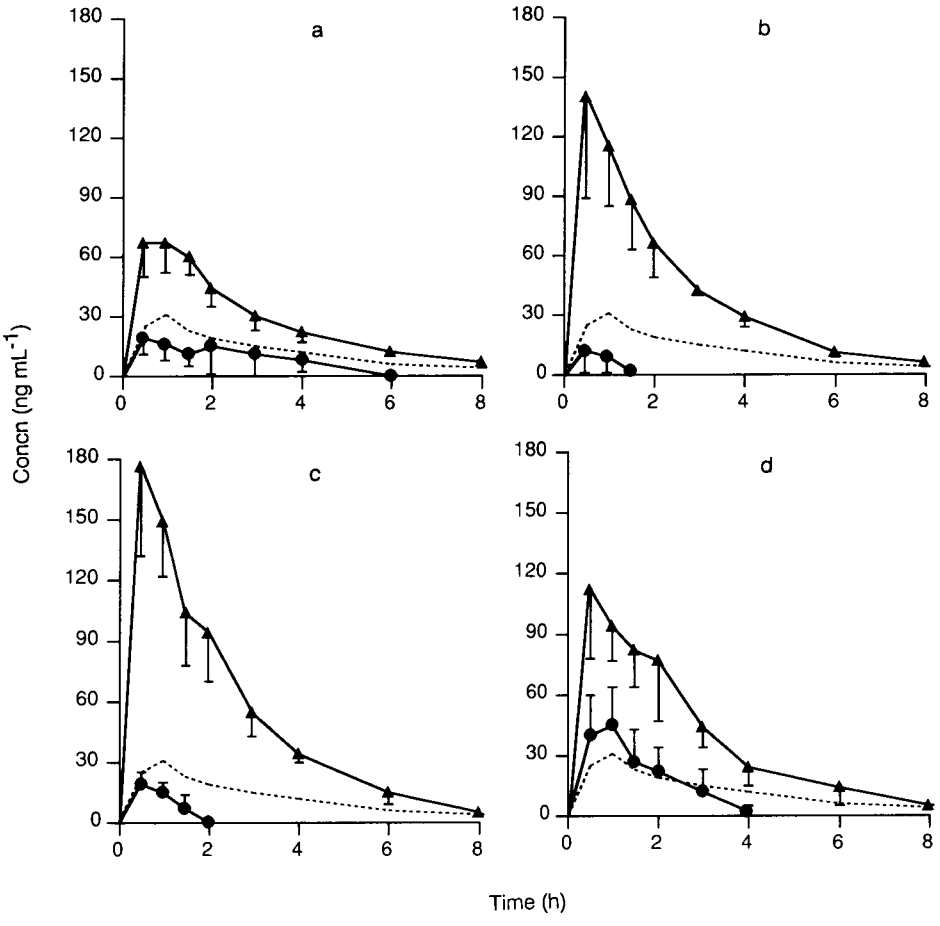


FIG 6. Mean plasma levels (±s.d., n=4) of propranolol and intact prodrug following oral administration (2 mg kg^{-1} propranolol HCl equivalents) of prodrugs to beagle dogs. a, prodrug I; b, prodrug III; c, prodrug VI; d, prodrug XII; ▲, propranolol liberated from prodrug; ●, intact prodrug; ---, average propranolol concentration obtained after 2 mg kg^{-1} propranolol HCl from Fig. 5.

decreased metabolic clearance of propranolol by blocking glucuronidation, and increased absorption rate of the prodrugs facilitated by their improved partition coefficient over propranolol. Fig. 7 shows good correlation (r=0·97) between the logarithm of partition coefficient and F values. However, the AUC values of prodrugs in Table 3 did not increase in the same order with their corresponding partition coefficients, probably due to the different degradation kinetics of the prodrugs influencing absorption. From the limited data points, there was an optimal hydrolysis rate of 0·10–0·15 min^{-1}. These data suggest that increase of lipophilicity in the prodrugs would be probably limited to provide better bioavailability of propranolol, but at the same time optimum hydrolysis in the liver is needed. Optimization of these two parameters would lead to better prodrugs.

The improvement in relative bioavailability using the hemisuccinyl prodrug was about 8- and 2·5-fold in dogs

Table 3. Bioavailability of propranolol and various ester prodrugs following oral administration to beagle dogs (±s.d., n=4).

Compound	AUC_{0-8}^{PL} (ng mL^{-1} h)	AUC_{0-8}^{PD} (ng mL^{-1} h)	F
Propranolol	100·73±20·73		0·08±0·01
I	219·21±35·21[a]	48·16±15·62	0·19±0·04[a]
III	332·49±70·45[a,c]	10·40±6·22[c,d]	0·28±0·05[a]
VI	412·74±60·35[a,c]	17·83±6·65[c,d]	0·36±0·04[a]
XII	283·71±95·44[b]	84·45±35·89	0·24±0·07[b]

AUC_{0-8}^{PL} (i.v.): 1178·27±185·89. [a]$P<0·01$ vs propranolol, [b]$P<0·05$ vs propranolol, [c]$P<0·05$ vs I, [d]$P<0·05$ vs XII.

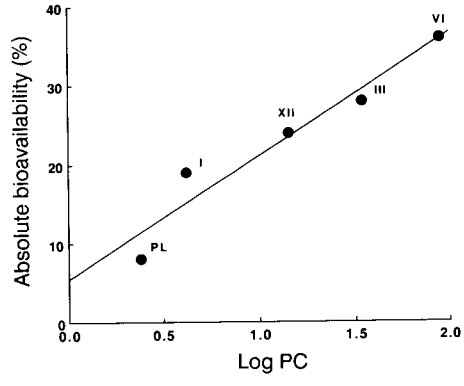


FIG. 7. Influence of lipophilicity of prodrugs on absolute bioavailability of propranolol.

(Garceau et al 1978) and in rats (Anderson et al 1988), respectively; the same result was also obtained for *O*-acetylpropranolol in rats. We found only a 2- to 4-fold increase in the AUC of propranolol from the prodrugs. These features suggest some species difference in the prodrug interaction which may be due to hepatic polymorphism or different protein binding. Our evaluation was at a lower dose (2 mg kg^{-1}) which is possibly in the dose range where nonlinear kinetics apply. The 40% bioavailability achieved maximally in this study indicated that a large amount of the propranolol must be normally subjected to presystemic loss due to decomposition of prodrugs by routes other than simply hydrolysis to propranolol or insufficient protection against *O*-glucuronidation; further evaluation monitoring the plasma level of 4-hydroxypropranolol and *O*-glucuronide is required.

It is concluded that prodrugs having higher chemical and enzymatic stability in the gastrointestinal tract and in blood but susceptible to hydrolysis in the liver are the most promising prodrugs of propranolol to avoid the first-pass metabolism which follows oral administration. The desirable properties for this kind of prodrug are suggested to be increased hydrophobicity and an optimum hydrolysis rate in the liver. *O*-Isovaleryl-propranolol (VI) was considered to be the most promising compound to ensure oral delivery of propranolol.

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