International Journal of Pharmaceutics, 33 (1986) 15–26
Elsevier

IJP 01095

Prodrugs of timolol for improved ocular delivery: synthesis, hydrolysis kinetics and lipophilicity of various timolol esters

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(Received 19 February 1986)
(Accepted 30 April 1986)

Key words: Timolol – Prodrug – Timolol ester – Partition coefficient – Chemical hydrolysis kinetics – Ocular hydrolysis

Summary

The O-acetyl, propionyl, butyryl and pivaloyl esters of timolol were synthesized and evaluated as prodrugs for potential in diminishing the systemic absorption and therefore side effects of topically applied timolol through increased corneal permeation. The esters showed a 14–525-fold increase in lipophilicity relative to timolol as determined by partition experiments in octanol–pH 7.4 buffer. They all were hydrolyzed to yield timolol in quantitative amounts in buffer solutions, human plasma and homogenates of the conjunctiva, corneal epithelium and iris–ciliary body of the pigmented rabbit. At pH 7.4 and 37°C, the half-lives of chemical hydrolysis were 28, 40, 50 and 215 min for the O-acetyl, propionyl, butyryl and pivaloyl esters, respectively. Human plasma did not catalyze the hydrolysis but showed instead a rate-decelerating effect. Such an effect was also observed with tromethamine (Tris) at neutral pH. However, the esters were 1.5–2 times more susceptible to hydrolysis in ocular tissue homogenates than in buffer. Hydrolysis was most rapid for the butyryl ester, followed, in turn, by the propionyl, acetyl and pivaloyl esters. For a given prodrug, hydrolysis proceeded most readily in the iris–ciliary body, followed by conjunctiva and corneal epithelium.

Introduction

Timolol [(S)-(+)-1-[(1,1-dimethyl)ethylamino]-3-[[4-(4-morpholino)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol](I) is a non-selective β-adrenergic receptor blocker widely used in the treatment of glaucoma. Its therapeutic usefulness is, however, potentially limited by a relatively high incidence of cardiovascular and respiratory side effects (for a review, see Munroe et al., 1985). These effects arise as a result of absorption of the topically applied drug into the systemic circulation (Alvan et al., 1980; Schmitt et al., 1980) and are essentially the same as those seen with oral timolol (Zimmerman et al., 1983).

A potentially useful approach to decrease the systemic absorption of topically applied timolol thereby diminishing its adverse effects may be the development of transient derivatives (prodrugs) with improved corneal absorption characteristics due to greater lipophilicity. This approach has already been applied to improve the ocular bioavailability of epinephrine (McClure, 1975; Hussain and Truelove, 1976; Bodor and Visor, 1984), nadolol (Duzman et al., 1982), various prostaglandins (Bito, 1984) and pilocarpine (Bundgaard et

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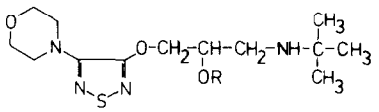
al., 1985). In particular, the more rapid and efficient corneal penetration of dipivefrin, a prodrug of epinephrine, permits the use of smaller doses than epinephrine resulting in a lower incidence of side effects (McClure, 1975).

The purpose of this study was to identify timolol prodrugs for improved ocular delivery. To this end, 4 aliphatic esters of timolol (II–V) were synthesized and evaluated with respect to lipophilicity as well as hydrolysis kinetics in buffers, plasma solutions and ocular tissue homogenates of the pigmented rabbit. The corneal transport and systemic absorption characteristics of these esters will be reported in a future communication.



I R = H

II R = -C-CH$_3$ (O)

III R = -C-CH$_2$-CH$_3$ (O)

IV R = -C-CH$_2$CH$_2$CH$_3$ (O)

V R = -C-C(CH$_3$)$_3$ (O)

Materials and Methods

Methods

^{1}H-NMR spectra were run on a Varian 360L instrument using tetramethylsilane as internal reference. Melting points were taken on a capillary melting-point apparatus and are uncorrected. Microanalyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark. High-performance liquid chromatography (HPLC) was performed with equipments mentioned below.

Chemicals

Timolol maleate was kindly provided by Leo Pharmaceutical Products, Denmark. Buffer substances and all other chemicals or solvents used were of reagent grade.

Preparation of timolol esters

Timolol maleate was converted to the hydrochloride salt as follows. Timolol maleate (6 g) was dissolved in water (100 ml). Sodium hydroxide (2 M) was added to give a pH of 10 and the mixture was extracted with ether (2 × 75 ml). The extracts were dried over anhydrous sodium sulphate and a slight excess of 3 M HCl in methanol was added. Petroleum ether was added and after standing overnight at 4°C the white crystalline precipitate formed was filtered off and recrystallized from acetone–ether to give 4.1 g (84%) of timolol hydrochloride, m.p. 130–131°C.

The timolol esters II–IV were prepared by reacting timolol hydrochloride with the corresponding acid chloride. Timolol hydrochloride (3 mmol, 1.05 g) was slurried in 12 ml of benzene. The appropriate acid chloride (10 mmol) was added and the mixture refluxed with stirring for 2 h. After cooling, the mixture was evaporated in vacuo. Benzene (10 ml) was added and the mixture evaporated again to remove traces of the acid chloride. The solid residue was slurried in ether, filtered off and washed with ether. Recrystallization of the hydrochloride salts of the timolol esters was performed using the solvents indicated in Table 1. Physical and analytical data for the compounds are also given in Table 1. The NMR and IR spectra of the compounds were consistent with their structures. Crowther and Smith (1968) have previously prepared the acetyl ester of propranolol hydrochloride under similar conditions.

Hydrolysis kinetics in aqueous solution

The hydrolysis of the timolol esters II–V was studied in aqueous buffer solutions at 37.0 ± 0.2°C. Hydrochloric acid, acetate, phosphate, borate, carbonate and sodium hydroxide were used as buffers; a constant ionic strength (μ) of 0.5 was generally maintained for each buffer by adding a calculated amount of potassium chloride.

The rates of hydrolysis were determined by using a reversed-phase HPLC procedure capable of separating the esters from timolol. The HPLC system consisted of a Kontron pump Model T-414, a fixed wavelength UV-detector (Type Kontrol Uvikon 740 LC) and a 20-μl loop injection valve. The column used, 100 × 3.0 mm, was packed with

TABLE 1

PHYSICAL AND ANALYTICAL DATA OF VARIOUS ESTERS (HYDROCHLORIDE SALTS) OF TIMOLOL

Compound	Yield (%)	Melting point (°C)	Recrystallization solvent [a]	Formula	Analysis (%)	
					Calculated	Found
II	92	203–204	A	$C_{15}H_{27}ClN_4O_4S$	C 46.62	45.64
					H 6.89	6.96
					N 14.19	14.18
					Cl 8.98	9.05
					S 8.12	8.02
III	90	187–188	A	$C_{16}H_{29}ClN_4O_4S$	C 46.99	47.04
					H 7.15	7.18
					N 13.70	13.68
IV	88	158–159	A	$C_{17}H_{31}ClN_4O_4S$	C 48.27	48.19
					H 7.39	7.40
					N 13.25	13.21
V	65	146–147	B	$C_{18}H_{33}ClN_4O_4S$	C 49.47	49.43
					H 7.61	7.65
					N 12.82	12.79

[a] Solvent of recrystallization: A, ethanol-ether; B, benzene-ethanol-petroleum ether.

CP SPHER C-8 (8-μm particles). The mobile phase was either 50% v/v (II and III), 60% v/v (IV) or 70% v/v (V) methanol in 0.02 M potassium dihydrogen phosphate (pH 4.5) at a flow rate of 1.0 ml/min. The column effluent was monitored at 280 nm. The retention times for the timolol esters under these conditions were as follows: II, 2.3 min; III, 3.8 min; IV, 4.2 min; V, 2.2 min. Timolol (I) had retention times of 1.8, 1.3 and 0.8 min at a methanol concentration of 50, 60 and 70% v/v, respectively. Quantitation of the esters as well as of timolol formed upon hydrolysis was done by measuring peak heights in relation to those of standards chromatographed under the same conditions.

The reactions were initiated by adding 20 μl of a stock solution of an ester in water to 10.0 ml of buffer solution, pre-heated at 37°C, in screw-capped test tubes, the final ester concentration in the reaction mixture being 0.08–0.1 mM. The solutions were kept in a water-bath at 37.0 ± 0.2°C. At appropriate times samples were taken and immediately chromatographed. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual timolol ester against time.

Hydrolysis in human plasma solutions

Hydrolysis of the timolol esters was studied in 0.01 M phosphate buffer (pH 7.4) containing 80% human plasma at 37°C. Initial concentrations of the compounds were 0.2–0.3 mM. At appropriate times, samples of 250 μl were withdrawn and deproteinized by mixing with 1000 μl of ethanol. After centrifugation for 2 min, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Hydrolysis in ocular tissue homogenates

Ten male, Dutch-belted pigmented rabbits (ABC Rabbitry, Pomona, CA) were killed by an overdose of intravenous pentobarbital solution (Eutha-5, Western Medical Supply, Arcadia, CA). The corneal epithelium, whole conjunctiva and iris–cilary body were excised sequentially from each eye. The specimens were stored frozen at −70°C and used within one month of collection.

On the day of an experiment, homogenates of a given tissue were prepared using a Potter-Elvejhem tissue homogenizer followed by centrifugation at 3020 × g in a Sorvall RC-5B refrigerated superspeed centrifuge (Dupont Instruments, Newton, CT) at 4°C for 10 min. The supernatant was

adjusted to a protein concentration of 1.18 mg/ml by adding 1.17% KCl prior to incubation with a freshly prepared timolol ester solution.

The incubation mixture consisted of 50 μl each of a tissue supernatant and of a 0.2 mM timolol ester solution in 10 mM Tris at pH 7.4. At selected times up to 360 min, the reaction was stopped by precipitating the proteins with 150 μl of acidified acetonitrile. After adding 50 μl of 25 μl/ml propranolol, the internal standard, the mixture was centrifuged at 4°C and 5–40 μl of the supernatant was injected, in duplicate, into the liquid chromatograph.

The HPLC system consisted of 2 Altex model 110A HPLC pumps, a Rheodyne model 7125 sample injector with a 100-μl loop, an Axiom model 710 HPLC controller, and an Altex Ultrasphere reverse-phase ODS C-18 column (4.6 × 250 mm, 5-μm particles). The mobile phase consisted of 34 parts of acetonitrile and 66 parts of water containing 1% triethylammonium hydrochloride (pH 3). The flow rate was 1 ml/min. Timolol and its esters were monitored at 294 nm using a Kratos 773 spectrophotometric detector. The retention times for timolol and its acetyl, propionyl, butyryl and pivaloyl esters were 3.8, 5.3, 7.3, 10.8 and 16.0 min, respectively, whereas the retention time for the internal standard was 7.9 min. The intra- and inter-run variations were less than 5 and 7.5%, respectively.

Determination of partition coefficients

The apparent partition coefficients (P) of timolol and its esters were determined in the system octanol –0.05 M phosphate buffer (pH 7.40) at 22°C. These two phases were mutually saturated at 22°C before use. The compounds were dissolved in the aqueous buffer phase and the octanol–buffer mixtures were shaken for 5 min to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution, could be measured readily using the HPLC method described under hydrolysis kinetics. At distribution equilibrium the two phases were separated by centrifugation for 2 min. During the entire procedure less than 2% timolol was formed from the esters as determined by HPLC. The partition coefficients were calculated from Eqn. 1:

$$P = \frac{C_i - C_w}{C_w} \times \frac{V_w}{V_o} \quad (1)$$

where C_i and C_w represent the solute concentrations in the aqueous phase before and after distribution, respectively; V_w represents the volume of the aqueous and V_o the volume of the octanol phase.

Results and Discussion

Conversion of timolol esters to timolol

Within the pH range investigated (pH 0.4–12.5), all 4 esters were found to be hydrolyzed quantitatively (100 ± 3%) to timolol. As evidenced by HPLC the disappearance of ester was accompanied by the progressive appearance of free timolol (Fig. 1). No other peaks in the chromatograms than that corresponding to timolol appeared during the degradation of the esters. Along with the quantitative formation of timolol this shows that a possible intramolecular aminolysis of the esters to give the corresponding N-acyl timolol derivatives (Scheme 1) does not take place to any significant extent. Intramolecular O → N acyl transfer reactions have previously been observed in esters of

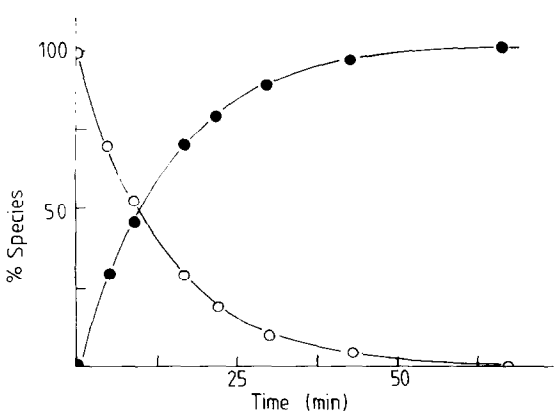
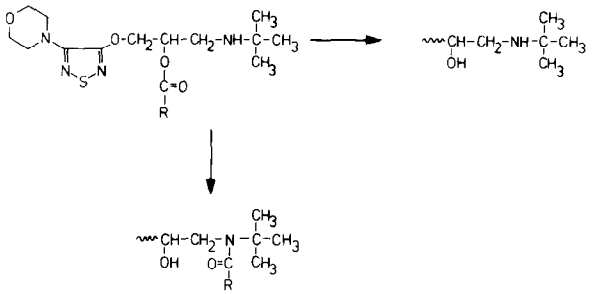


Fig. 1. Time-courses of disappearance of O-acetyltimolol (○) and appearance of timolol (●) in a 0.05 M borate buffer solution (pH 8.25) of O-acetyltimolol at 37°C.



Scheme 1

various β-aminoalcohols, including O-acetylethanolamine (Martin and Parcell, 1961; Hansen, 1963, Martin et al., 1964; Schmir, 1968), O-acetylserine (Caswell et al., 1981), O-acetylephedrine (Welsch, 1947; Fodor et al., 1949) and O-nicotinoylethanolamine (Nagai et al., 1984). In fact, for these compounds, intramolecular aminolysis predominates over hydrolysis in neutral and alkaline solutions. The inability of the timolol esters to undergo intramolecular aminolysis may most likely be ascribed to steric hindrance exhibited by the bulky tertiary butylamino group. The quantitative conversion of the timolol esters II–V to timolol was also observed in 80% human plasma and ocular tissue homogenates of the pigmented rabbit.

Kinetics and mechanism of chemical hydrolysis of timolol esters (II–V)

At constant pH and temperature strict first-order kinetics was observed for the hydrolysis of all 4 timolol esters for several half-lives. Typical first-order plots are shown in Fig. 2. In all cases the pseudo-first-rate constants (k_{obs}) determined on the basis of measuring the remaining ester agreed within 4% of the rate constants determined from measurement of timolol formed.

At buffer concentrations less than 0.05 M no significant catalysis by the buffer substances used to maintain constant pH was observed (Table 2). As described below Tris (tris(hydroxymethyl)aminomethane) buffers showed a remarkable inhibiting effect.

The influence of pH on the rates of hydrolysis at 37°C is shown in Fig. 3 in which the logarithm

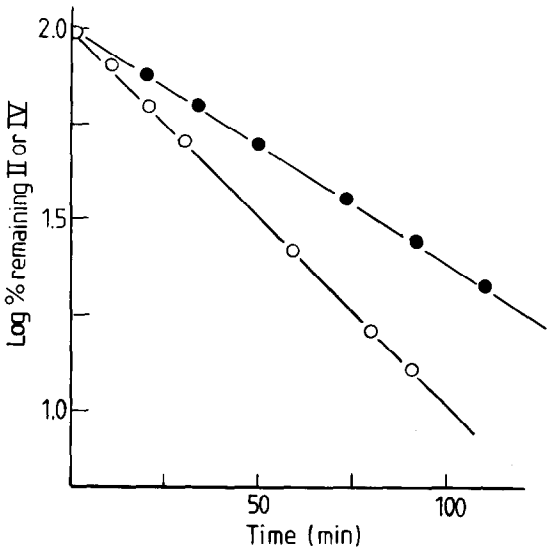


Fig. 2. First-order plots for the hydrolysis of O-acetyltimolol (○) and O-butyryltimolol (●) in 0.02 M phosphate buffer solution of pH 7.40 at 37°C.

of k_{obs} has been plotted against pH. The shape of the pH-rate profiles indicates that: (i) the free base and the protonated forms of the esters undergo hydrolysis at different rates; and (ii) the hydrolysis can be described in terms of specific base-catalyzed reactions involving both species as well as a specific acid-catalyzed and a spontaneous reaction involving the protonated ester

TABLE 2

EFFECT OF BUFFER CONCENTRATION ON THE RATE OF HYDROLYSIS OF O-ACETYLTIMOLOL (II) AT 37°C ($\mu = 0.5$)

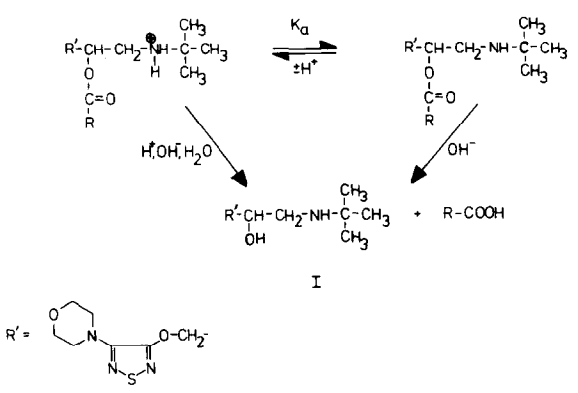
Buffer			k_{obs} (min^{-1})
Acetate	pH 4.00	0.02 M	5.3×10^{-5}
		0.05 M	5.2×10^{-5}
Phosphate	pH 6.01	0.02 M	1.2×10^{-3}
		0.05 M	1.1×10^{-3}
Phosphate	pH 7.50	0.02 M	3.7×10^{-2}
		0.05 M	3.4×10^{-2}
Borate	pH 8.35	0.02 M	0.105
		0.05 M	0.116
Borate	pH 9.40	0.02 M	0.315
		0.05 M	0.309

(Scheme 2). Mathematically,

$$k_{obs} = k_H a_H \frac{a_H}{a_H + K_a} + k_o \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (2)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activities, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total ester in the protonated and free base forms, respectively, and K_a is the apparent ionization constant of the protonated NH-group in the esters. The rate constant k_o refers to the spontaneous or water-catalyzed hydrolysis of the protonated form of the ester, k_H is the specific acid-catalyzed rate constant for protonated ester, and k_{OH} and k'_{OH} are the second-order rate constants for the apparent attack of hydroxide ion on the protonated and unprotonated ester species, respectively. Due to rapid rate of hydrolysis at pH > 10 a value of k'_{OH} was determined only for the O-pivaloyl ester (V).

The various rate and ionization constants derived from the pH–rate profiles are listed in Table 3. Using these constants, the solid curves in Fig. 3 were constructed. The good agreement between calculated and experimental data demonstrates that Eqn. 2 and, accordingly Scheme 2, adequately describe the hydrolytic mechanism. As shown in



Scheme 2

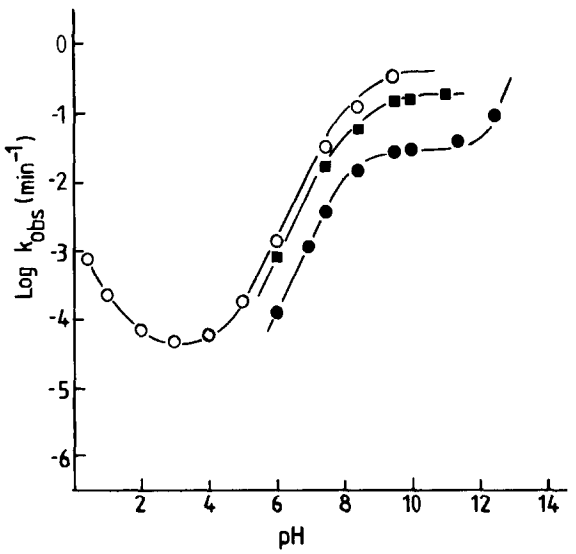


Fig. 3. The pH-rate profiles for the degradation of various timolol esters in aqueous solution at 37°C. Key: ○, O-acetyltimolol (II); ■, O-butyryltimolol (IV); ●, O-pivaloyltimolol (V). The profile for O-propionyl ester is near to the profiles for O-acetyl and O-butyryl esters and is omitted for clarity.

Table 3, the kinetically derived pK_a values agreed satisfactorily with those determined by titrimetry. The esters are less basic than timolol, which has a pK_a of 9.21 at 35°C (Schoenwald and Huang, 1983), due to the electron-withdrawing effect of the ester moiety. Timolol undergoes protonation at the side-chain NH nitrogen only (Nieminen et al., 1984).

Based on the values of k_{OH} and k'_{OH} for compound V and on the shape of the pH-rate profiles, it appears that the ester with a protonated amino group is almost 10^4 times more susceptible to hydrolysis than the free base form. This increased reactivity of the protonated ester, which also has been observed previously for other esters of β-aminoalcohols (Zaslowsky and Fisher, 1963; Hansen, 1963; Chu and Mautner, 1966; Bruice and Mautner, 1973), may be attributed to either intramolecular general acid-catalyzed hydroxide ion attack (mechanism a in Scheme 3) or intramolecular general base catalysis by the unprotonated amino group of water attack on the ester group (mechanism b in Scheme 3). These mechanisms are kinetically equivalent and cannot be dis-

TABLE 3

IONIZATION CONSTANTS AND RATE DATA FOR THE HYDROLYSIS OF VARIOUS TIMOLOL ESTERS ($\mu = 0.5$; 37°C)

Compound	k_H (M^{-1} min^{-1})	k_o (min^{-1})	k_{OH} (M^{-1} min^{-1})	k'_{OH} (M^{-1} min^{-1})	pK_a [a]
II	1.8×10^{-3}	3.5×10^{-5}	5.2×10^{4}		8.4
III			3.6×10^{4}		8.4
IV			3.0×10^{4}		8.4
V			4.7×10^{3}	1.3	8.4 (8.4) [b]

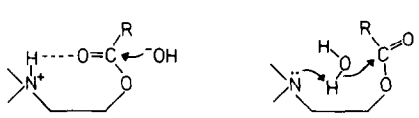
[a] Kinetically determined values.

[b] Determined by titration at 37°C ($\mu = 0.5$).

tinguished from one another using the present kinetic data. On the other hand, the great reactivity of the timolol esters in neutral and weakly alkaline solutions cannot be ascribed to intramolecular nucleophilic attack by the unprotonated amino group on the ester moiety, since such a reaction should result in the formation of stable N-acylated timolol derivatives. As noted earlier such compounds are not formed.

The effect of ionic strength (μ) on the hydrolysis rate was examined with the acetyl ester (II) in 0.02 M phosphate buffer solutions of pH 7.45. The stability increased with increasing ionic strength as revealed from the following observed half-lives: $\mu = 0.04$, $t_{1/2} = 17$ min; $\mu = 0.5$, $t_{1/2} = 28$ min; $\mu = 1.0$, $t_{1/2} = 40$ min. This finding is consistent with reactions involving ions of opposite charges and thus is indicative of mechanism a in Scheme 3.

The reactivity of the esters is a function of steric and polar factors. The polar effects of the acyl groups in compounds II–V are almost identical and the observed differences in reactivity in neutral and alkaline solution can solely be ascribed to differences in the steric properties as shown in Fig. 4, where the logarithm of the half-lives at pH 7.40 is plotted against the steric substituent parameter ν (Charton, 1977).



a b

Scheme 3

Rate retarding effect of Tris

As described above phosphate and the other buffer substances used did not influence the rate of hydrolysis of the timolol esters at the concentrations studied (0.01–0.05 M). It was therefore surprising to find that in Tris buffers (pH 6–7.5) the rate of hydrolysis was depressed, even at very small Tris concentrations ($< 10^{-4}$ M). Table 4 shows that 0.005 M Tris at pH 7.4 increased the half-lives of the timolol esters by a factor of 1.5–2. As shown in Fig. 5 the observed pseudo-first-order rate constants for the hydrolysis of O-acetyltimolol (II) in 0.02 M phosphate buffer of pH 7.40 at 37°C is not a linear function of Tris concentration. Rather it asymptotically approaches a minimum value at high Tris con-

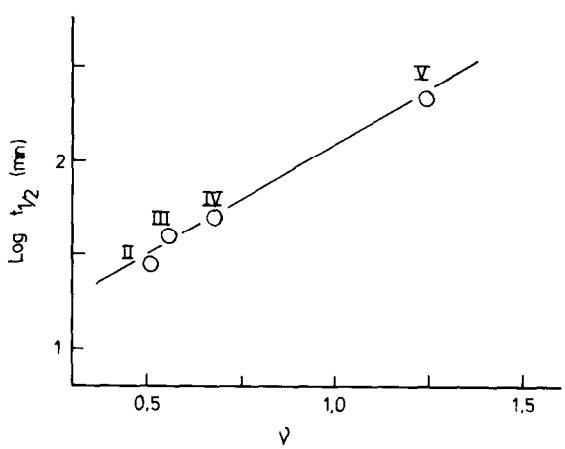


Fig. 4. Plot of log $t_{1/2}$ (at pH 7.40 and 37°C) vs the steric parameter (ν) for various timolol esters. The ν values refer to the alkyl moiety in the acyl groups.

TABLE 4

EFFECT OF 0.005 M TRIS AND 80% HUMAN PLASMA ON THE RATE OF HYDROLYSIS OF TIMOLOL ESTERS (0.1 mM) IN 0.02 M PHOSPHATE BUFFER SOLUTIONS (pH 7.4) AT 37°C.

Compound	Half-life (min)		
	Buffer	(+) Tris	(+) Plasma
II	28	71	35
III	40	53	45
IV	50	83	106
V	215	310	525

centrations. This saturation behaviour suggests that the decelerating rate effect of Tris is due to complex formation. Fitting the data of Fig. 5 to the model shown in Scheme 4 reveals that the complexation constant (K) was 4.5×10^4 M^{-1} and that the hydrolytic rate constant of the complexed form of O-acetyltimolol was 1.9-times smaller relative to the uncomplexed form.

$$\begin{array}{ccc} \text{Timolol ester} + \text{Tris} & \overset{K}{\rightleftharpoons} & \text{Timolol ester} - \text{Tris} \\ \downarrow k & & \downarrow k' \\ \text{Timolol} & & \text{Timolol} \end{array}$$

Scheme 4

The rate-decelerating effect of Tris decreased with increasing pH, indicating that the protonated rather than the unprotonated Tris species participated in complexation (pK_a of Tris is 8.05 at 37°C and $\mu = 0.5$).

At a total Tris concentration of 10^{-3} M, the rate-decelerating effect amounted to a factor of 2.0 at pH 6.5, 1.9 at pH 6.9, 1.8 at pH 7.4, 1.3 at pH 8.4 and 1.2 at pH 9.1. The rate-decelerating effect is seen to decrease with increasing pH, i.e. with decreasing proportion of the protonated Tris form. The stabilizing effect of Tris was observed for all esters studied. Rate data obtained at pH 7.40 (37°C) in the presence of 0.005 M Tris are listed in Table 4.

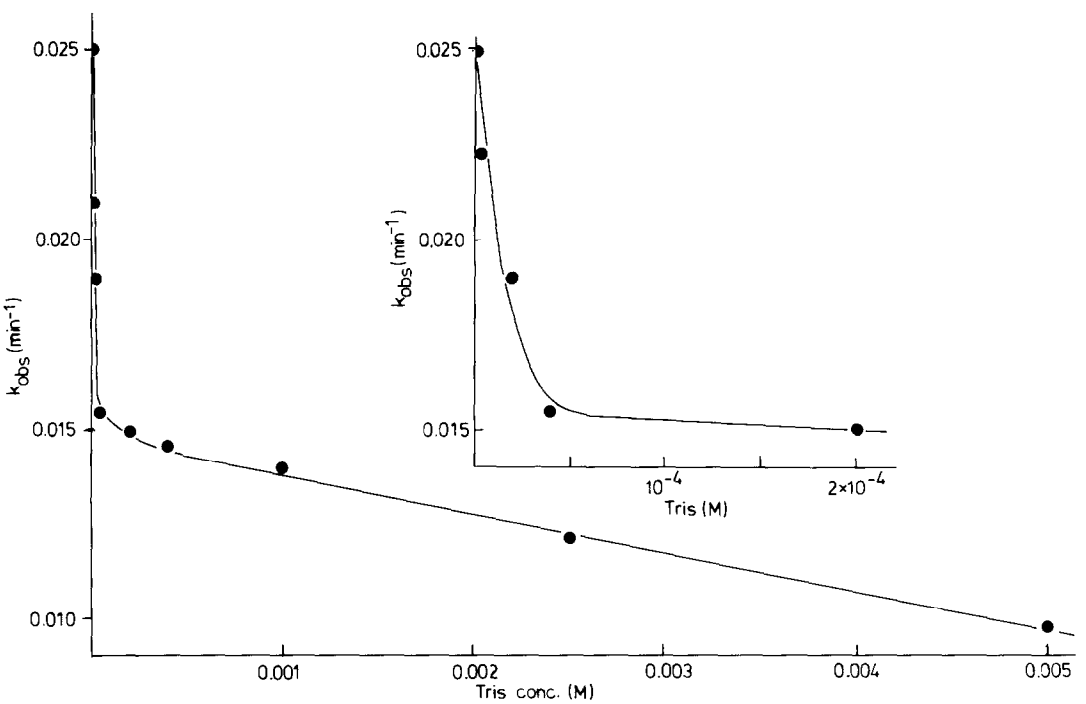


Fig. 5. Plot showing the influence of Tris concentration on the rate of hydrolysis of O-acetyltimolol in 0.02 M phosphate buffer of pH 7.40 ($\mu = 0.5$; 37°C). The inset is an enlargement of the data at low Tris concentrations.

The stabilizing effect of Tris appears solely to be confined to hydroxide ion-catalyzed hydrolysis of the timolol esters. Thus, the pseudo-first-order rate constant for the hydrolysis of O-acetyltimolol in 0.5 M hydrochloric acid containing 10^{-3} M Tris was found to be 7.6×10^{-4} min^{-1} at 37°C whereas the rate constant in 0.5 M hydrochloric acid without Tris was 8.0×10^{-4} min^{-1}.

To learn more about the specificity of the effect of Tris, the rate of hydrolysis of O-acetyltimolol was determined in the presence of a number of substances structurally related to Tris, i.e. ethanolamine, diethanolamine, triethanolamine, 2-amino-1-propanol and glycerol. The reaction solutions consisted of 0.05 M phosphate buffer of pH 7.40 ($\mu = 0.5$) containing each of these substances at a concentration of 10^{-3} M. In all cases the observed half-lives of ester hydrolysis (28–31 min) were similar to the half-life (28 min) in the phosphate buffer alone. It was also found that the addition of disodium edetate (10^{-4} M) to the phosphate buffer of the combined phosphate-Tris solutions had no effect upon the hydrolysis rate, thus eliminating the possibility of an influence from trace metals.

Concerning the site of the postulated complexation between timolol esters and protonated Tris, the morpholino-thiadiazolyl moiety of the timolol molecule is most likely involved. This is in agreement with a lack of effect of Tris on the rate of hydrolysis of esters of various other β-blockers including propranolol (unpublished findings).

Hydrolysis in plasma

As shown in Table 4, the rate of ester hydrolysis was reduced in 80% human plasma. This rate-retarding effect may be due to binding of the esters to plasma proteins in much the same way as observed for Tris, the bound ester being protected against enzymatic attack. Nevertheless, this lack of ester hydrolysis in plasma observed in vitro may not hold in vivo. Indeed, bopindolol, the benzoyl ester of the β-blocker 4-(2-hydroxy-3-tert-butylaminopropoxy)-2-methylindole, which contains an amino side chain similar to that in timolol, has been reported to be rather stable in human plasma in vitro although it is rapidly hydrolyzed in vivo (Oddie et al., 1983).

Interestingly, the rate-decelerating effect of human plasma on timolol ester hydrolysis appears to occur only at high protein concentrations. At very low protein concentrations (0.2–2.5 mg/ml), the rate of ester hydrolysis increased linearly with protein concentration. This is illustrated in Fig. 6.

Ocular hydrolysis of timolol esters

Table 5 shows the first-order rate constants for hydrolysis of timolol esters in homogenates of the conjunctiva, corneal epithelium and iris–ciliary body of the pigmented rabbit at a protein concentration of 1.18 mg/ml. A significant fraction of hydrolysis was due to chemical hydrolysis, especially so for O-acetyltimolol. Similar to 1- and 2-naphthyl esters (Lee, 1983), all 4 timolol esters were most readily hydrolyzed in homogenates of the iris–ciliary body, followed by conjunctiva and then corneal epithelium. This finding indicates that timolol prodrugs will be hydrolyzed to regenerate timolol both during absorption across the corneal epithelium and upon reaching the iris–ciliary body, where timolol acts. It also indicates that these prodrugs will be hydrolyzed to varying extents during absorption into the systemic circulation via the blood vessels in the conjunctiva.

In the iris–ciliary body and, to a lesser extent,

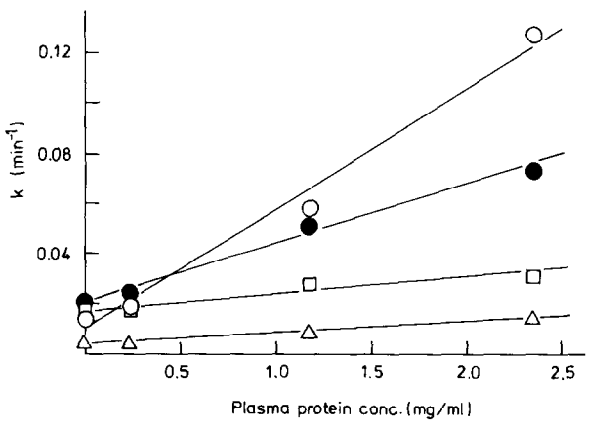


Fig. 6. Influence of human plasma protein concentration on the pseudo-first-order hydrolytic rate constants of various timolol esters at pH 7.40 and 37°C. Key: □, O-acetyltimolol; ●, O-propionyltimolol; ○, O-butyryltimolol; △, O-pivaloyltimolol.

TABLE 5

FIRST-ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF 0.02 mM TIMOLOL ESTERS IN 1.17% KCl AND IN HOMOGENATES OF CONJUNCTIVA, CORNEAL EPITHELIUM, AND IRIS-CILIARY BODY OF THE PIGMENTED RABBIT AT 37°C [a]

Compound	First-order rate constant (10^2/min^{-1}) [b]			
	1.17% KCl	Conjunctiva	Corneal epithelium	Iris-ciliary body
II	1.8 ±0.03	2.2 ±0.09 (18%)	2.1 ±0.09 (14%)	2.4 ±0.1 (25%)
III	2.1 ±0.2	3.1 ±0.09 (32%)	2.7 ±0.2 (22%)	4.1 ±0.1 (49%)
IV	1.4 ±0.2	3.3 ±0.2 (58%)	2.4 ±0.2 (42%)	8.0 ±0.3 (83%)
V	0.42 ± 0.02	0.65 ± 0.02 (35%)	0.49 ± 0.01 (14%)	0.89 ± 0.04 (53%)

[a] The protein concentration was 1.18 mg/ml.

[b] Mean ± S.E.M. for triplicate determinations. Figures in parentheses refer to percent contribution of enzymatic hydrolysis to total hydrolysis.

the conjunctiva and corneal epithelium, significant increases in enzymatic hydrolytic rate were observed with increasing number of carbons in the alkyl side chain. This finding is consistent with the chain length dependence of ocular ester prodrug hydrolysis reported previously (Chang and Lee, 1983). The enhanced stability of the O-pivaloyl ester to hydrolysis in ocular tissue homogenates, relative to the companion esters, is most probably due to steric hindrance to attack by acetyl- and butyrylcholinesterases present in these ocular tissues (Lee et al., 1985).

Lipophilicity of the timolol esters

Partition coefficients for the timolol esters and timolol between octanol and aqueous phosphate buffer of pH 7.40 are listed in Table 6. The results obtained show that the derivatives are all more lipophilic than the parent timolol. This is both a result of decreased pK_a values, affording a greater proportion of the lipophilic free base form at pH 7.4, and due to conversion of the hydroxyl group to an ester group.

The lipophilicity of the derivatives was also evaluated by means of reversed-phase HPLC. In this method the capacity factor (k′) of a solute is taken as a measure for the relative lipophilicity (cf. e.g. Hafkenscheid and Tomlinson, 1983):

$$k' = (t_r - t_o)/t_o \qquad (3)$$

where t_r is the retention time of the solute and t_o is the elution time of the solvent. With methanol–0.03 M phosphate pH 4.5 (1 : 1 v/v) as mobile phase the compounds I–V showed the k′ values given in Table 6. These data also demonstrate the higher lipophilicity of the esters in comparison with timolol.

O-Acyl esters of timolol as prodrugs

The results obtained show that esterification of timolol may be a potentially useful approach to obtain prodrugs with the ultimate goal to improve corneal drug absorption, thereby reducing systemic drug load. The aliphatic esters studied possess a greater lipophilicity than the parent drug, and as indicated in preliminary experiments, they show enhanced corneal permeability characteristics in comparison with timolol. While the esters are hydrolyzed appreciably to regenerate timolol in ocular tissue homogenates, they suffer from chemical instability. The esters are most stable at

TABLE 6

PARTITION COEFFICIENTS (P) AND CAPACITY FACTORS (k') OF TIMOLOL AND VARIOUS TIMOLOL ESTERS AT 22°C

Compound	log P [a]	log k′
Timolol	−0.04	0.38
II	1.12	0.56
III	1.62	0.81
IV	2.08	0.99
V	2.68	1.20

[a] P is the partition coefficient between octanol and 0.05 M phosphate buffer (pH 7.4).

pH about 3 but even at this pH the stability is so limited that aqueous solutions with practical shelf-lives cannot be made. Formulation of these prodrugs into polymeric matrices for administration to the eye may be an approach to overcome this solution instability problem. Studies are ongoing to further examine the basis of this instability and to develop other bioreversible derivatives with improved chemical stability.

Acknowledgement

This work was supported in part by Grants EY03816 and BRSG S07RR05792 from the National Institutes of Health, Bethesda, Maryland, U.S.A.

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