How an Increase in the Carbon Chain Length of the Ester Moiety Affects the Stability of a Homologous Series of Oxprenolol Esters in the Presence of Biological Enzymes

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Abstract □ β-Blockers including timolol and propranolol are administered in eye-drops for the treatment of glaucoma. Due to high incidence of cardiovascular and respiratory side-effects, their therapeutic value is limited. As a result of poor ocular bioavailability, many ocular drugs are applied in high concentrations, which give rise to both ocular and systemic side-effects. Therefore, some methods have been employed to increase ocular bioavailability such as (a) the development of drug delivery devices designed to release drugs at controlled rates, (b) the use of various vehicles that retard precorneal drug loss, and (c) the conversion of drugs to biologically reversible derivatives (prodrugs) with increased corneal penetration properties, from which the active drugs are released by enzymatic hydrolysis. A series of structurally related oxprenolol esters were synthesized and investigated as potential prodrugs for improved ocular use. The stability of each ester was studied in phosphate buffer (pH 7.4), also in the presence of (a) 30% human plasma, (b) aqueous humor, and (c) corneal extract at pH 7.4 and at 37 °C. An account is given of how the stability of a homologous series of oxprenolol esters in the presence of biological enzymes is affected by an increase in the carbon chain length of the ester moiety.

1. Introduction

Esters are the best known prodrugs due to the predominance of carboxylic and hydroxyl substituents in drug molecules along with the availability of enzymes in living systems capable of hydrolyzing them. Therefore, considerable attention has been focused on the use of bioreversible (prodrugs) in order to improve the delivery characteristics of various drugs.[1] A fundamental requisite for the usefulness of the prodrug approach is the ready availability of chemical derivatives satisfying the prodrug requirements, principally reconversion of the prodrug to the parent drug in vivo.

In previous studies, esters of timolol have been developed to potentially diminish the systemic absorption of topically added timolol through increased corneal absorption. The cardiovascular and respiratory side-effects[2−4] are thereby reduced. However, these esters are unstable in aqueous solutions. In respect of this, a series of esters of both propranolol[5−7] and timolol[8−10] were synthesized and the kinetics of degradation of the prodrugs in aqueous solution studied.

The degradation of esters of the β-adrenergic blocker timolol was studied.[8] They were all 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. A series of propranolol esters were synthesized[5,11] and their rates of hydrolysis were measured in 0.02 M phosphate buffer (pH 7.4) in the presence of 80% human plasma.[5]

Propranolol and oxprenolol are used in the treatment of glaucoma, and hence the ability of their respective esters to regenerate the parent compound at a reasonable rate in ocular tissue homogenates is an important factor when considering propranolol and oxprenolol esters as promising prodrugs for ocular delivery.

The susceptibility of the esters of propranolol and oxprenolol to undergo conversion to their respective parent compounds was studied in vitro. The degradation of each ester was studied in phosphate buffer (pH 7.4), also in the presence of (a) 30% human plasma, (b) aqueous humor, and (c) corneal extract at pH 7.4 and at 37 °C.[12] The reason this homologous series of esters was synthesized is because the parent compound oxprenolol, due to poor ocular bioavailability, needs to be applied in high concentrations thereby giving rise to both ocular and systemic side-effects.[7] Therefore, these prodrugs were produced in the hope of improving targeting to particular tissues, to decrease the incidence of side-effects and hopefully to provide a stable formulation.

This paper gives an account of how the stability of a homologous series of oxprenolol esters is affected by an increase in the carbon chain length of the ester moiety in the presence of biological enzymes.

2. Materials and Methods

2.1. Chemicals—Samples of oxprenolol hydrochloride were obtained from both Ciba-Geigy and Sigma (UK). The acid chlorides were obtained from Aldrich (UK). All solvents used were either high-performance liquid chromatography (HPLC) grade or distilled before use. Solid reagents were analytical or reagent grade and were used as supplied or were recrystallized before use.

All solvents used in HPLC (i.e. acetonitrile, methanol, acetone, and tetrahydrofuran) were HPLC grade. All buffer substances were of reagent or analytical grade. The ionic strength of each buffer solution was adjusted to 0.5 by adding a specific quantity of analytical grade potassium chloride.

2.2. Synthesis of Oxprenolol Esters—The homologous series of oxprenolol esters were prepared by heating oxprenolol hydrochloride (1 g) under reflux for a specified length of time with the appropriate acid chloride. Excess acid chloride was removed under vacuum. A solid product was obtained with the *O*-pivaloyl derivative. An oily residue was obtained in all other cases. The oil was crystallized by adding 5 mL of acetone followed by 30 mL of petroleum ether. Continuous removal of the acetone and petroleum ether under vacuum and repetition of the process resulted in the formation of a milky precipitate which was recrystallized from 2-propanol.

2.3. Preparation of Biological Samples—*2.3.1. Human Plasma*—Human plasma was obtained from the Blood Transfusion Board, Dublin, and stored frozen.

2.3.2. Aqueous Humor—Eyes were obtained from freshly killed cattle in Red Meats, Dublin. The aqueous humor was extracted from each eye using a 1 mL sterile disposable syringe fitted with

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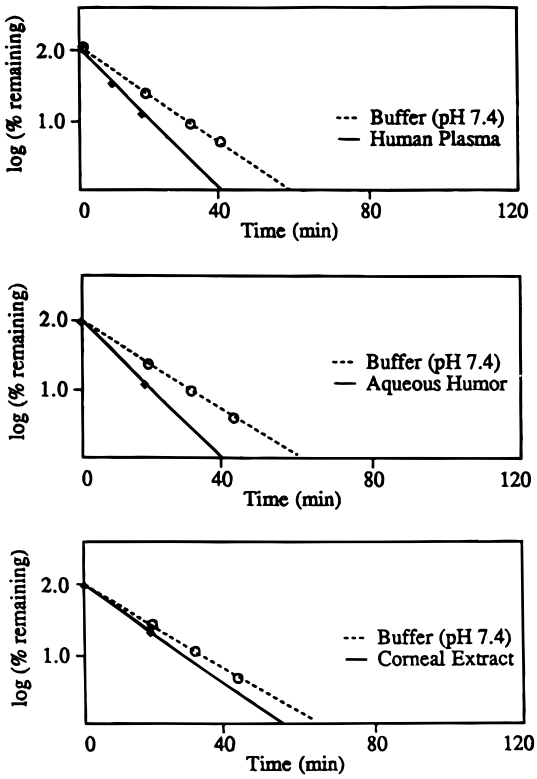


Figure 1—First-order plots for the degradation of *O*-acetyloxprenolol in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 30% human plasma, (b) aqueous humor, and (c) corneal extract, at the same pH and at 37 °C.

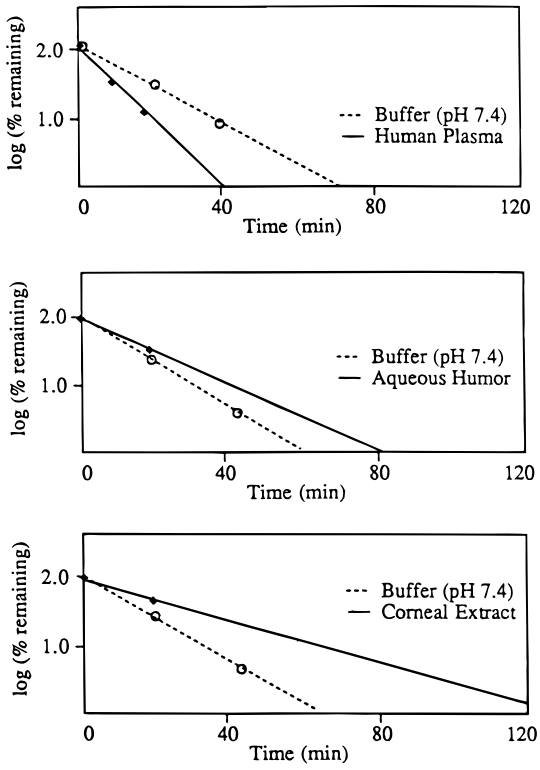


Figure 2—First-order plots for the degradation of *O*-propionyloxprenolol in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 30% human plasma, (b) aqueous humor, and (c) corneal extract, at the same pH and at 37 °C.

a brown 25 G 3/8 in. needle. Each eye contained approximately 1 mL of aqueous humor, and it was refrigerated in sterile vials at 4 °C until used.

2.3.3. Corneal Extract—An incision was made in the cornea using a scalpel, and the cornea was dissected using a forceps and scalpel. This transparent tissue was then chopped into small pieces using a pair of scissors and was put into a 4 in. mortar that contained approximately 3 g of hydrochloric acid washed sand and approximately 5 mL of ice-cold 0.05 M phosphate buffer (pH 7.4). The contents were homogenized using a Heto motor-driven tissue grinder. The ocular tissue homogenate was then poured into centrifuge tubes and centrifuged at 12 000 rpm for approximately 45 min in an AGB bench centrifuge. The clear supernatant was decanted off and stored in sterile vials at 4 °C. The corneal extract and aqueous humor were only stored for a maximum of 3 days at 4 °C. After that time fresh ocular tissue was obtained.

2.4. Apparatus—HPLC was carried out using a system consisting of a Waters 501 HPLC pump, a variable wavelength detector attached to a Houston omniscribe recorder and a 20 μL Rheodyne loop injection valve. The column used, 100 × 4.6 mm, was packed with Spherisorb C-8 (5 μm particles). A precolumn, 50 × 4.6 mm, was similarly packed. A 10 μL sample was introduced by means of a Hamilton syringe.

The pH value of each solution was determined using a Radiometer M-26 pH meter fitted with a glass electrode (Radiometer G-202B) and a calomel reference electrode (Radiometer K-401). Reference buffers were Radiometer standard solutions (pH 4.00/22 °C, pH 6.97/22 °C, and pH 8.86/22 °C). A Heto thermostat water-bath with a Heto contact thermometer were used in all experiments.

The corneal extract contents were homogenized using a Heto motor-driven tissue grinder.

2.5. Stability Studies—A 20% stock solution of each ester was prepared in HPLC grade, distilled water. To a 3 mL stock solution of each ester, 4 mL of phosphate buffer (0.05 M, pH 7.4) was added, and then 3 mL of aqueous humor or corneal extract was added. Before immersing the solution in a Heto temperature-controlled water-bath at 37 °C ± 0.2 °C, the mixing was agitated by hand for approximately 2 min in order to ensure complete mixing. At

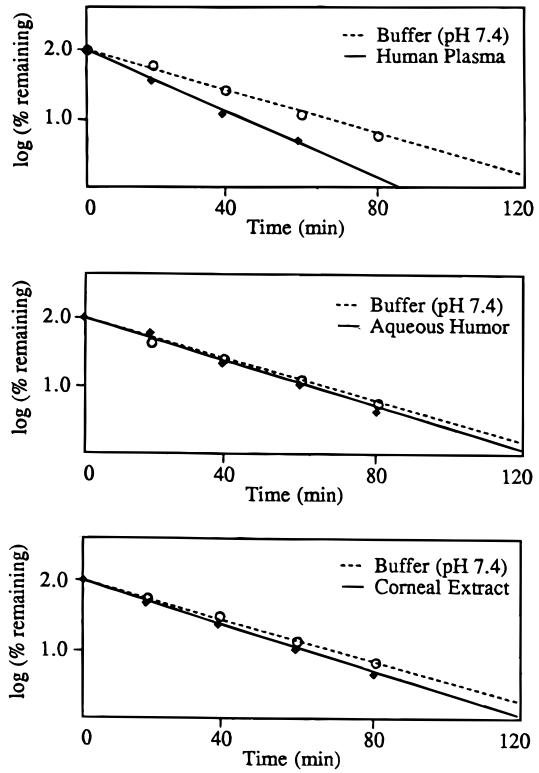


Figure 3—First-order plots for the degradation of *O*-butyryloxprenolol in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 30% human plasma, (b) aqueous humor, and (c) corneal extract, at the same pH and at 37 °C.

20 min intervals a 10 μL sample was removed and immediately chromatographed.

To a 3 mL stock solution of each ester, 4 mL of phosphate buffer (0.05 M, pH 7.4) was added, and then 3 mL of human plasma was

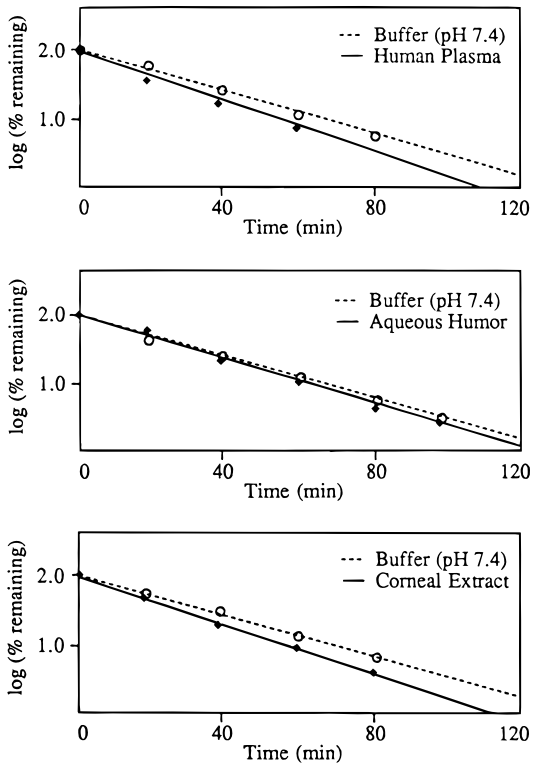


Figure 4—First-order plots for the degradation of *O*-valeryloxprenolol in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 30% human plasma, (b) aqueous humor, and (c) corneal extract, at the same pH and at 37 °C.

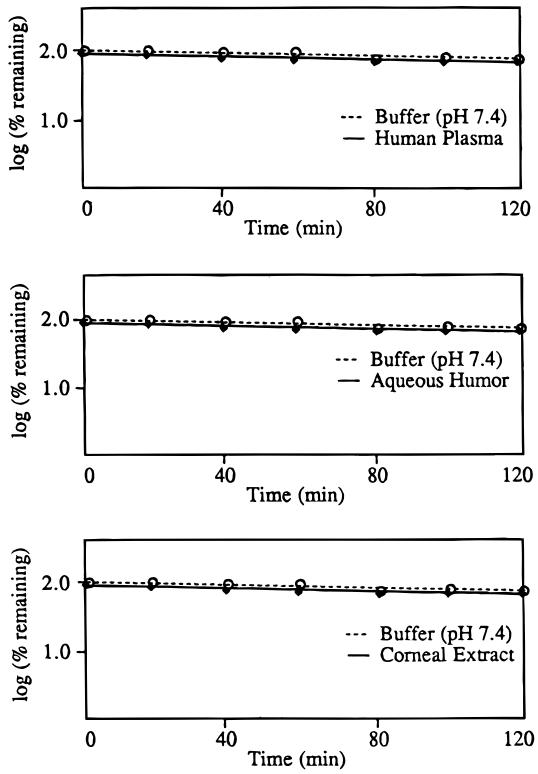


Figure 5—First-order plots for the degradation of *O*-pivaloyloxprenolol in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 30% human plasma, (b) aqueous humor, and (c) corneal extract, at the same pH and at 37 °C.

added. It was kept in a temperature-controlled Heto water bath at 37 °C ± 0.2 °C. At 20 min intervals, the solution was agitated quickly by hand and a 250 μL sample was withdrawn using a micropipet and added to a 1000 μL of analytical grade ethanol in order to deproteinize the plasma. After mixing and centrifugation at 12 000 rpm for 2 min in an AGB bench centrifuge, 10 μL of the clear supernatant liquid was removed using a Hamilton syringe and immediately chromatographed.

A control was used in each experiment, and it consisted of ester sample and 0.05 M phosphate buffer (pH 7.4) only. It was also maintained at 37 °C ± 0.2 °C, and at 20 min intervals, a 10 μL sample was withdrawn and immediately chromatographed.

3. Results and Discussion

Quantitation of the esters was determined by measuring the peak heights in relation to those of standards chromatographed under identical conditions or by measuring the peak areas. A plot of the log of residual concentration (log % remaining) against time (min) was drawn for each ester (Figures 1−5). Pseudo-first-order rate constants (k_{obs}) were calculated from the slopes of these linear plots using eq 1

$$\text{slope} = -k_{obs}/2.303 \quad (1)$$

Those rate constants were also calculated for the overall degradation of the esters in 0.05 M phosphate buffer only (pH 7.4), and also in the presence of (a) human plasma, (b) aqueous humor, and (c) corneal extract. Their half-lives and shelf lives were calculated from the rate constants using eqs 2 and 3, respectively

$$t_{0.5} = 0.693/k_{obs} \quad (2)$$

$$t_{90} = 0.105/k_{obs} \quad (3)$$

where k_{obs} = the observed pseudo-first-order-rate constant

Table 1—Observed Pseudo-First-Order Rate Constants (k_{obs}) for the Degradation of a Homologous Series of Oxprenolol Esters in 0.05 M Phosphate Buffer (pH 7.4) and Also in the Presence of (a) 30% Human Plasma, (b) Aqueous Humor, and (c) Corneal Extract, at the Same pH and at 37 °C (μ = 0.5)

	k_{obs} (min^{-1}) × 10^{-2}			
ester	buffer (pH 7.4)	human plasma (30%)	aqueous humor	corneal extract
O-acetyl	7.5	16.0	11.1	7.1
O-propionyl	6.6	10.7	4.7	6.1
O-butyryl	3.6	5.3	3.7	5.3
O-valeryl	3.2	4.2	3.5	4.2
O-pivaloyl	0.03	0.26	0.10	0.18

Table 2—The Ratio of the Hydrolysis Rates in Different Media Relative to That in Buffer (pH 7.4) and at 37 °C (μ = 0.5)

ester	human plasma (30%)	aqueous humor	corneal extract
O-acetyl	2.13	1.48	0.95
O-propionyl	1.62	0.71	0.93
O-butyryl	1.47	1.02	1.47
O-valeryl	1.31	1.09	1.31
O-pivaloyl	8.66	3.33	6.0

of the ester at a particular pH. These data are represented in Tables 3 and 4, respectively. Fresh ocular tissue only was used in these experiments, as only fresh extract provided maximal esterase activity. Every effort was made to use the ocular tissue on the day it was obtained, or if this was not possible, ocular tissue that was stored at 4 °C for no more than 3 days would be used. Ocular tissue that had been stored for longer than 3 days at 4 °C was inactive and could not be used in experiments. Preliminary experiments carried out with inactive ocular tissue (boiled to over

Table 3—Calculated Half-Life ($t_{0.5}$) Values for a Homologous Series of Oxprenolol Esters in 0.05 M Phosphate Buffer (pH 7.4) and Also in the Presence of (a) 30% Human Plasma, (b) Aqueous Humor, and (c) Corneal Extract, at the Same pH and at 37 °C ($\mu = 0.5$)

	$t_{0.5}$ (min)			
ester	buffer (pH 7.4)	human plasma (30%)	aqueous humor	corneal extract
O-acetyl	9.1	4.3	6.1	9.6
O-propionyl	10.4	6.4	14.6	11.3
O-butyryl	19.1	12.8	18.3	13.0
O-valeryl	21.1	16.2	19.6	16.4
O-pivaloyl	2035.5	263.2	687.8	375.5

Table 4—Calculated Shelf-Life (t_{90}) Values for a Homologous Series of Oxprenolol Esters in 0.05 M Phosphate Buffer (pH 7.4) and Also in the Presence of (a) 30% Human Plasma, (b) Aqueous Humor, and (c) Corneal Extract, at the Same pH and at 37 °C ($\mu = 0.5$)

	t_{90} (min)			
ester	buffer (pH 7.4)	human plasma (30%)	aqueous humor	corneal extract
O-acetyl	1.4	0.65	0.93	1.4
O-propionyl	1.5	0.97	2.2	1.7
O-butyryl	2.9	1.9	2.7	1.9
O-valeryl	3.1	2.4	2.9	2.4
O-pivaloyl	308.4	39.8	104.2	56.7

40 °C and allowed to cool before use) produced results similar to those obtained in buffer only.

The homologous series of oxprenolol esters degrade faster in ocular tissue homogenate than in buffer. This is due to the presence of many esterases in the ocular tissues to catalyze the ocular hydrolysis, particularly acetylcholinesterase and the predominant butyrylcholinesterase enzymes. These esterases succeed in exhibiting their catalytic effect over the chemically stable *O*-pivaloyloxprenolol derivative, thereby overcoming the steric hindrance induced by its tertiary butyl ester group and hence speeding up its rate of ocular hydrolysis. This same derivative of oxprenolol exhibits a faster rate of hydrolysis in ocular tissue than in buffer only. This phenomenon is probably due to the overriding effect of the $OCH_2CH{=}CH_2$ group in the ortho position of the benzene ring of oxprenolol, which suppresses the resistance of this sterically hindered ester group to enzymatic catalysis. Therefore, the ocular esterases present are allowed to exert their catalytic effect, hence resulting in an increased rate of ocular hydrolysis for this ester. For the homologous series of oxprenolol esters, their rates of ocular hydrolysis decrease as the chain length increases (Table 1). This is in sharp contrast to research done on adult albino rabbits.[18] They found that the ocular hydrolysis of a series of α- and β-naphthyl esters increased as the ester chain length increased. The ratio of the hydrolysis rates in different media relative to that in buffer (pH 7.4) and at 37 °C ($\mu = 0.5$) was determined (Table 2). From the results obtained, one could speculate that the *O*-propionyl, *O*-butyryl, and *O*-valeryl esters would reach the intracular tissues most effectively because of their moderate rates of hydrolysis. In contrast, the *O*-acetyl prodrug is too unstable and the *O*-pivaloyl derivative too stable to gain entry effectively. As the rate of hydrolysis of each ester was not measured in rabbit ocular tissue, one might expect the ocular hydrolysis rate to increase as the ester chain length increased. These results would compare favorably with the results of research done on adult albino rabbits.[18]

There are a number of reasons why the rate of ocular hydrolysis increases as the lipophilicity of the ester prodrug

Table 5—Yields of Oxprenolol Formed (%) during the Degradation of a Homologous Series of Oxprenolol Esters in 0.05 M Phosphate Buffer (pH 7.4) and Also in the Presence of (a) 30% Human Plasma, (b) Aqueous Humor, and (c) Corneal Extract, at the Same pH and at 37 °C after 120 min ($\mu = 0.5$)

	oxprenolol formed (%)			
ester	buffer (pH 7.4)	human plasma (30%)	aqueous humor	corneal extract
O-acetyl	96	95	99	92
O-propionyl	95	90	98	94
O-butyryl	98	91	96	95
O-valeryl	97	92	97	97
O-pivaloyl	98	98	95	96

Table 6—Partition Coefficients (log *P*), Apparent Partition Coefficients ($\log P)_{app}$, and Capacity Factors ($\log k^1$) of a Homologous Series of Oxprenolol Esters at 22 °C

compound[a]	log *P*	log P_{app} (pH 7.4)[b]	log k^1 (pH 7.4)[c,d]
O-acetyl	3.82	2.52	0.43
O-propionyl	3.76	2.58	0.58
O-butyryl	3.95	3.29	0.74
O-valeryl	4.0	3.57	0.90
O-pivaloyl	3.54	2.78	0.95

[a] All of the *O*-acyl esters synthesized were soluble in aqueous solvents, i.e., water and buffers, and also in organic solvents such as methanol, chloroform, ethanol, ethyl acetate, acetone and petroleum ether. However, the *N*-acetyl and *N*-propionyl derivatives were also synthesized, and they were completely insoluble in aqueous solvents but soluble in organic solvents. [b] pH 7.4, calculated from log *P*. [c] pH 7.4. [d] k^1 = capacity factor and is defined by the following equation: $k^1 = t_r - t_o/t_o$, where t_r is the retention time of the solute and t_o represents the elution time of the solvent.

increases. The lipophilic nature of the corneal epithelium severely constrains the ability of topically applied ophthalmic drugs, the polar ones especially, to traverse this barrier. For a drug to gain entry to the intraocular tissues, it must be lipophilic enough to partition from the tears to the corneal epithelium.[19] Therefore, the basis for improvement in ocular absorption by prodrugs is an increase in the lipophilicity of the prodrugs relative to their compound, which favors their uptake by, and diffusion across, the lipophilic corneal epithelium. It should be anticipated that corneal prodrug absorption may be altered when the corneal epithelial barrier is breached. This is because the prodrug would then be exposed to the corneal stroma which differs from the epithelium in both lipophilicity and esterase activity.[20] A number of explanations can be put forward as to why the ocular hydrolysis of the homologous series of oxprenolol esters decreases as the chain length and their lipophilicity increases (Table 6), since the chemical nature of the parent compound can significantly influence the magnitude of the acylation rate of the enzyme and in turn the hydrolytic rate of the associated ester prodrug. Therefore, possibly the *O*-propionyl, *O*-butyryl, and *O*-valeryl derivatives of oxprenolol are not as susceptible to attack by the acetylcholinesterase and butyrylcholinesterase present in the ocular tissues, because of the effect exerted by the ortho substituent on the benzene ring of oxprenolol which slows down the interaction between the esterases in the ocular tissue and the ester moiety, thereby resulting in a reduced rate of ocular hydrolysis. The amount of interference would increase as the chain length of the ester increased.

Another possible explanation is that perhaps there were not enough esterases present in the ocular tissue, e.g. butyrylcholinesterase, to increase the ocular hydrolysis of the prodrugs, and therefore chemical hydrolysis was the dominant reaction taking place. Therefore, it could be

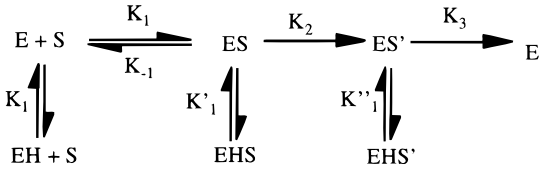
proven that as fresh ocular tissue was used in those experiments, there were enough esterases present to increase the ocular hydrolysis of the oxprenolol prodrugs.

Finally, the most valid explanation for the reduced rate of ocular hydrolysis of oxprenolol esters in bovine eye tissue is due to the dependence of the rate of ester hydrolysis in ocular tissue homogenates on ester chain length. Maximum hydrolytic rate was achieved with the valerate ester when a series of 1- and 2-naphthyl esters were incubated with the ocular tissue homogenates of albino rabbits. However, the opposite trend was observed, when the hydrolytic rates of 1- and 2-naphthyl acetate, propionate, and valerate esters were measured in microsomal fractions of the corneal epithelium and iris-ciliary body of bovine eyes.[16] Therefore, a similar trend is probably occurring here, where the rate of ocular hydrolysis decreases as the ester chain length and lipophilicity increases (Tables 1 and 6, respectively).

In contrast to a decrease in the rate of ocular hydrolysis as the ester chain length increases for a homologous series of oxprenolol esters, the opposite was observed for a similar series of timolol esters. The ocular hydrolysis of the timolol esters was measured in homogenates of the conjunctiva, corneal epithelium, and iris-ciliary body of the pigmented rabbit.[8]

Significant increases were observed in the enzymatic ocular hydrolytic rate as the number of carbons in the alkyl side chain increased and also with increasing lipophilicity for a homologous series of timolol esters.[8] Unlike the *O*-pivaloyl derivatives of propranolol[12] and oxprenolol, *O*-pivaloyltimolol showed enhanced stability to hydrolysis in ocular tissue homogenates, relative to the companion esters. This high stability is most probably due to steric hindrance to attack by acetylcholinesterase and butyrylcholinesterase present in these ocular tissues.[21] The timolol esters hydrolyzed readily in ocular tissue like those of propranolol and oxprenolol, and they also displayed a higher hydrolytic rate in corneal extract than in aqueous humor.[22] Intramolecular aminolysis did not take place in ocular tissue homogenates or in buffer-only solutions for timolol esters; this is probably due to the high stability of the tertiary butylamino side-chain of the timolol molecule, which inhibits formation on the *N*-acyltimolol derivative. Therefore the percentage of timolol formed was the same during ocular hydrolysis as during hydrolysis in buffer only, because there was no *N*-acyl derivative formed to interfere with the amount of parent compound formed.[8]

Esterases play a major role in the pharmacology of prodrugs, which are enzymatically labile derivatives of drugs designed to improve the pharmacokinetics of their parent compounds.[13] It is reasonable to expect that an understanding of the esterase composition in various ocular tissues would facilitate ester prodrug design. From a study carried out using albino and pigmented rabbits,[14] the esterase activities in their corneas, irises, ciliary bodies, and aqueous humor were delineated. It was found that at least some of the already named esterases present in the aqueous humor were different from those in the cornea, iris, and ciliary body. It was also shown that the esterase activity was highest in the iris-ciliary body followed by the cornea and then the aqueous humor. Specifically, the esterase activity in the cornea and aqueous humor was, respectively, 50 and 2−5% of that seen in the iris-ciliary body. The higher level of esterase activity in the iris and ciliary may be due to the fact that these tissues are more cellular than both the cornea and aqueous humor and therefore more abundant in esterases. Even though the cornea is not as enzymatically active as the iris and ciliary body, it is still in a strategic position to determine the amount of intact drug ultimately reaching the internal eye



Scheme 1—E and EH are the active and inactive forms of the enzyme; S, is the substrate; ES and EHS are the active and inactive forms of the enzyme–substrate complex; ES′ and EHS′ are the corresponding forms of the acyl enzyme intermediate.

from topical application. It was found that in both breeds of rabbits, esterase activity was the highest in the iris-ciliary body followed by the cornea and then the aqueous humor.[15] However, on comparing results on the esterase activity in the bovine eye with those obtained in the rabbit, it is apparent that significant species differences in ocular esterase activity exist.[16] The bovine and rabbit eyes differ in a number of ways. First, the esterase activity is 2−40 times higher in the rabbit than in bovine eye with the most prominent difference in the corneal epithelium. Second, whereas, in the rabbit the corneal epithelium possesses twice as much esterase activity as the stroma, this rank order is reversed in the bovine eye.[17] It was also found that the cornea was richer in esterases than the aqueous humor. As there were no experiments carried out on rabbit eye tissue, one can only speculate from the above information that the rate of ocular hydrolysis would be greater in rabbit eye tissue for all esters. This is because ocular esterase activity is 2−40 times higher in rabbit eye tissue than in bovine eye.

For the homologous series of oxprenolol esters, i.e., from the *O*-acetyl to the *O*-valeryl derivatives, it was observed that the rate of degradation decreased as the chain length of the *O*-acyl group increased (Table 1). This occurred with and without plasma. It is relevant in this case to explain why it occurs in the presence of plasma. There are a number of enzymes present in plasma which are capable of hydrolyzing esters. It is possible to predict which enzyme is involved in the in vivo hydrolysis of a prodrug with an ester functional group as its reversible group[18] by carrying out experiments with plasma selectively enriched with specific enzymes, and one might obtain a reasonably good idea of which enzyme is responsible for the major portion of the hydrolysis. Furthermore, data for the variation of ester reactivity with structure is not available for most of these enzymes. However, ester prodrug hydrolysis catalyzed by plasma enzymes is believed to follow the path shown below[23] (Scheme 1):

The rate-determining step in this type of reaction is deacylation. Thus, the rate of deacylation is accelerated by electron acceptors and by increasing hydrophobicity.[24] It is decelerated in the case of the oxprenolol esters already mentioned, because of the increasing chain length of the ester moiety and also because of increasing lipophilicity and the electron-releasing character of the *O*-acyl groups. This gives rise to a decrease in the rate of hydrolysis as the ester group becomes larger (Table 1).

Like *O*-pivaloylpropranolol,[12] the *O*-pivaloyloxprenolol derivative exhibits a very low rate constant at pH 7.4 with or without plasma, in comparison with those of the other oxprenolol esters. This is due to the steric hindrance exhibited by the bulky tertiary butyl group of the *O*-acyl moiety which makes this ester very stable. Due to their increased rates of hydrolysis in plasma, their half-lives and shelf lives are decreased (Tables 3 and 4). Enzymatic hydrolysis of the oxprenolol esters is accompanied by intramolecular aminolysis. The percentage of oxprenolol formed during enzymatic hydrolysis of each ester is shown in Table 5. A slight decrease in the formation of oxprenolol

is observed in some cases, and this is possibly due to an increase in the formation of the *N*-acyl derivative.

4. Conclusion

One could conclude that the stability of the homologous series of oxprenolol esters increased as the carbon chain length of the ester moiety increased. This phenomenon was observed in phosphate buffer (pH 7.4) only, also in the presence of biological enzymes, thereby resulting in increased half-lives and shelf lives. Enzymatic hydrolysis is accompanied by intramolecular aminolysis in the case of each ester. From the results of this study, the *O*-propionyl, *O*-butyryl, and *O*-valeryl prodrugs are the most effective because of their moderate hydrolysis rates and would most probably reach the iris-ciliary body most effectively, whereas the *O*-acetyl is too unstable and the *O*-pivaloyl is too stable. The *O*-pivaloyloxprenolol derivative displays a very high stability in the presence of biological enzymes due to the steric hindrance exhibited by the bulky tertiary butyl group of the *O*-acyl moiety which makes this ester very stable. This conclusion about the stability of the esters was drawn from physicochemical data such as the partition coefficients (log *p*) and the capacity factors (log k^1) (Table 6). As in vivo studies were not carried out with these esters, it would be very interesting to find out the best ways of administering them, to be able to comment on the relative ease of stabilizing the formulations, and to compare the rates of hydrolysis in the iris-ciliary body. This undoubtedly would make very interesting future work.

References and Notes

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