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# Studies showing the effect of enzymes on the stability of ester prodrugs of propranolol and oxprenolol in biological samples

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**Abstract**

A number of $\beta$-adrenergic blockers, including timolol and propranolol, are administered in eye-drops for the treatment of glaucoma. Their therapeutic value is limited by a relatively high incidence of cardiovascular and respiratory side-effects. Because of poor ocular bioavailability, many ocular drugs are applied in high concentrations, which give rise to both ocular and systemic side-effects. Among the methods employed to increase ocular bioavailability are (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 esters of propranolol and oxprenolol were synthesised 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) 25% human plasma, (b) aqueous humor and (c) corneal extract at pH 7.4 and at 37°C.

*Keywords:* Propranolol; Oxprenolol; Prodrugs; Enzymes; Stability; Human plasma; Aqueous humor; Corneal extract

## 1. Introduction

Considerable attention has been focused on the use of bioreversible derivatives (prodrugs) in order to improve the delivery characteristics of various drugs (Bundgaard, 1985). A fundamental requisite for the usefulness of the prodrug approach is the ready availability of chemical derivative types satisfying the prodrug requirements, principally reconversion of the prodrug to

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the parent drug in vivo. 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 hydrolysing them.

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 (Velde and Kaiser, 1983; Munroe et al., 1985; Nelson et al., 1978) are thereby reduced. However, these esters are unstable in aqueous solutions. In respect of this a series of esters of both propranolol (Buur et al., 1988; Irwin and Belaid, 1988a) and timolol (Bundgaard et al., 1986, 1988; Chang et al., 1987) were synthesised and the kinetics of degradation of the prodrugs in aqueous solution studied.

The degradation of esters of the $\beta$-adrenergic blocker timolol was studied by Bundgaard et al., 1986. They were all hydrolysed 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 synthesised by Buur et al., 1988, and their rates of hydrolysis were measured in 0.02 M phosphate buffer (pH 7.4) in the presence of 80% human plasma.

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) 25% human plasma, (b) aqueous humor and (c) corneal extract at pH 7.4 and at 37°C.

## 2. Materials and methods

### 2.1. Chemicals

Samples of propranolol hydrochloride were obtained from Orsynetics (UK) and 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 recrystallised before use.

All solvents used in HPLC (i.e. acetonitrile, methanol, acetone and tetrahydofuran) 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 propranolol and oxprenolol esters

Propranolol esters were synthesised according to the method described by Quigley et al., 1994 and oxprenolol esters were synthesised according to the method described by Jordan et al., 1992.

### 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 a brown 25 G 3/8 inch 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 inch 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 homogenised 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 UV detector attached to a Houston omniscribe recorder and a 20 $\mu$l Rheodyne loop injection valve. The column used, 100 × 4.6 mm, was packed with Spherisorb C-8 (5 $\mu$m particles). A pre-column, 50 × 4.6 mm, was similarly packed. A 10 $\mu$l 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 was used in all experiments.

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

### 2.5. Degradation studies

A 20 mg % stock solution of each ester was prepared in HPLC grade, distilled water. To a 3 ml stock solution of each ester, 5 ml of phosphate buffer (0.05 M, pH 7.4) was added and then 2 ml of aqueous humor or corneal extract was added. Before immersing the solution in a Heto temperature controlled water-bath at 37 ± 0.2°C, the mixture was agitated by hand for approximately 2 min in order to ensure complete mixing. At 20 min intervals a 10 $\mu$l sample was removed and immediately chromatographed.

To a 4 ml stock solution of each ester, 3 ml of phosphate buffer (0.05 M, pH 7.4) was added and then 3 ml of human plasma was added. It was kept in a temperature-controlled Heto water bath at 37 ± 0.2°C. At 20 min intervals, the solution was agitated quickly by hand and a 250 $\mu$l sample was withdrawn using a micropipette and added to a 1000 $\mu$l 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 $\mu$l 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 ± 0.2°C and at twenty minute intervals, a 10 $\mu$l 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 (Figs. 1 and 2). Pseudo-first-order rate constants ($k_{obs}$) were calculated from the slopes of these linear plots using Eq. (1)

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

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.

$$t_{0.5} = \frac{0.693}{k_1} \quad (2)$$

$$t_{90} = \frac{0.105}{k_1} \quad (3)$$

where $k_1$ = the observed pseudo-first-order rate constant of the ester at a particular pH.

The high stability exhibited by the *O*-pivaloyl-propranolol derivative is most probably due to the resistance of the bulky *O*-pivaloyl analogue to enzymatic hydrolysis (Table 1). There are many enzymes present in human plasma, e.g. chymotrypsins, peptidases, acetylcholinesterase and butyrycholinesterase (Bodor et al., 1988). These may all exert a catalytic effect on the rates of hydrolysis of propranolol esters. The rates of

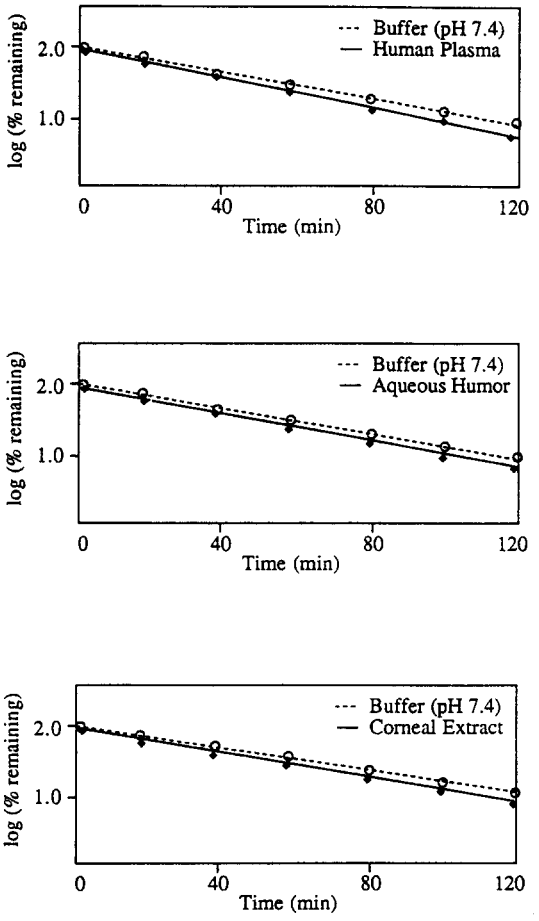



Fig. 1. First-order plots for the degradation of *O*-isobutyryl-propranolol in 0.05 M phosphate buffer (pH 7.4), and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C.

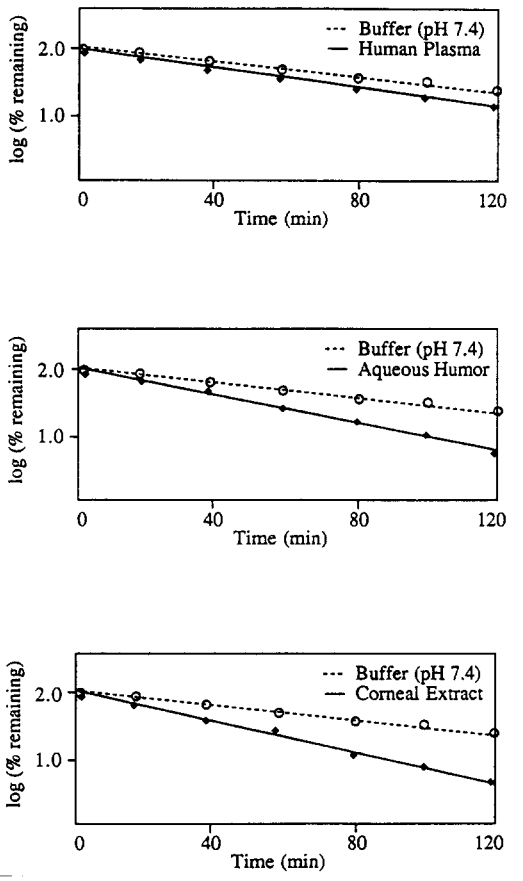



Fig. 2. First-order plots for the degradation of *O*-isobutyryloxprenolol in 0.05 M phosphate buffer (pH 7.4), and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C.

hydrolysis in the presence of plasma should have been higher for the propranolol esters. The rates of hydrolysis in the presence of plasma should have been higher for the propranolol esters compared with those obtained by Buur et al., 1988 for the *O*-acetyl and *O*-pivaloyl derivatives. This suppression of hydrolysis is possibly due to the high concentration of phosphate buffer (0.05 M, pH 7.4) used in this research in comparison with the relatively low buffer concentration (0.01 M, pH 7.4) used by Buur et al., 1988. The difference between the degradation rates for *O*-crotonylpropranolol in buffer only and in the presence of human plasma is large compared with those obtained for the *O*-cyclopropanoyl derivative. This

Table 1
Observed pseudo-first-order rate constants ($k_{obs}$) for the degradation of various propranolol esters in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C ($\mu = 0.5$)

| Ester | $k_{obs}$ ($min^{-1}$) $\times 10^{-2}$ | | | |
|---|---|---|---|---|
| | Buffer (pH 7.4) | Human plasma (25%) | Aqueous humor | Corneal extract |
| *O*-acetyl | 11.10 | 13.8 | 10.5 | 11.5 |
| *O*-isobutyryl | 1.7 | 2.1 | 1.8 | 1.6 |
| *O*-crotonyl | 1.2 | 1.8 | 1.3 | 1.5 |
| *O*-cyclopropanoyl | 1.1 | 1.5 | 1.0 | 0.92 |
| *O*-pivaloyl | 0.03 | — | 0.13 | 0.25 |
| *O*-*p*-nitrobenzoyl | 0.26 | 0.48 | 0.13 | 0.09 |

— indicates no degradation.

Table 2
Calculated half-life ($t_{0.5}$) values for various propranolol esters in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C ($\mu = 0.5$)

| Ester | $t_{0.5}$ (min) | | | |
|---|---|---|---|---|
| | Buffer (pH 7.4) | Human plasma (25%) | Aqueous humor | Corneal extract |
| *O*-acetyl | 6.2 | 5.0 | 6.5 | 6.0 |
| *O*-isobutyryl | 39.7 | 32.7 | 38.2 | 41.3 |
| *O*-crotonyl | 55.3 | 37.0 | 52.3 | 44.4 |
| *O*-cyclopropanoyl | 58.5 | 45.5 | 64.5 | 75.2 |
| *O*-pivaloyl | 2310.0 | — | 495.6 | 271.8 |
| *O*-*p*-nitrobenzoyl | 258.8 | 142.8 | 509.1 | 732.7 |

— indicates no degradation.

is because of the steric hindrance exhibited by the cyclic *O*-acyl group. This retards the catalytic effect exerted by the plasma enzymes, thereby resulting in a slight increase in the rate of hydrolysis in the presence of plasma (Table 1). There is also a small difference observed for the hydrolysis rates in the case of the *O*-*p*-nitrobenzoyl derivative, this is because of the bulkiness of the aromatic ester group which slows down hydrolysis in the presence of plasma.

For the *O*-pivaloylpropranolol derivative, the rate of ocular hydrolysis is faster than the rate of hydrolysis in buffer only. This is most probably due to the enhancement of hydrolysis by the enzymes present in the ocular tissues. There are many esterases present in ocular tissues, e.g. acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and peptidases (Lee et al., 1985). 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 (Grass and Robinson, 1988). 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 by Lee (1983), using albino and pigmented rabbits, 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 body maybe due to the fact that these tissues are more cellular than both the cornea and the aqueous humor and therefore are more abundant in esterases. Eventhough 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 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 (Chien et al., 1991). 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 (Lee et al., 1983). 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 (Chien et al., 1988). It was also found that the cornea was richer in esterases than the aqueous humor. Therefore, in this research on ocular hydrolysis of propranolol esters in bovine eye tissue, it was generally found that the rate of ocular hydrolysis was higher in the presence of corneal extract than in the presence of aqueous humor. This is due to the presence of a large number of esterases, e.g. butyrylcholinesterase to catalyse the hydrolysis reaction. BuChE is the dominant cholinesterase present in both the corneal extract and the aqueous humor of the bovine eye (Lee et al., 1983).

The rate of ocular hydrolysis of the *O*-cyclopropanoyl and *O*-*p*-nitrobenzoyl propranolol derivatives is slow compared with that in buffer only. This is most probably due to steric hindrance exhibited by their ester groups, hence their resistance to attack by AChE and BuChE present in these ocular tissues. Therefore, the half-lives and shelf-lives of those two esters are higher in the presence of ocular tissues than in buffer only whereas, the half-lives and shelf-lives of the other propranolol esters are lower in the presence of ocular tissue (Tables 2 and 3).

It is of interest to note that the enzyme catalysed degradation of the esters involved, not only ester hydrolysis but also intramolecular aminolysis (Bundgaard, 1976). The percentage of propranolol formed in plasma, aqueous humor, corneal extract and buffer only solutions is shown in Table 4.

In contrast to *O*-pivaloylpropranolol, the rate of hydrolysis of *O*-pivaloyloxprenolol was faster in the presence of plasma than in buffer only at the same pH and temperature. 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 the bulky *O*-pivaloyl analogue to enzymatic catalysis. As a result, the enzymes present in the plasma are allowed to exert their catalytic effect, thereby resulting in an increased rate of hydrolysis.

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 5). 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 hydrolysing esters. It is therefore impossible to predict which enzyme is involved in the in vivo hydrolysis of a prodrug with an ester functional group as its reversible group (Chang and Lee, 1983). Furthermore, data for the variation of ester reactivity with structure is not available for most of these enzymes. However, ester prodrug hydrolysis catalysed by plasma enzymes is believed to follow the path shown below (Charton, 1977) (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 (Huang et al., 1983a). 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-releas-

Table 3
Calculated shelf-life ($t_{90}$) values for various propranolol esters in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C ($\mu = 0.5$)

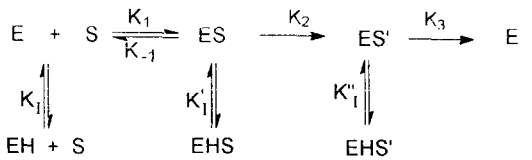
| Ester | $t_{90}$ (min) | | | |
|---|---|---|---|---|
| | Buffer (pH 7.4) | Human plasma (25%) | Aqueous humor | Corneal extract |
| *O*-acetyl | 0.94 | 0.76 | 0.99 | 0.91 |
| *O*-isobutyryl | 6.0 | 4.9 | 5.7 | 6.2 |
| *O*-crotonyl | 8.3 | 5.6 | 7.9 | 6.7 |
| *O*-cyclopropanoyl | 8.8 | 6.9 | 9.7 | 11.3 |
| *O*-pivaloyl | 350.0 | — | 75.1 | 41.1 |
| *O*-*p*-nitrobenzoyl | 39.2 | 21.6 | 77.1 | 111.0 |

— indicates no degradation.

Table 4
Yields of propranolol formed (%) during the degradation of various propranolol esters in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C after 120 min

| Ester | Propranolol formed (%) | | | |
|---|---|---|---|---|
| | Buffer (pH 7.4) | Human plasma (25%) | Aqueous humor | Corneal extract |
| *O*-acetyl | 94 | 88 | 94 | 90 |
| *O*-isobutyryl | 98 | 89 | 92 | 87 |
| *O*-crotonyl | 91 | 84 | 90 | 87 |
| *O*-cyclopropanoyl | 96 | 92 | 94 | 92 |
| *O*-pivaloyl | 92 | 100 | 95 | 97 |
| *O*-*p*-nitrobenzoyl | 50 | 42 | 60 | 79 |

ing character of the *O*-acyl groups. This gives rise to a decrease in the rate of hydrolysis as the ester group becomes larger (Table 5). This reasoning may also be applied to the *O*-cyclopropanoyl, *O*-crotonyl and *O*-*p*-nitrobenzoyl derivatives, because of the large size of their *O*-acyl groups and their low rates of hydrolysis in buffer only and in the presence of plasma.




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.

Like *O*-pivaloylpropranolol, 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. In the case of the *O*-cyclopropanoyl derivative of oxprenolol, the plasma catalysed hydrolysis is slowed down somewhat because of the steric effect of the cyclic *O*-acyl group, this was also noticed in *O*-cyclopropranolol (Irwin and Belaid, 1988b). Due to their increased rates of hydrolysis in plasma, their half-lives and shelf-lives are decreased (Tables 6 and 7).

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 8. A slight decrease in the formation of oxprenolol is

Table 5
Observed pseudo-first-order rate constants ($k_{obs}$) for the degradation of various oxprenolol esters in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C ($\mu = 0.5$)

| Ester | $k_{obs}$ ($min^{-1}$) $\times 10^{-2}$ | | | |
|---|---|---|---|---|
| | Buffer (pH 7.4) | Human plasma (25%) | 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 |
| *O*-isobutyryl | 1.0 | 1.4 | 2.2 | 2.2 |
| *O*-cyclopropanoyl | 1.6 | 1.4 | 1.3 | 1.2 |
| *O*-crotonyl | 0.98 | 1.3 | 1.7 | 1.5 |
| *O*-*p*-nitrobenzoyl | — | 0.20 | 0.03 | 0.04 |

— indicates no degradation.

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

The 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 catalyse the ocular hydrolysis, particularly AChE and the predominant BuChE 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. In contrast to the *O*-*p*-nitrobenzoyl propranolol derivative, 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 decreases as the chain length

Table 6
Calculated half-life ($t_{0.5}$) values for various oxprenolol esters in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C ($\mu = 0.5$)

| Ester | $t_{0.5}$ (min) | | | |
|---|---|---|---|---|
| | Buffer (pH 7.4) | Human plasma (25%) | 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 |
| *O*-isobutyryl | 65.2 | 46.5 | 31.2 | 31.3 |
| *O*-cyclopropanoyl | 64.8 | 46.5 | 50.5 | 54.0 |
| *O*-crotonyl | 70.4 | 50.6 | 40.3 | 44.0 |
| *O*-*p*-nitrobenzoyl | - | 330.4 | 2006.3 | 1604.9 |

— indicates no degradation.

Table 7
Calculated shelf-life ($t_{90}$) values for various oxprenolol esters in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C ($\mu = 0.5$)

| Ester | $t_{90}$ (min) | | | |
|---|---|---|---|---|
| | Buffer (pH 7.4) | Human plasma (25%) | 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 |
| *O*-isobutyryl | 9.8 | 7.0 | 4.7 | 4.7 |
| *O*-cyclopropanoyl | 9.8 | 7.0 | 7.6 | 8.1 |
| *O*-crotonyl | 10.6 | 7.6 | 6.1 | 6.6 |
| *O*-*p*-nitrobenzoyl | — | 50.07 | 303.9 | 243.1 |

— indicates no degradation.

increases. This is in sharp contrast to research done by Chang and Lee, 1983 on adult albino rabbits. They found that the ocular hydrolysis of a series of $\alpha$- and $\beta$-naphthyl esters increased as the ester chain length increased.

There are a number of reasons why the rate of ocular hydrolysis increases as the lipophilicity of the ester prodrug 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 (Bodor and El-Koussi, 1991). Therefore, the basis for improvement in ocular absorption by prodrugs is an increase in the lipophilicity of the prodrugs relative to their parent compound, which favours 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 (Lee et al., 1982). A number of explanations can be put forward as to why the ocular hydrolysis of the homologous series of

Table 8
Yields of oxprenolol formed (%) during the degradation of various oxprenolol esters in 0.05 M phosphate buffer (pH 7.4) and also in the presence of (a) 25% human plasma, (b) aqueous humor and (c) corneal extract, at the same pH and at 37°C after 120 min ($\mu = 0.5$)

| Ester | Oxprenolol formed (%) | | | |
|---|---|---|---|---|
| | Buffer (pH 7.4) | Human plasma (25%) | 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 |
| *O*-isobutyryl | 97 | 91 | 98 | 95 |
| *O*-cyclopropanoyl | 93 | 92 | 94 | 99 |
| *O*-crotonyl | 99 | 99 | 95 | 90 |
| *O*-*p*-nitrobenzoyl | 94 | 97 | 99 | 91 |

oxprenolol esters decreases as the chain length and their lipophilicity increases. 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 associate ester prodrug. Therefore, possibly the *O*-propionyl, *O*-butyryl and the *O*-valeryl derivatives of oxprenolol are not as susceptible to attack by the ZChE and BuChE 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. BuChE to increase the ocular hydrolysis of the prodrugs, and therefore chemical hydrolysis was the dominant reaction taking place. Preliminary experiments carried out with inactive ocular tissue (i.e. boiled to over 40°C and allowed to cool down before use) produced results similar to those obtained in buffer only. 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 (Lee et al., 1983). Therefore, a similar trend is probably occurring here, where the rate of ocular hydrolysis decreases as the ester chain length and lipophilicity increases (Table 5).

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 (Bundgaard et al., 1986).

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 (Bundgaard et al., 1986) Unlike the *O*-pivaloyl derivatives of propranolol 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 AChE and BuChE present in these ocular tissues (Lee et al., 1985). The timolol esters hydrolysed 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 (Chang et al., 1988). 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 of the *N*-acyl timolol 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 (Bundgaard et al., 1986).

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