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Permeability of prostaglandin $F_{2\alpha}$ and prostaglandin $F_{2\alpha}$ esters across cornea in vitro

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Summary

The permeability and absorption rate for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its methyl and benzyl esters through isolated pig cornea were investigated in vitro. A connection between the partition coefficient (octanol–water) and the permeability was observed. The permeability rate and absorption decreased in the same order as the partition coefficient, $PGF_{2\alpha}$ benzyl ester > $PGF_{2\alpha}$ methyl ester ≫ $PGF_{2\alpha}$. TLC and GC-MS analyses showed that both esters were hydrolyzed to $PGF_{2\alpha}$ during passage through the cornea. Incubation of the esters with cornea homogenates indicated that the main site for hydrolysis of the esters was the epithelium. Incubation with carbonic anhydrase or acetylcholinesterase did not affect the esters. Butyrylcholinesterase had a greater effect and both esters were rapidly hydrolyzed to $PGF_{2\alpha}$. Introduction of a cholinesterase inhibitor reduced the enzyme activity. A parallel situation was observed when the inhibitor was added to the cornea homogenate. These findings suggest that the corneal hydrolysis of $PGF_{2\alpha}$ esters is due to butyrylcholinesterase, or enzymes with butyrylcholinesterase activity.

Introduction

Glaucoma is one of the most serious diseases of the eye. Three groups of drugs (miotics, adrenergic agonists and carbonic anhydrase inhibitors) are used in the treatment of this disease (Akers et al., 1977). They all have a good pharmacodynamic effect on the intraocular pressure, but are associated with disadvantages. First, they all have a short therapeutic effect which leads to frequent administration. Second, and perhaps the most difficult problem to overcome when working with ophthalmic drugs, is incomplete absorption which partly prevents the drug from reaching its intraocular site of action. The low bioavailability depends on a low corneal permeability of the drug and a rapid drainage from the application site (Chrai et al., 1973; Chrai et al., 1974). Another side-effect of topically administered ophthalmic drugs is a general systemic absorption.

During the last few years there has been considerable research effort to overcome these bioavailability problems. For this purpose new ophthalmic vehicles and dosage forms have been proposed. In order to lengthen the contact time with the eye surface, experiments with vehicles containing viscosity-increasing agents have been performed (Green and Downs, 1975). A membrane-controlled drug delivery system, Ocusert, is another method that has been used to minimize the frequency of eye drug applications (Quigley et al., 1975). None

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of these formulations have given the expected success. One way which seems more hopeful is formulation of prodrugs. An example is the concept with dipivefrin, a pivalic acid diester of epinephrine (Kass et al., 1979). This compound reduces the intraocular pressure (IOP) in humans and rabbits at doses 5–10% of the commonly used epinephrine compound (Kaback et al., 1976). The esterfication has made the molecule more lipophilic which increases its corneal permeability with subsequent higher concentration in the eye (Wei et al., 1978). It has recently been shown that prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) and some of its esters have an IOP lowering effect in animals. The effect has been shown to be at least similar or in some cases better than the effect of commonly used antiglaucoma drugs. One remarkable finding was the prolonged lowering of IOP by $PGF_{2\alpha}$ as compared with the commercially available drugs. The effect of $PGF_{2\alpha}$ had a duration of 24 h after a single administration. However, the effect of different $PGF_{2\alpha}$ esters was not predictable and in some cases short-lasting (Camras and Bito, 1981; Stern and Bito, 1982; Bito, 1984).

The purpose of this study was to measure the corneal permeability rate of $PGF_{2\alpha}$, $PGF_{2\alpha}$ methyl ester and $PGF_{2\alpha}$ benzyl ester, across an excised pig cornea and also to investigate possible enzymatic hydrolysis of the esters and in which part of the cornea it takes place.

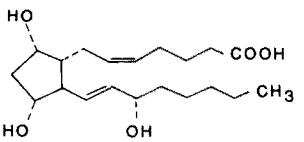
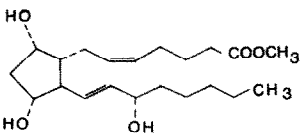
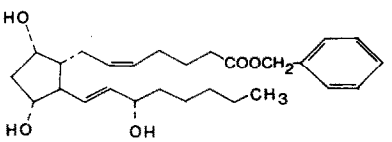
Materials and Methods

The corneas used in this work were obtained from pigs of the Yorkshire × Swedish Landrase, aged 6–7 months. The eyes were rapidly excised at the slaughter-house (Farmek, Uppsala) and transported to the laboratory packed on ice. They were used within 1 h of death of the animal.

[9-^{3}H]$PGF_{2\alpha}$ was purchased from Amersham International plc, Amersham, UK. The radioactivity was 507 GBq/mmol (13.7 Ci/mmol). [9-^{3}H]$PGF_{2\alpha}$ methyl ester (857 MBq/mmol; 23.2 Ci/mol) and [9-^{3}H]$PGF_{2\alpha}$ benzyl ester, (389 MBq/mmol; 10.5 Ci/mol) were obtained from Pharmacia AB, Sweden (Table 1). The radiochemical purity was checked by direct measurements of

TABLE 1

Chemical structures, molecular weights and log partition coefficients of $PGF_{2\alpha}$, $PGF_{2\alpha}$ methyl ester and $PGF_{2\alpha}$ benzyl ester

	mol wt	log PC
 $PGF_{2\alpha}$	354.5	1.10
 $PGF_{2\alpha}$ methyl ester	368.5	3.07
 $PGF_{2\alpha}$ benzyl ester	444.5	4.11

thin-layer chromatograms (run in three different systems) with a Berthold radiochromatogram scanner (LB 2723). The esters had a radiochemical purity higher than 97%, and contained less than 0.5% of free $PGF_{2\alpha}$. Carbonic anhydrase (EC 4.2.1.1), acetylcholinesterase (EC 3.1.1.7), butyrylcholinesterase (EC 3.1.1.8) and acetazolamide (Lot 64F-0327) were purchased from Sigma Chemicals, U.S.A. and synstigmine methyl sulphate (Neostigmin) was obtained from Leo AB, Sweden. All other chemicals used were of analytical grade.

Radioactivity measurements

Radioactivity measurements were made in a Packard Liquid Scintillation Counter, Tri-Carb, Model 2450. The samples were mixed with scintillation cocktail PCS II, Amersham, and adapted to the dark and appropriate temperature for at least 12 h before they were counted. The counting efficiency (generally more than 40%) was calculated from standard samples and an external standard. The metabolic conversion of the drugs was tested

on precoated TLC plates, Silica Gel 60, F-254 Merck, with a mobile phase consisting of diethyl ether–methanol (10:2). The plates were analyzed by radiochromatogram scanning.

Partition coefficient studies

Distilled water and octanol were mixed and allowed to stand for 24 h at 22°C before use. The two phases were then separated into a water phase saturated with octanol (W_o) and an octanol phase saturated with water (O_w). The compounds were dissolved in the O_w phase and the partition coefficient (PC) was determined by shaking the O_w phase intermittently with the W_o phase for 30 min at 22°C to reach a partition equilibrium. The two phases were then separated by centrifugation (3 000 rpm for 2 h). The volume of each phase was chosen so that the drug concentration in both phases could be measured with confidence after extraction. Radioactivity of the compounds was measured in the O_w phase before and after extraction, and in the W_o phase after extraction.

In vitro corneal permeability

Pig eyes with no visually epithelial defects were used within 1 h of death of the animal. The whole eye was placed in a holder to facilitate dissection. The cornea with a 2 mm ring of the sclera was carefully cut out with a pair of scissors and mounted in the perfusion apparatus according to Camber (1985).

During this moment great care was taken to avoid damage of the cornea. The perfusion apparatus, made of acrylic plastic, was placed on an electric heater previously adjusted to maintain the solution temperature within 34–35°C. The perfusion model consists of two compartments separated by the cornea. The compartment adjacent to the endothelial surface of the cornea has a volume of 6 ml, whereas the compartment adjacent to the epithelial side has a volume of 1 ml. Due to the pressure difference between the compartments, the cornea is easily held in position and is also prevented from buckling during the experiment. Preheated glutathione bicarbonate Ringer solution (GBR) (O'Brien and Edelhauser, 1977; Schoenwald and Huang, 1983) was added to the epithelial (1 ml) and endothelial (6 ml) compartments. The solutions were gassed with a mixture of 95% O_2–5% CO_2 to maintain a pH of 7.65. Ten minutes after the start of the experiments the GBR solution on the epithelial side was substituted for an O_2/CO_2 saturated GBR solution containing 9.3 μM or 18.6 μM $PGF_{2\alpha}$, $PGF_{2\alpha}$ methyl or benzyl ester. Samples of 100 μl were taken every 40 min during a period of 4 h from the endothelial side. Each aliquot was replaced immediately with an equal volume of GBR. The liquid levels were checked throughout the experiment. An equalization of the liquid levels between the compartments indicates leakage and such experiments were discarded. Stirring in both compartments were obtained by bubbling (about 3 bubbles/s).

The metabolic alteration of the $PGF_{2\alpha}$ esters were examined in the following way. When the permeability experiment was completed, the solutions from the two compartments were collected. After addition of 0.1 M HCl to obtain pH 2, the solutions were extracted twice with ethyl acetate. The phases were separated by centrifugation (3 000 rpm for 5 min) and the resulting organic phases were pooled and evaporated at room temperature, by directing a gentle stream of nitrogen gas onto the surface of the solution. The residue was dissolved in methanol and subjected to TLC analysis. Controls were prepared by placing equal concentrations of the esters in GBR and bubbling with 95% O_2–5% CO_2 for 4 h at 35°C. The results obtained from TLC were compared with GC-MS analysis. No discrepancies in results between the methods were seen.

Uptake of compounds by the cornea

After permeability experiments as described above, the corneas were weighed and placed in scintillation vials. Tissue solubilizer (2 ml of Soluene-350, United Technologies, Packard) was added to each vial. Treatment at 37°C for 24 h yielded a clear solution, and isopropanol (0.4 ml) and hydrogen peroxide 30% (0.8 ml) were added. A scintillation cocktail with chemiluminescence inhibitor, (10 ml of Dimilume-30, United Technologies, Packard) was then added. The vials were then kept for 24 h in the dark, to eliminate non-radioactive induced luminescence, before being counted.

Isolation and preparation of corneal homogenate

Fresh corneas were cut into pieces and homogenized in 1 ml of ice-cold GBR in a Potter-Elvehjelm homogenizer. In some experiments, the epithelial layer was stripped off, dispersed in 1 ml of GBR and homogenized. The remaining stroma/endothelium was treated in a similar way. One ml of $PGF_{2\alpha}$ or ester prodrugs (18.6 μM) were added to the homogenates and incubated at 37°C. At intervals (10, 20, 40, 80 and 120 min) the enzymatic conversion of the compounds was investigated by TLC.

Gas chromatography-mass spectrometry (GC-MS) analysis of $PGF_{2\alpha}$

$PGF_{2\alpha}$ was determined by a GC-MS procedure described in the literature (Axen et al., 1971; Gréen et al., 1973). In brief, the buffer samples from the permeability experiments with $PGF_{2\alpha}$ benzyl ester were acidified with 2 M HCl to pH 2–3 and extracted with ethyl acetate. A deuterated $PGF_{2\alpha}$ standard was added to the organic phase and the tris(trimethylsilyl ether) methyl ester derivatives were prepared. Analysis on a LKB 2091 GC-MS showed that the benzyl ester had been completely hydrolyzed during the penetration of the cornea, and no other metabolites could be detected.

Results

Partition coefficient (PC)

The PC for $PGF_{2\alpha}$, $PGF_{2\alpha}$ methyl ester and $PGF_{2\alpha}$ benzyl ester are expressed as log(octanol–water partition coefficients) (Table 1). They were calculated by:

$$PC = \frac{\text{conc. of drug in the } O_w \text{ phase}}{\text{conc. of drug in the } W_o \text{ phase}}$$

Since prostaglandin $F_{2\alpha}$ is ionizable, the distribution coefficient, DC, was used instead. The DC for $PGF_{2\alpha}$ was converted to a PC value. The PC values of the $PGF_{2\alpha}$ methyl and benzyl esters are about 100 and 1 000 times as high as the value of $PGF_{2\alpha}$, showing the increased lipophilicity of the prodrugs, mediated by the esterfication.

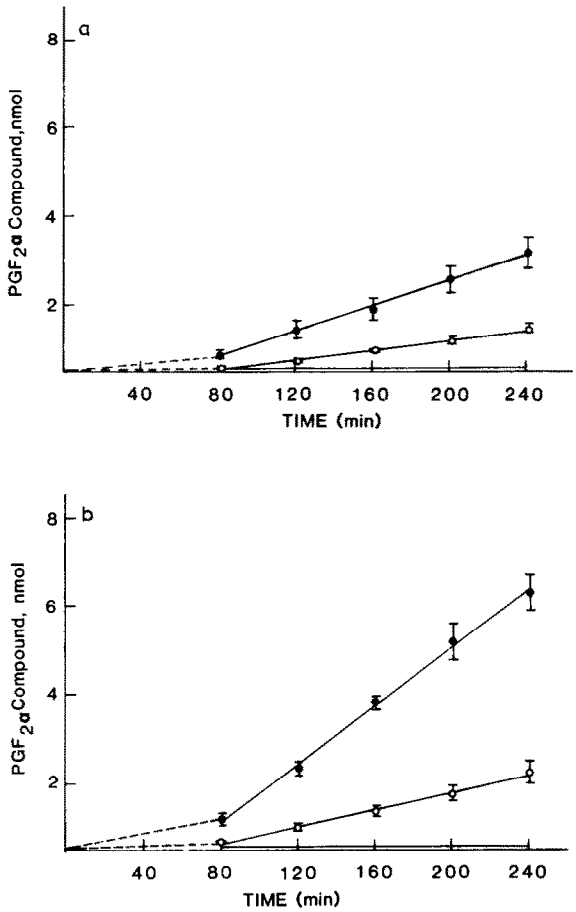


Fig. 1. Rate of permeability of prostaglandin $F_{2\alpha}$ compounds across excised pig cornea. The compounds used were: (●) $PGF_{2\alpha}$ benzyl ester; (○) $PGF_{2\alpha}$ methyl ester; and (–) $PGF_{2\alpha}$. The points were fitted by regression analysis and each point represents a mean of 6 determinations. The vertical bars indicate standard error of the mean. The initial concentrations of the compounds used in these experiments were: (a) 9.3 μM and (b) 18.6 μM.

Corneal permeability and absorption

The rate of permeability of the ester prodrugs and $PGF_{2\alpha}$ through the cornea are shown in Fig. 1a and b. The increase of radioactivity in the endothelial compartment was linear against time. When drug concentration was increased 2-fold, from 9.3 to 18.6 μM, on the epithelial side, the same magnitude of increase was seen on the endo-

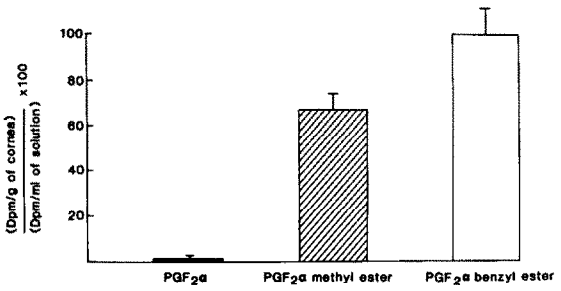


Fig. 2. Radioactivity in the cornea after 4 h perfusion. The compounds had the same initial concentration, 18.6 μM, on the epithelial side. 10 corneas were used for each compound. The bars represent standard error of the mean.

thelial side. The apparent permeability coefficient (P_{app}, cm/s) was determined according to:

$$P_{app} = \frac{\Delta Q}{\Delta t \cdot 60 \cdot A \cdot C_0}$$

where $\Delta Q/\Delta t$ is the permeability rate (i.e. steady-state flux) of radioactive molecules across each excised cornea, C_0 is the initial drug concentration (in these experiments 9.3 and 18.6 μM), A is the corneal surface area (in this study 1.33 cm^2), and 60 is the conversion of minutes to seconds. Corrections to C_0 were made to account for the sample volume removed over time and subsequent replacement with blank solution. For the two ester prodrugs used, the following P_{app} were determined: 8.87×10^{-6} cm/s and 26.20×10^{-6} cm/s for $PGF_{2\alpha}$ methyl and benzyl ester, respectively.

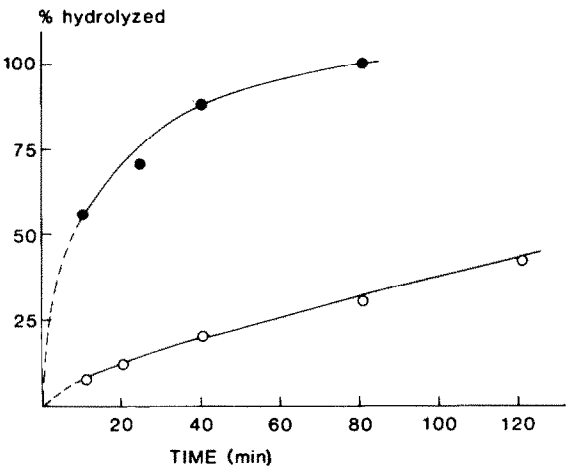


Fig. 3. Hydrolysis of $PGF_{2\alpha}$ benzyl ester by corneal epithelium (●) or stroma/endothelium (○). Each point represents the mean value of 3 determinations. The lines are drawn to better illustrate the hydrolytic capacity of the tissues.

These results indicate that the permeability rate of $PGF_{2\alpha}$ benzyl ester is about 3-fold faster than for $PGF_{2\alpha}$ methyl ester. No value was calculated for $PGF_{2\alpha}$ because of the very low permeability rate. The radioactivity measured was not significantly higher than the background. TLC and GC-MS analyses showed that both $PGF_{2\alpha}$ esters had been totally hydrolyzed during the passage through the cornea and no ester was detected on the endothelial side. Furthermore, no other metabolites except $PFG_{2\alpha}$ could be seen. Analysis of samples from the epithelial side showed both intact benzyl ester and free $PGF_{2\alpha}$. Approximately 50% of the benzyl ester had been hydrolyzed after 4 h perfusion, probably due to reflux of $PGF_{2\alpha}$ from the epithelium. Control experiments revealed that both esters were chemically and radiochemically stable in the perfusion medium (GBR and pH 7.65). No hydrolysis was seen after incubation for 4 h at 37°C and all radioactivity was found in the ethyl acetate phase after extraction. The amount of $PGF_{2\alpha}$ and its esters absorbed by the cornea after 4 h perfusion are shown in Fig. 2. It can be seen that the uptake is highly dependent of the lipophilicity of the compound used. Thus, both the methyl and the benzyl ester (measured as radioactivity) are efficiently concentrated in the cornea. About 25 and 40 times higher uptake is seen for the methyl and the benzyl ester, respectively, as compared with $PGF_{2\alpha}$.

Homogenate experiments

Incubation of the different cornea homogenates with 18.6 μM $PGF_{2\alpha}$ ester solutions indicated that hydrolysis of the esters occurred mainly in the epithelium. This is clearly shown with $PGF_{2\alpha}$ benzyl ester (Fig. 3). Hydrolysis of the $PGF_{2\alpha}$ methyl ester was faster than for the benzyl ester (Fig. 4a and b). Heat treatment of the homogenate eliminated the enzymatic activity completely.

Enzymatic, in vitro, hydrolysis of $PGF_{2\alpha}$ methyl and benzyl esters

To investigate the stability of the prostaglandin

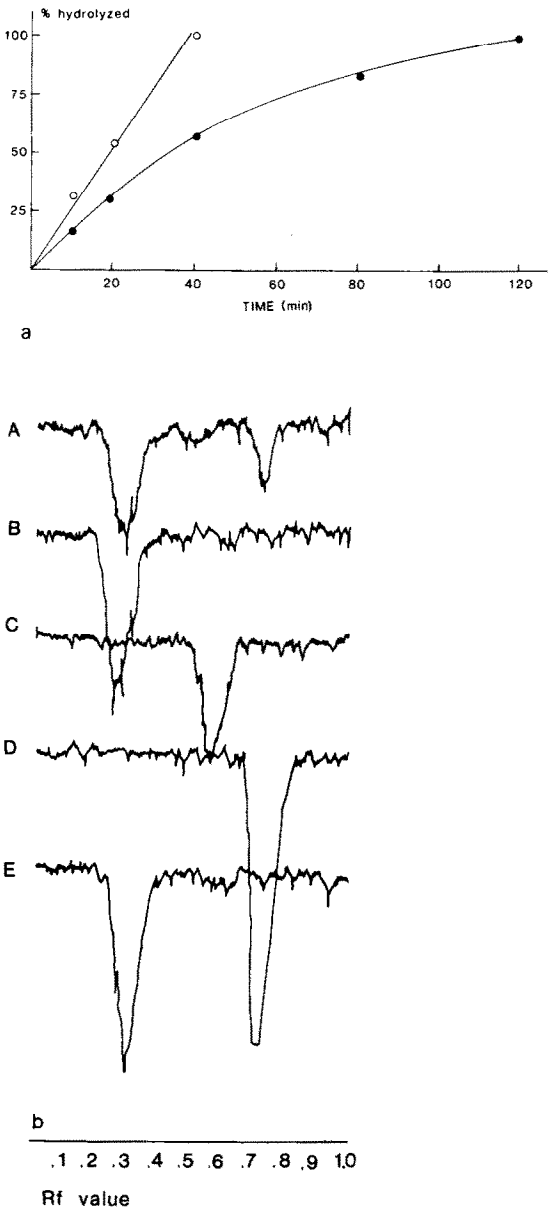


Fig. 4. Hydrolysis of $PGF_{2\alpha}$ methyl ester and benzyl ester by cornea homogenate. The same amounts of the compounds and the homogenate were used in each determination. Each point represents the mean value of two or three determinations. (a) shows the hydrolysis rate of (○) $PGF_{2\alpha}$ methyl ester and (●) $PGF_{2\alpha}$ benzyl ester. (b) reveals scans of thin-layer radiochromatograms and R_f values at 80 min incubation. A: $PGF_{2\alpha}$ benzyl ester. B: $PGF_{2\alpha}$ methyl ester incubated with homogenate. C: $PGF_{2\alpha}$ methyl ester. D: $PGF_{2\alpha}$ benzyl ester incubated without homogenate. E: $PGF_{2\alpha}$.

esters and also to identify a potential candidate responsible for the enzymatic hydrolysis of the esters during their passage through the cornea, some experiments were performed with carbonic anhydrase, acetylcholinesterase, and butyrylcholinesterase. These enzymes have unspecific esterase activity and it is known from the literature that carbonic anhydrase and cholinesterase activities are present in the cornea (Lönnerholm, 1974). Incubation with carbonic anhydrase did not affect the esters, and no traces of free $PGF_{2\alpha}$ were seen. Similar results were obtained with acetylcholinesterase. Butyrylcholinesterase, on the other hand, rapidly hydrolyzed both esters. Addition of a cholinesterase inhibitor, synstigimine methyl sulphate, reduced the enzyme activity. A parallel situation was seen when the inhibitor was added to the corneal homogenate.

Discussion

The present study shows that the methyl and benzyl esters of prostaglandin $F_{2\alpha}$ are more rapidly taken up by the pig cornea, in vitro, than the parent $PGF_{2\alpha}$.

The uptake is dependent on the lipophilicity of the substance. The benzyl ester with the greatest octanol–water partition coefficient has, as expected, the highest uptake. Not surprisingly, $PGF_{2\alpha}$ has a low permeability rate across the cornea. $PGF_{2\alpha}$ with a pK_a value of 4.9, will be almost completely ionized at the pH used. Molecules which are ionized at physiological pH do not pass biological membranes, except when hydrophilic canals or pores are available. Under normal conditions the passage of an ionizable substance across a membrane is determined mainly by the fraction of the substance that is unionized. As a result, the permeability of a completely ionized compound is negligible (Francoeur et al., 1983).

Our results, showing a relationship between the corneal permeability and the partition coefficient, are comparable with earlier work by Schoenwald and Ward (1978), Mosher and Mikkelson (1979) and Schoenwald and Huang (1983). Schoenwald and coworkers determined the permeability of steroids and β-blocking agents across rabbit cornea

and showed a relationship between log(permeability rate) and log(octanol–water partition coefficient). Mosher and Mikkelson used another method for depicting the lipophilicity. They plotted log(permeability) of *n*-alkyl-*p*-aminobenzoate esters across isolated rabbit corneas against the *n*-alkyl chain length.

Due to the high esterase activity in the cornea, the $PGF_{2\alpha}$ esters are hydrolyzed and only $PGF_{2\alpha}$ reaches the endothelial side of the perfusion apparatus. The rate of permeability of the two $PGF_{2\alpha}$ esters demonstrates linear kinetics, clearly shown in Fig. 1a and b. Furthermore, when the concentration of $PGF_{2\alpha}$ ester was increased 2-fold on the epithelial side, a concomitant increase of $PGF_{2\alpha}$ was seen on the endothelial side. No saturation was found at the highest concentration of esters, indicating a non-facilitated diffusion of the esters across the corneal epithelium. Similar results have been reported for a large number of compounds, e.g. pilocarpine, trifluorothymidine, idoxuridine and adenine arabinoside (O'Brien and Edelhauser, 1977; Francoeur et al., 1983).

It has been shown by Stern and Bito (1982), that topically administered $PGF_{2\alpha}$ decreases the IOP in cats and rhesus monkeys. As discussed previously, $PGF_{2\alpha}$ is an amphiphilic compound which is ionized at physiological pH, resulting in a low corneal permeability. Bito (1984), also used some derivatives of $PGF_{2\alpha}$ (e.g. the methyl ester) to investigate a possible increase in the ocular hypotensive potency. He found that $PGF_{2\alpha}$ esters reduce the IOP at doses which are much lower than for $PGF_{2\alpha}$ and assumed that the esters were hydrolyzed during their passage through the cornea. Our study has clearly shown that $PGF_{2\alpha}$ methyl and benzyl ester are effectively taken up by the cornea due to their lipophilicity and are then readily hydrolyzed by cornea-associated enzymes.

It is well documented that esterases are present in the cornea, especially in the epithelium (Redell et al., 1983). Experiments with homogenates from the pig cornea, the epithelium and the stroma/endothelium have shown that most of the esterase activity is located in the epithelium. The $PGF_{2\alpha}$ benzyl ester is more slowly hydrolyzed than the methyl ester and a plausible explanation might be that steric effects from the bulky benzyl group affect the rate of enzymatic hydrolysis. Nevertheless, both esters used in this study are mainly hydrolyzed in the epithelium and only free $PGF_{2\alpha}$ reaches the endothelial side, as shown by both GC-MS and TLC. Carbonic anhydrase and different cholinesterases have been reported to be present in the eye (Petersen et al., 1965; Lönnerholm, 1974). Since these enzymes have a documented unspecific esterase activity, we carried out some experiments to see if the above-mentioned enzymes contributed to the ester hydrolysis of $PGF_{2\alpha}$ methyl and benzyl ester. Carbonic anhydrase and acetylcholinesterase had no effect on the esters. Butyrylcholinesterase had a large effect and both esters were hydrolyzed to $PGF_{2\alpha}$. Addition of Neostigmine to cornea homogenate reduced the hydrolytic activity. Furthermore, heat treatment of the homogenate destroyed the esterase activity. These findings taken together indicate that the esterase activity is mediated via a heat-labile enzyme, probably butyrylcholinesterase, which is partially inhibited by cholinesterase inhibitors. Although there are reports of other metabolic processes in the eye, we could not find any other metabolites of the $PGF_{2\alpha}$ esters than the free acid.

In summary, $PGF_{2\alpha}$ esters are functional prodrugs increasing the permeability across the cornea. Furthermore, the esters are principally hydrolyzed by esterases located in the epithelium giving $PGF_{2\alpha}$ on the endothelial side.

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References

Akers, M.J., Schoenwald, R.D. and McGinity, J.W., Practical aspects of ophthalmic drug development. Drug. Dev. Ind. Pharm., 3 (1977) 185–217.

Axen, U., Gréen, K., Hörlin, D. and Samuelsson, B., Mass spectrometric determination of picomole amounts of prostaglandins E_2 and $F_{2\alpha}$ using synthetic deuterium labeled carriers. Biochem. Biophys. Res. Commun., 45 (1971) 519–525.

Bito, L.Z., Comparison of the ocular hypotensive efficacy of Eicosanoids and related compounds. Exp. Eye. Res., 38 (1984) 181–194.

Camber, O., An in vitro model for determination of drug permeability through the cornea. Acta Pharm. Suec., 22 (1985) 335–342.

Camras, C.B. and Bito, L.Z., Reduction of intraocular pressure in normal and glaucomatous primate (*Aotus trivirgatus*) eyes by topically applied prostaglandin $F_{2\alpha}$. Curr. Eye Res., 1 (1981) 205–209.

Chrai, S.S., Patton, T.F., Mehta, A. and Robinson, J.R., Lacrimal and instilled fluid dynamics in rabbit eyes. J. Pharm. Sci., 62 (1973) 1112–1121.

Chrai, S.S., Makoid, M.C., Eriksen, S.P. and Robinson, J.R., Drop size and initial dosing frequency problems of topically applied ophthalmic drugs. J. Pharm. Sci., 63 (1974) 333–338.

Francoeur, M., Ahmed, I., Sitek, S. and Patton, T.F., Age-related differences in ophthalmic drug disposition III. Corneal permeability of pilocarpine in rabbits. Int. J. Pharm., 16 (1983) 203–213.

Green, K. and Downs, S.J., Ocular penetration of pilocarpine in rabbits. Arch. Ophthalmol., 93 (1975) 1165–1168.

Green, K., Granström, E., Samuelsson, B. and Axen, U., Methods for quantitative analysis of $PGF_{2\alpha}$, PGE_2, 9α, 11α-dihydroxy-15-keto-prost-5-enoic acid 9α,11α,15-trishydroxy-prost-5-enoic acid from body fluids using deuterated carriers and gas chromatography-mass spectrometry. Anal. Biochem., 54 (1973) 434–453.

Kaback, M.B., Podos, S.M., Harbin, T.S., Jr., Mandell, A. and Becker, B., The effect of dipivalyl epinephrine on the eye. Am. J. Ophthalmol., 81 (1976) 768–772.

Kass, M.A., Mandell, A.I., Goldberg, I., Paine, J.M. and Becker, B., Dipivefrin and epinephrine treatment of elevated intraocular pressure. A comparative study. Arch. Ophthalmol., 97 (1979) 1865–1866.

Lönnerholm, G., Carbonic anhydrase in the cornea. Acta physiol. scand., 90 (1974) 143–152.

Mosher, G.L. and Mikkelson, T.J., Permeability of the *n*-alkyl *p*-aminobenzoate esters across the isolated corneal membrane of the rabbit. Int. J. Pharm., 2 (1979) 239–243.

O'Brien, W.J. and Edelhauser, H.F., The corneal penetration of trifluorothymidine, adenine arabinoside, and idoxuridine: a comparative study. Invest. Ophthalmol., 16 (1977) 1093–1103.

Petersen, R.A., Lee, K-J. and Donn, A., Acetylcholinesterase in the rabbit cornea. Arch. Ophthalmol., 73 (1965) 370–377.

Quigley, H.A., Pollack, I.P. and Harbin, T.S., Pilocarpine Ocuserts, Long term clinical trials and selected pharmacodynamics. Arch. Ophthalmol., 93 (1975) 771–775.

Redell, M.A., Yang, D.C. and Lee, V.H.L., The role of esterase activity in the ocular disposition of dipivalyl epinephrine in rabbits. Int. J. Pharm., 17 (1983) 299–312.

Schoenwald, R.D. and Huang, H.-S., Corneal penetration behavior of β-blocking agents. I: Physiochemical factors. J. Pharm. Sci., 72 (1983) 1266–1272.

Schoenwald, R.D. and Ward, R.L., Relationship between steroid permeability across excised rabbit cornea and octanol-water partition coefficients. J. Pharm. Sci., 67 (1978) 786–788.

Stern, F.A. and Bito, L.Z., Comparison of the hypotensive and other ocular effects of prostaglandins E_2 and $F_{2\alpha}$ on cat and rhesus monkey eyes. Invest. Ophthalmol., 22 (1982) 588–598.

Wei, C.-P., Anderson, J.A. and Leopold, I., Ocular absorption and metabolism of topically applied epinephrine and a dipivalyl ester of epinephrine. Invest. Ophthamol., 17 (1978) 315–321.