ELSEVIER

International Journal of Pharmaceutics 127 (1996) 85–94

international journal of pharmaceutics

Rate control of ocular pilocarpine delivery with bispilocarpic acid diesters

Pekka Suhonen[a,*], Tomi Järvinen[b], Kari Lehmussaari[c], Timo Reunamäki[c], Arto Urtti[a]

[a]*Department of Pharmaceutics, University of Kuopio, PO Box 1627, FIN-70211 Kuopio Finland*
[b]*Department of Pharmaceutical Chemistry, University of Kuopio, PO Box 1627, FIN-70211 Kuopio Finland*
[c]*Leiras OY, PO Box 33, FIN-33721 Tampere Finland*

Received 27 March 1995; revised 12 June 1995; accepted 25 June 1995

Abstract

Ocular delivery of pilocarpine as bispilocarpic acid diester prodrugs was studied in albino rabbits using miosis as a bioassay of pilocarpine availability in the iris. Bispilocarpic acid diester fumarate eyedrops, in a dose equivalent to 0.5% pilocarpine, were administered at pH 5.0. Bispilocarpic acid diesters increased the duration of miosis and decreased the peak miotic response of pilocarpine. The time of peak miosis was delayed from 40 min to 55–135 min with the prodrugs. The duration of action was extended with seven compounds from 3 h to 4–5 h. Plateauing responses indicating sustained release of pilocarpine from the prodrug were seen in some cases. Compared with 1% pilocarpine, the prodrugs showed either increased, decreased or equal biphasic availability of pilocarpine in the iris. Neither lipophilicity nor enzymatic lability of prodrug alone could explain the miosis profile of pilocarpine. Eye irritation increased with increasing lipophilicity of the prodrugs. It appears that the ocular bioavailability of pilocarpine and its duration of action can be improved by bispilocarpic acid diesters, but in predicting their performance both lipophilicity and prodrug cleavage rate should be taken into account.

Keywords: Pilocarpine; Pilocarpine prodrugs; Bispilocarpic acid diesters; Miotic activity; Irritation; Ocular drug delivery; Ocular bioavailability

1. Introduction

Pilocarpine eyedrops are widely used in the treatment of glaucoma (Newell, 1986). Due to its hydrophilicity and rapid precorneal elimination, only a small fraction of pilocarpine absorbs into the eye (Chrai and Robinson, 1974), and therefore, relatively high drug concentrations, up to 4% must be administered. Poor absorption and rapid elimination from the inner eye necessitate frequent administration, three to six times daily. These factors increase the frequency and intensity of ocular and systemic side-effects, i.e. browache, miosis, accommodation problems, salivation, and even respiratory problems (Brown et al., 1976;

* Corresponding author.

SSDI 0378-5173(95)04202-L

Zimmerman, 1981). Frequent administration and side-effects of pilocarpine eyedrops impair patient compliance (Norell, 1981).

The need to improve the properties of ophthalmic pilocarpine has stimulated the research for long acting sustained release ophthalmic dosage forms. These dosage forms include gels (March et al., 1982), thermosetting eyedrops (Gurny et al., 1987), liposomes (Benita et al., 1984), nanoparticles (Diepold et al., 1989), polymer matrices (Maichuk, 1975; Urtti et al., 1984), and Ocusert reservoir inserts (Armaly and Rao, 1973). The prolonged acting dosage forms of pilocarpine improve the ocular bioavailability and duration of action, and in some cases, they release the drug at a controlled rate so that high pilocarpine peak concentrations and the related side-effects are decreased. Due to pharmaceutical and practical difficulties, e.g. poor retention of Ocuserts in the eye, the controlled release systems have not gained widespread use, but many advantages of controlled ocular pilocarpine delivery are evident.

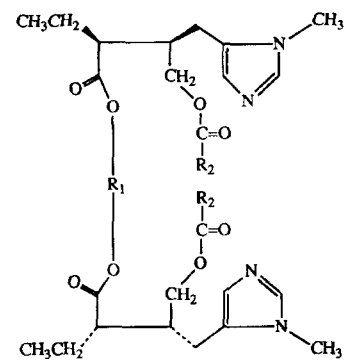
Prodrugs have been used to improve the ocular absorption of pilocarpine (Mosher et al., 1987), epinephrine (Hussain and Truelove, 1976; Bodor and Visor, 1984), phenylephrine (Chien and Schoenwald, 1986; Schoenwald and Chien, 1988), prostaglandins (Bito, 1984) and timolol (Bundgaard et al., 1988). Over the past few years we have synthesized and studied in vitro several pilocarpine prodrugs that showed improved corneal permeability (Suhonen et al., 1991a; Suhonen et al., 1991b). These prodrugs, bispilocarpic acid diesters, contain two pilocarpic acids linked with a spacer chain. Pilocarpine is released from the prodrug in sequential manner, when an enzymatic esterase catalyzed reaction first exposes the labile monoester intermediate, which is further hydrolyzed, and upon closure of the lactone ring pilocarpine is formed (Järvinen et al., 1991b).

In this study, we evaluated corneal permeability in vitro and pilocarpine delivery in vivo to the rabbit eyes with bispilocarpic acid diesters. The miotic response in albino rabbits was used as a bioassay of ocular pilocarpine delivery.

2. Materials and methods

2.1. Materials

Pilocarpine hydrochloride was obtained from Leiras Pharmaceuticals, Tampere, Finland and isopilocarpine nitrate was purchased from Aldrich Chemie (Germany). Synthesis and analysis of bispilocarpic acid diester fumarates have been described previously (Järvinen et al., 1991a; Järvinen et al., 1995b). The structures of prodrugs studied in this study are shown in Fig. 1. They are *O,O'*-(1,2-ethylene) bispilocarpic acid diesters (I, II and



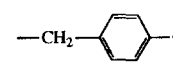
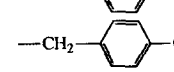
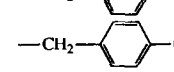
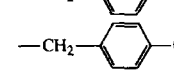
Compound	R_1	R_2
I	—CH_2-CH_2—	—CH_2-CH_3
II	—CH_2-CH_2—	
III	—CH_2-CH_2—	—C(CH_3)$_2$-CH_3
IV	—CH_2-CH_2-CH_2—	—CH_2-CH_3
V	—CH_2-CH_2-CH_2—	
VI	—CH_2-CH_2-CH_2—	
VII	—CH_2-  -CH_2—	—CH_2-CH_3
VIII	—CH_2-  -CH_2—	—CH_2-CH_2-CH_3
IX	—CH_2-  -CH_2—	
X	—CH_2-  -CH_2—	—C(CH_3)$_2$-CH_3
XI	—CH_2-$(CH_2)_4$-CH_2—	

Fig. 1. The chemical structures of bispilocarpic acid diesters studied.

III), *O,O'*-(1,3-propylene) bispilocarpic acid diesters (IV, V and VI), *O,O'*-(1,4-xylylene) bispilocarpic acid diesters (VII, VIII, IX and X) and *O,O'*-(1,6-hexylene) bispilocarpic acid diester (XI). HPLC-grade methanol was from J.T. Baker (Deventer, The Netherlands). All other chemicals were reagent grade. Glutathione buffered Ringer's (GBR) solution was prepared as described previously (Suhonen et al., 1991a).

2.2. In vitro drug corneal penetration experiment

Both sexes of adult New Zealand albino rabbits, weighing 2.3–4.2 kg were used. After euthanization by intravenous injection of T-61 vet. (Hoechst, Munich, Germany), excised rabbit corneas were mounted on holding rings and placed between two diffusion chambers (Suhonen et al., 1991a). GBR solution (3.4 ml), preadjusted to pH 7.65, was added to the receiver chamber (endothelial side), and immediately thereafter, 3.2 ml of GBR containing 150–572 μM of pilocarpine or 39–148 μM of prodrug, were added to the donor chamber (epithelial side). Otherwise the permeability study including the calculation of the permeability coefficient, and pilocarpine and prodrug analysis (high performance liquid chromatography, HPLC) was carried out as described earlier (Suhonen et al., 1991a).

2.3. Apparent partition coefficients

The 1-octanol/phosphate buffer (0.16 M, pH 5.0) apparent partition coefficients were determined by using the previously described method (Järvinen et al., 1991b).

2.4. Release of pilocarpine from the prodrugs

Pilocarpine is released in a two-step process. First esterase catalyzed cleavage of the double prodrug to monoester. Thereafter, the monoester is chemically hydrolyzed. The rate of enzymatic hydrolysis was determined in vitro as described earlier (Järvinen et al., 1995b). Briefly, rabbit corneas were homogenized, to 500 μl of homogenate 0.12 μmol of prodrug in Tris buffer (0.05 M, pH 7.4) was added, and the mixture was incubated at 37°C and sampled at fixed intervals. The samples were centrifuged and the prodrug concentration was determined using RP-HPLC. The half-lives ($t_{1/2}$) of the prodrugs in the homogenate were calculated from the linear regression slopes of semi-logarithmic plots.

Time of 50% pilocarpine formed ($f_{50\%}$) was determined using STELLA 2.2.2. simulation software (High Performance Systems Inc., Hanover, USA). In the model, double prodrug is first degraded to monoester and then further to pilocarpine. In the simulations the rate constants for enzymatic and chemical hydrolysis steps were from the experiments, where the rates of enzymatic hydrolysis of diester and chemical hydrolysis of monoester were followed in human plasma (Järvinen et al., 1995b) and in buffer solution (pH 7.4), respectively (Järvinen et al., 1992). When these hydrolysis rates are known the rate of pilocarpine formation from bispilocarpic acid diester was simulated.

2.5. Eyedrop preparation

Bispilocarpic acid diester fumarate was dissolved in 0.5% NaCl solution, and the solution was adjusted to pH 5.0, and isotonicity. Osmolality of the buffer solutions were measured with Auto-Osmometer OSMOSTAT OM-6020 (Kyoto Daiichi, Kagaku Co. Ltd, Japan). The prodrug solutions were equivalent to 0.5% pilocarpine (i.e. contain the same amount of pilocarpine molecules), except *O,O'*-dibutyryl (1,4-xylylene) bispilocarpic acid diester (VIII) and *O,O'*-dipivaloyl (1,4-xylylene) bispilocarpic acid diester (X), which were equivalent to 0.25% pilocarpine due to the low aqueous solubility of the prodrugs. The concentration range of the prodrug solutions was 6.26–12.47 mg/ml. Pilocarpine hydrochloride (equivalent to 1% pilocarpine base) was dissolved in 0.5% NaCl and the solution was adjusted to pH 5.0 and isotonicity. The control solution was 0.9% NaCl at pH 5.0. Distilled water was used in all formulations.

2.6. Miosis tests

Male and female New Zealand white rabbits,

weighing 1.9–4.2 kg, were used without any special pretreatment diets. Six rabbits were used in each experiment. All measurements were carried out in the same room at constant level of illumination. The rabbits were accustomed to this environment for 1 h before the experiment. All animal experiments were carried out in accordance with the ARVO Resolution on the Use of Animals in Experimentation.

Eyedrops of 25 μl were instilled onto the superior sclera of the right eye, while the left eye remained as a control. During instillations the upper lid was slightly pulled away from the globe. Washout of at least 48 h was allowed between experiments. The experiments were carried out in a 6 × 6 masked cross-over and age-randomized manner using two groups of six rabbits. The eyes were photographed before the drug administration and at fixed intervals during 6 h. The negatives were enlarged with a microfilm reflector and the pupillary diameters were measured from magnified images. The changes were expressed as percentage decrease of the mean of horizontal and vertical pupillary diameters.

Peak miosis (I_{max}), its time of appearance (peak time) and the area under the miotic response vs. time curve ($AUC_{0-\infty}$) were determined. I_{max} and peak time were determined from the actual data points. $AUC_{0-\infty}$ was calculated using the trapezoidal method (Gibaldi and Perrier, 1982). Duration of miosis was the period with miosis of 3% or more.

2.7. Irritation test

Discomfort of the rabbit eyes was graded, so that slight irritation was characterized by half-closed eyelids and severe irritation by firm closure of the eye. The eyelid closure was expressed as the sum of full closure and half-closure times of the eyes. Mucoidal discharge was scored from 0 to 2, where 0 is normal, any clear discharge different from normal is 1, and milky discharge moistening the lids scores 2.

2.8. Statistical analysis

The statistical significance of the differences in the pharmacokinetic parameters between pilocarpine and the prodrugs were evaluated by Fisher's PLSD test ($P < 0.05$).

3. Results

3.1. Lipophilicity and prodrug cleavage

At pH 5.0 apparent log PC of pilocarpine was − 1.74. All prodrugs had greater lipophilicity than pilocarpine. Their log PC values ranged from − 0.77 to 2.49 at pH 5.0 (Table 1).

In corneal homogenates the prodrugs hydrolyzed at half-lives ranging from 3 min to 97 min, and in the pH 7.4 buffer the intermediate monoesters hydrolyzed at half-lives 10–150 min. Values of $f_{50\%}$ were 17–172 min (Table 1).

3.2. In vitro permeability

The apparent permeability coefficient of pilocarpine was $2.8 \pm 0.3 \times 10^{-6}$ cm/s. Pilocarpine permeability, when given as bispilocarpic acid diesters was $1.7–20.2 \times 10^{-6}$ cm/s (Table 1) depending on the prodrug structure. The best permeability (compound VII) is more than 7-fold compared with pilocarpine. HPLC analysis showed that bispilocarpic acid diesters completely hydrolyzed in the cornea. The corneal permeabilities of the prodrugs increased with increasing lipophilicity (Fig. 2). According to the previously published results with greater range of lipophilicity of pilocarpine prodrugs (Suhonen et al., 1991a,b), a parabolic relationship between the corneal permeabilities and lipophilicity was shown. Therefore parabolic fit of the data was carried out (Fig. 2). The optimal lipophilicity for improving corneal permeability, in terms of log PC, was 0.5–1 at pH 5.0. Our results show that in vitro corneal permeability is increased when pilocarpine formation rate is increased. However, the rate of pilocarpine formation alone from prodrugs ($f_{50\%}$) does not predict the corneal permeability ($r^2 = 0.18$) (Fig. 3).

3.3. Miotic response

Fig. 4 shows the plots of the mean observed changes in pupillary diameter as a function of

Table 1
Lipophilicity (log PC), enzymatic lability ($t_{1/2}$ in cornea homogenate and at pH 7.4 phosphate buffer, and $f_{50\%}$) and in vitro apparent corneal permeability (P_{app}) of pilocarpine and pilocarpine prodrugs

Compound	LogPC[a] (pH 5.0)	$t_{1/2}$ in cornea[b] homogenate (min)	$t_{1/2}$ at pH 7.4[c] buffer (min)	$f^{d}_{50\%}$ (min)	$P_{app} \times 10^{-6}$[e] (cm/s)
Pilocarpine	−1.74	-	-	-	2.8 ± 0.3
I	−0.77	14	10	29	1.7 ± 0.3
II	0.30	4	10	17	9.0 ± 0.9
III	0.80	97	10	113	6.5 ± 0.5
IV	−0.61	10	72	88	3.5 ± 0.2
V	−0.30	16	72	98	3.2 ± 0.7
VI	0.60	4	72	78	14.7 ± 1.5
VII	0.66	3	18	23	20.2 ± 1.2
VIII	1.69	3	18	23	14.3 ± 1.7
IX	1.04	16	18	41	10.4 ± 1.9
X	2.49	39	18	67	-
XI	0.88	14	150	172	6.4 ± 0.8

[a]Apparent partition coefficients between octanol and phosphate buffer solution of pH 5.0. Data from Järvinen et al., 1995b, except compounds III and X.[b]Half-lives of bispilocarpic acid diester degradation at pH 7.4 and 37°C. Data from Järvinen et al., 1995b, except compounds I–III and X.[c]Half-lives of monoester prodrugs at 0.16 M phosphate buffer at 37°C. Data from Järvinen et al., 1992.[d]$f_{50\%}$ equals simulated times, when 50% of total pilocarpine is formed. $f_{50\%}$ values for the prodrugs were calculated by the Stella® simulation program.[e]Apparent corneal permeability (mean ± S.E., n=2-6).

time following the instillation of 25 μl of pilocarpine and prodrug solutions. Pilocarpine caused rapid miotic response peaking at 40 ± 5 min. The maximal response was 25 ± 4%. $AUC_{0-\infty}$ was 2547 ± 922% · min. After the peak miosis the response decreased rapidly to the baseline so that the duration of action (> 3%) was 3 h (Table 2).

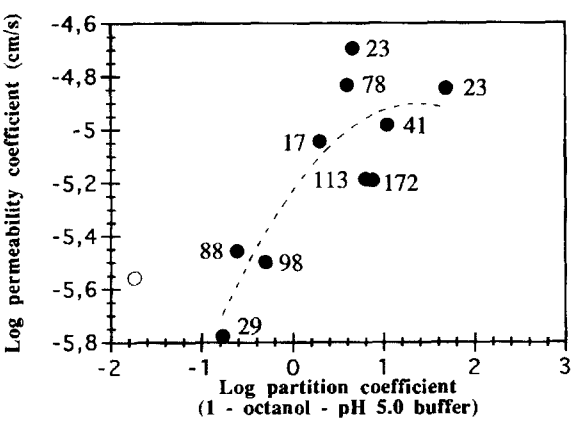


Fig. 2. The relationship between the log corneal permeability coefficient (pH 7.65) of pilocarpine (○) and prodrogs (●) and their respective log apparent partition coefficient (1-octanol-pH 5.0 phosphate buffer). The numbers beside the dots represent the $f_{50\%}$ values (time when 50% of total pilocarpine is formed).

All prodrugs showed delayed and decreased peak miosis, when compared with pilocarpine (Table 2). In two cases (III and X) both the duration of activity and $AUC_{0-\infty}$ were increased significantly (Table 2). The general shapes of the miosis-time profiles are different and the apparent elimination phases from various prodrug derivatives suggest different rates of pilocarpine delivery behaviour. The magnitude of miotic response and the duration of miotic activity to pilocarpine prodrugs were significantly altered by changing the substituents R_1 and R_2 (Fig. 1). The ocular bioavailability of bispilocarpic acid diesters as measured by $AUC_{0-\infty}$ and their duration of activity appear to increase with increasing lipophilicity ($r^2 = 0.67$ and 0.54, respectively) (Fig. 5 and Fig. 6).

With different spacer chains (R_1) (Fig. 1), it was possible to obtain bispilocarpic acid diesters with various lipophilicities and different corneal absorptions. For example, changing the spacer chain (R_1), lipophilicities of the *O,O'*-dipropionyl bispilocarpic acid diesters appear to increase from

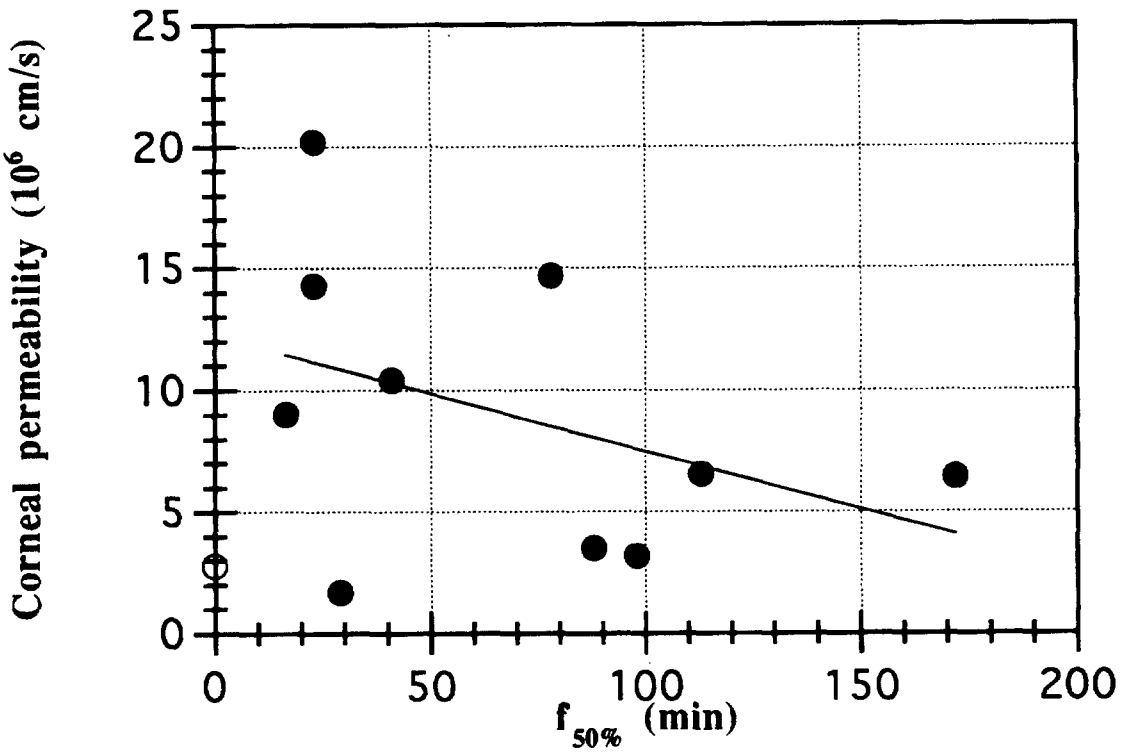


Fig. 3. The relationship between corneal permeability coefficient of pilocarpine (○) and prodrugs (●) and $f_{50\%}$ (time when 50% of total pilocarpine is formed).

−0.77 (I) to −0.61 (IV) and 0.66 (VII). Similar rank order was found in their corneal permeabilities. Also, for *O,O'*dicyclopropionyl bispilocarpic acid diesters (V, IX, and XI), similar rank orders were found in their lipophilicities, permeabilities and $AUC_{0-\infty}$ values. Also, the R_2 moiety affects the lipophilicity and absorption of the pilocarpine prodrugs (Table 1 and Table 2).

Enzymatic degradation of bispilocarpic acid diesters can be controlled by changing the R_2 moiety ($t_{1/2} = 3 - 97$ min) (Table 1), but at the same time also lipophilicity is changed, which makes differentiation of the effects on $AUC_{0-\infty}$ difficult (Table 1 and Table 2). Neither the enzymatic degradation rate nor lipophilicity of the prodrugs determines the miotic response alone. Miotic response is susceptible to both of them. $AUC_{0-\infty}$ and permeability appear to increase with decreasing $t_{1/2}$ of diester degradation ($r^2 = 0.30$ and 0.47, respectively) and decreasing $f_{50\%}$ ($r^2 = 0.003$ and 0.18, respectively). In spite of these tendencies, the miotic $AUC_{0-\infty}$ showed poor correlation with permeability ($r^2 = 0.10$).

Due to low solubility, the compounds VIII and X were prepared equimolar to 0.25% pilocarpine. Despite the smaller concentration, compound X produced the highest $AUC_{0-\infty}$ and the longest duration of action. A plateauing response of 2 h was observed for compound X (Fig. 4). Interestingly, prodrugs I and XI showed relative inactivity. Compound I was the most hydrophilic of the prodrugs and not able to absorb sufficiently to the lipophilic corneal epithelium (Table 1 and Table 2). On the other hand, compound XI has greater lipophilicity than pilocarpine (log PC 0.88), but enzymatic and chemical hydrolysis are too slow (14 and 150 min (Table 1), respectively) in order to achieve adequate miotic response (Table 2).

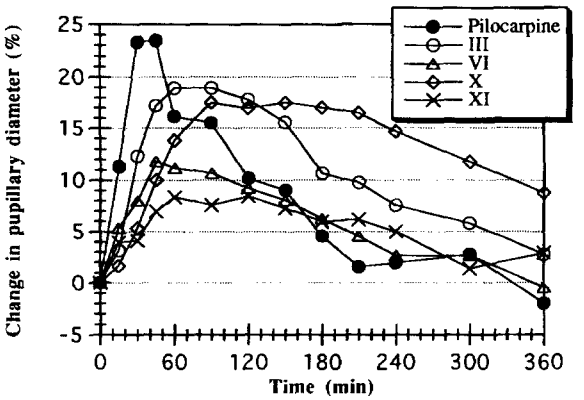


Fig. 4. Plot of change in pupillary diameter (%) as a function of time following a 25 μl topical administration of unbuffered 1% pilocarpine hydrochloride (Pilocarpine), and unbuffered 0.5% prodrugs *O,O'*-dipivaloylcarbonyl (1,2-ethylene) bispilocarpate (compound III), *O,O'*-dicyclobutyrylcarbonyl (1,3-propylene) bispilocarpate (VI), *O,O'*-dipivaloylcarbonyl (1,4-xylylene) bispilocarpate (X) and *O,O'*-dicyclopropylcarbonyl (1,6-hexylene) bispilocarpate (XI). S.E. lines were omitted for clarity. All S.E. values were smaller than ± 5.1%, $n = 6$.

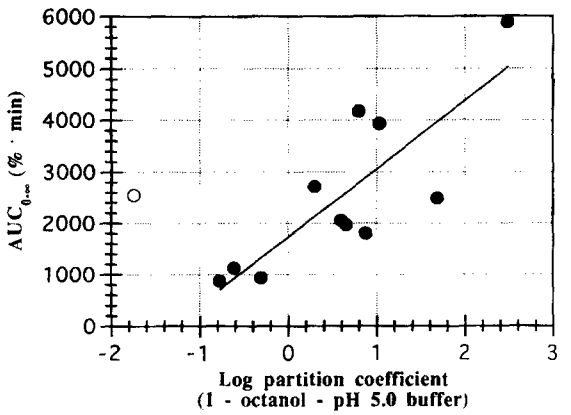


Fig. 5. The relationship between log apparent partition coefficient (1-octanol-pH 5.0 phosphate buffer) and the area under the curve ($AUC_{0-\infty}$, % · min) for pilocarpine (○) and prodrugs (●). The prodrug solutions were equivalent to 0.5% pilocarpine, except compounds VIII and X, which were equivalent to 0.25% pilocarpine.

3.4. Irritation

Pilocarpine (1%) caused some ocular irritation (Table 2). Prodrugs I and IV were better tolerated than 1% pilocarpine. The rabbits did not close their eyes and the eyes were half-closed for, at most, 0.2 min. All the other compounds showed similar or stronger (X) irritation than 1% pilocarpine at pH 5.0 (Table 2).

Pilocarpine and prodrugs II, V and VII did not cause mucoidal discharge in the rabbits. Slight

Table 2
Pharmacokinetic parameters of the miotic response and eyelid closure after topical administration of the compounds tested. Each value represents the mean ± S.E. of six to seven experiments. Pilocarpine indicates 1% pilocarpine as a control

Compound	T^{a}_{max} (min)	I^{b}_{max} (%)	$AUC^{c}_{0-\infty}$ (% · min)	Duration[d] (min)	Eyelid closure[e] (min)
Pilocarpine	40±5	25±4	2547±922	184±45	2.8±1.0
I	90±17*	8±1*	869±269*	171±31	0.2±0.1*
II	60±7	16±3*	2709±744	216±40	1.0±0.4
III	78±7	20±2	4172±513*	329±18*	1.5±0.5
IV	81±13*	9±2*	1124±154	139±34	0.1±0.1*
V	83±13*	8±1*	935±307*	172±33	1.9±1.1
VI	55±7	13±1*	2055±255	250±33	3.1±1.3
VII	135±17*	16±2*	1976±242	223±21	3.6±1.0
VIII	73±8	15±2*	2484±508	240±35	3.4±1.1
IX	71±17	15±2*	3931±456	310±26	1.8±0.4
X	120±15*	18±2*	5886±803*	339±4 *	6.9±1.9*
XI	68±13	9±1*	1800±384	245±34	4.0±2.6

[a]The time at which the maximum miosis was obtained.[b]The maximum change in pupillary diameter.[c]The area under the miosis vs. time curve.[d]The duration of the miotic effect was calculated as 3% change over the baseline.[e]Eyelid closure was expressed as the sum of full closure and half-closure times of the eyes.*Significantly different from the values of pilocarpine by Fisher's PLSD test ($P < 0.05$).

mucoidal discharge (mean value $\leq$ 0.5) was caused by I, IV and IX. More severe discharge followed after instillation of III, VI, VIII, X and XI, the score values being 0.6 $\pm$ 0.3, 0.9 $\pm$ 0.4, 1.3 $\pm$ 0.4, 0.6 $\pm$ 0.2 and 1.5 $\pm$ 0.3, respectively.

4. Discussion

Miotic activity was used in our experiments as a bioassay of pilocarpine availability in the iris sphincter muscle. Thus, if prodrug delivers active pilocarpine into the eye, it should cause miotic response. Pilocarpine formation from the bispilocarpic acid diesters has been shown previously by Järvinen et al. (1991b). In this study we assume that neither prodrug nor its intermediate hydrolytic products show muscarinic activity and that observed miotic activities reflect pilocarpine concentration in the iris.

Pilocarpine prodrugs produce different miotic response profiles than pilocarpine (Fig. 4). This is understood on the basis of the underlying kinetic processes. Pilocarpine is absorbed through the cornea into the eye and the corneal epithelium serves both as a barrier and depot regulating the diffusion of pilocarpine (Chrai et al., 1973). In the case of bispilocarpic acid diesters, however, the rate of pilocarpine delivery into the aqueous humor and iris is slowed by the enzymatic and chemical hydrolysis of the prodrug. Therefore, the peak time is delayed and I_{max} is decreased with the prodrugs compared with pilocarpine (Table 2). Pilocarpine delivery into the iris was controlled by the formation of pilocarpine from the prodrugs. These kinetics appear to be favourable, since the peak pilocarpine concentrations in the eye are decreased and, probably, the associated side-effects may be reduced. Also, the longer duration of activity is an advantage over pilocarpine (Table 2). It should, however, be remembered that extrapolation of these results (peak response, duration of miosis) to the clinical situation and to the treatment of glaucoma is difficult and further studies are required.

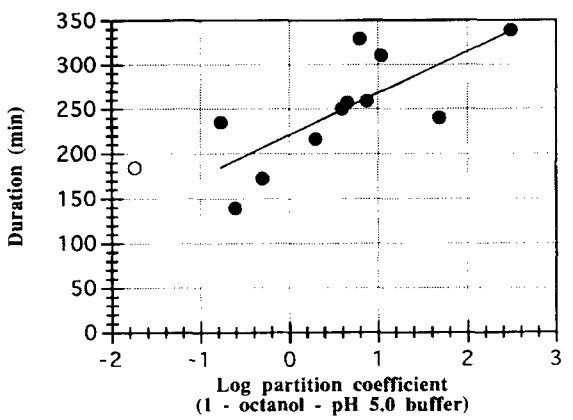


Fig. 6. The relationship between log apparent partition coefficient (1-octanol-pH 5.0 phosphate buffer) and duration (the time when miosis is 3% or more) for pilocarpine (○) and prodrugs (●). The prodrug solutions were equivalent to 0.5% pilocarpine, except compounds VIII and X, which were equivalent to 0.25% pilocarpine.

Increased lipophilicity of the prodrugs (Table 1) is expected to increase the absorption of prodrug into the eye. Increased partitioning of the prodrugs to the cornea at neutral pH has been shown in our previous studies (Suhonen et al., 1991b). Comparison of in vitro permeability and absorption of the prodrugs in vivo is complex, because the eyedrop neutralizes on the ocular surface and pH affects the partitioning of the prodrug to the cornea. Therefore, the in vitro permeability does not necessarily predict prodrug absorption in vivo.

The half-lives of the prodrugs in cornea homogenates vary between 3 and 97 min (Table 1). Interestingly, our results suggest a poor correlation between the half-life of the prodrugs in the cornea homogenate and corneal permeability ($r^2 = 0.47$) and miotic $AUC_{0-\infty}$ ($r^2 = 0.30$). This suggests that the enzymatic degradation does not control the amount of pilocarpine available in the iris. Furthermore, enzymatic degradation in the corneal homogenate did not correlate with the duration of miotic activity ($r^2 = 0.34$), also pointing out that enzymatic degradation alone is a poor predictor of corneal penetration. Correlations were also attempted between the $AUC_{0-\infty}$ and the pilocarpine formation rate ($f_{50\%}$), but the correlation was poor ($r^2 = 0.30$). Also the rate of chemical hydrolysis did not correlate with the $AUC_{0-\infty}$ ($r^2 = 0.003$) or with the duration of miotic activity ($r^2 = 0.01$). Consequently, the biophasic availability of pilocarpine in the iris seems

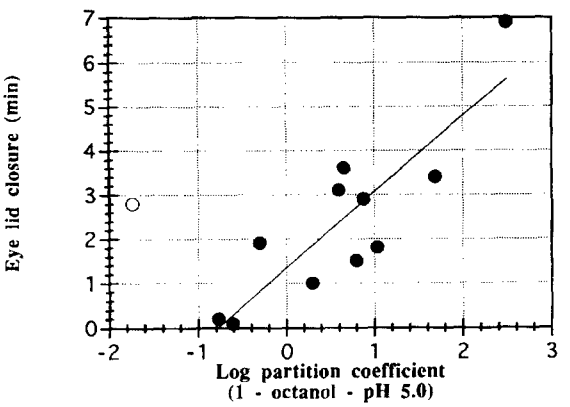


Fig. 7. The relationship between log apparent partition coefficient (1-octanol-pH 5.0 phosphate buffer) and irritation (the sum of full closure and half-closure times of the eyes) for pilocarpine (○) and prodrugs (●).

to be a very complex phenomenon that cannot be predicted with a single parameter. It seems, however, that log PC is a reasonably good predictor. Deviations from parabolic relationships seem to be related to the kinetics of pilocarpine formation.

A fairly good correlation was observed between the eyelid closure (half-open plus closed) times with the lipophilicity of the compound (Fig. 7). Irritation limits the usefulness of the increased prodrug lipophilicity as a means to improve ocular bioavailability of pilocarpine. Eye irritation observed after topical administration of the prodrugs may be due to rapid absorption and possible precipitation of the lipophilic prodrugs in the precorneal lacrimal fluid, when pH is neutralized after eyedrop application. In accordance, eye irritation due to the pilocarpine prodrugs can be decreased by buffering of the eyedrop and by cyclodextrin coadministration (Järvinen et al., 1995a; Suhonen et al., 1995).

In conclusion, bispilocarpic acid diesters show rate control in pilocarpine delivery to the eye as suggested by the increase in the duration of miosis and reduction of the peak miosis. The miosis versus time profiles of pilocarpine prodrugs are dependent on the lipophilicity and enzymatic lability of the prodrugs.

Acknowledgements

Leiras OY (Finland), The Technology Development Centre (Finland) and Finnish Cultural Foundation are gratefully acknowledged for their financial support. The authors wish to thank Ms. Pirjo Hakkarainen and Ms. Päivi Perttula for their skilful technical assistance. Arto Urtti and Tomi Järvinen are grateful for the support by the Academy of Finland.

References

Armaly, M.F. and Rao, K.R., The effect of pilocarpine Ocusert with different release rates on ocular pressure. *Invest. Ophthalmol.*, 12 (1973) 491–496.

Benita, S., Plenecassagne, J.D., Gave, G., Drovin, D., Le Hao Dong, D. and Sincholle, D., Pilocarpine hydrochloride liposomes: Characterization in vitro and preliminary evaluation in vivo in rabbit eye. *J. Microencaps.*, 1 (1984) 203–216.

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

Bodor, N. and Visor, G., Improved delivery through biological membranes. XVIII. A site specific chemical delivery system as a short-acting mydriatic agent. *Pharm. Res.*, 1 (1984) 168–173.

Brown, H.S., Meltzer, G., Merril, R.C., Fisher, M., Ferré, C. and Place, V.A., Visual effects of pilocarpine in glaucoma. *Arch. Ophthalmol.*, 94 (1976) 1716–1719.

Bundgaard, H., Buur, A., Chang, S.C. and Lee, V.H.L., Timolol prodrugs: Synthesis, stability and lipophilicity of various alkyl, cycloalkyl and aromatic esters of timolol. *Int. J. Pharm.*, 46 (1988) 77–88.

Chien, D.S. and Schoenwald, R.D., Improving the ocular absorption of phenylephrine. *Biopharm. Drug Dispos.*, 7 (1986) 453–462.

Chrai, S.S. and Robinson, J.R., Corneal permeation of topical pilocarpine nitrate in the rabbit. *Am. J. Ophthalmol.*, 77 (1974) 735–739.

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.

Diepold, R., Kreuter, J., Himber, J., Gurny, R., Lee, V.H.L., Robinson, J.R., Saettone, M.F. and Schnaudigel, O.E., Comparison of different models for the testing of pilocarpine eyedrops using conventional eyedrops and a novel depot formulation (nanoparticles). *Graefe's Arch. Clin. Exp. Ophthalmol.*, 227 (1989) 188–193.

Gibaldi, M. and Perrier, D. *Pharmacokinetics*, Marcel Dekker, New York, 1982, p. 445–449.

Gurny, R., Ibrahim, H., Aebi, A., Buri, P., Wilson, C.G., Washington, N., Edman, P. and Camber, O., Design and

evaluation of controlled release systems for the eye. *J. Control. Rel.*, 6 (1987) 367–373.

Hussain, M.A. and Truelove, J.E., Prodrug approaches to enhancement of physicochemical properties of drugs. IV. Novel epinephrine prodrug. *J. Pharm. Sci.*, 65 (1976) 1510–1512.

Järvinen, T., Suhonen, P., Auriola, S., Vepsäläinen, J., Urtti, A. and Peura, P., O,O'-(1,4-Xylylene) bispilocarpic acid esters as new potential double prodrugs of pilocarpine for improved ocular delivery. I. Synthesis and analysis. *Int. J. Pharm.*, 75 (1991a) 249–258.

Järvinen, T., Suhonen, P., Urtti, A. and Peura, P., O,O' -(1,4-Xylylene) bispilocarpine acid esters as new potential double prodrugs of pilocarpine for improved ocular delivery. II. Physicochemical properties, stability, solubility and enzymatic hydrolysis. *Int. J. Pharm.*, 75 (1991b) 259–269.

Järvinen, T., Suhonen, P., Urtti, A. and Peura, P., Bispilocarpic acid monoesters as prodrugs of pilocarpine: II. Physicochemical properties and kinetics of hydrolysis in aqueous solution. *Int. J. Pharm.*, 79 (1992) 243–250.

Järvinen, T., Järvinen, K., Urtti, A., Thompson, D. and Stella, V.J., Sulfobutyl ether β-cyclodextrin (SBE4-β-CD) in eyedrops improves the tolerability of a topically applied pilocarpine prodrug in rabbits. *J. Ocul. Pharmacol.*, 11 (1995a) 95–106.

Järvinen, T., Poikolainen, M., Suhonen, P., Vepsälänen, J., Alaranta, S. and Urtti, A., Comparison of enzymatic hydrolysis of pilocarpine prodrugs in human plasma, rabbit cornea, and butyrylcholinesterase solutions. *J. Pharm. Sci.*, 84 (1995b) 656–660.

Maichuk, Y.F., Ophthalmic drug inserts. *Invest. Ophthalmol.*, 14 (1975) 87–90.

March, W.F., Stewart, R.M., Mandell, A.I. and Bruce, L.A., Duration of effect of pilocarpine gel. *Arch. Ophthalmol.*, 100 (1982) 1270–1271.

Mosher, G.L., Bundgaard, H., Falch, E., Larsen, C. and Mikkelson, T.J., Ocular bioavailability of pilocarpic acid mono- and diester prodrugs as assessed by miotic activity in the rabbit. *Int. J. Pharm.*, 39 (1987) 113–120.

Newell, F.W. *Ophthalmology. Principles and Concepts.* C.V. Mosby, Princeton, 1986, pp. 3–108.

Norell, S.E., Monitoring compliance with pilocarpine therapy. *Am. J. Ophthalmol.*, 92 (1981) 727–731.

Schoenwald, R.D. and Chien, D.S., Ocular absorption and disposition of phenylephrine and phenylephrine oxazolidine. *Biopharm. Drug Dispos.*, 9 (1988) 527–538.

Suhonen, P., Järvinen, T., Peura, P. and Urtti, A., Permeability of pilocarpic acid diesters across albino rabbit cornea in vitro. *Int. J. Pharm.*, 74 (1991a) 221–228.

Suhonen, P., Järvinen, T., Rytkönen, P., Peura, P. and Urtti, A., Improved corneal pilocarpine permeability with O,O'-(1,4-xylylene) bispilocarpic acid ester double prodrugs. *Pharm. Res.*, 8 (1991b) 1539–1542.

Suhonen, P., Järvinen, T., Lehmussaari, K., Reunamäki, T. and Urtti, A., Ocular absorption and irritation of pilocarpine prodrug is modified with buffer, polymer, and cyclodextrin in the eyedrop. *Pharm. Res.*, 12 (1995) 529–533.

Urtti, A., Salminen, L., Kujari, H. and Jäntti, V., Effect of ocular pigmentation on pilocarpine pharmacology in the rabbit eye. II. Drug response. *Int. J. Pharm.*, 19 (1984) 53–61.

Zimmerman, T.J., Pilocarpine. *Ophthalmologica*, 88 (1981) 85–88.