# Improved Corneal Pilocarpine Permeability with *O,O'*-(1,4-Xylylene) Bispilocarpic Acid Ester Double Prodrugs

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*O,O'*-(1,4-Xylylene) bispilocarpic acid esters are pilocarpine prodrugs containing two pilocarpic acid monoesters linked with one pro-moiety. Each mole of prodrug forms two pilocarpine moles in the presence of esterases. Corneal uptake and permeability of various bispilocarpic acid diesters were investigated *in vitro* using isolated albino rabbit corneas. The permeability coefficient of pilocarpine was $2.8 \times 10^{-6}$ cm/sec, whereas for bispilocarpic acid diesters, despite their large molecular weights (between 638 and 722), permeability coefficients were $6.5–20.2 \times 10^{-6}$ cm/sec. Only pilocarpine, and no intact prodrug, was observed at the endothelial side. Corneal uptake was increased with increasing lipophilicity, but a parabolic relationship between the logarithm of the apparent partition coefficient (1-octanol–pH 7.4 phosphate buffer) (log PC) and the corneal permeability was noticed. Corneal permeability and the rate of enzymatic hydrolysis of the compounds correlated well. The corneal permeability of pilocarpine given as lipophilic bispilocarpic acid diester (log PC $\geq 3$) prodrugs seems to be controlled by the formation of pilocarpine in the corneal epithelium rather than by the absorption of prodrugs into the epithelium or their epithelium–stroma transport rate.

KEY WORDS: pilocarpine; prodrug; bispilocarpic acid diesters; corneal permeability; drug delivery.

## INTRODUCTION

Pilocarpine is a widely used drug for the treatment of chronic, simple, or wide-angle glaucoma (1). Despite its good pharmacodynamic effect on the intraocular pressure, pilocarpine has some disadvantages. Due to the rapid precorneal loss and poor corneal permeability, only 1–2% of the instilled dose penetrates into the eye (2–6). Poor absorption and rapid intraocular elimination necessitates three to six instillations of pilocarpine eye drops. This may impair patient compliance (7).

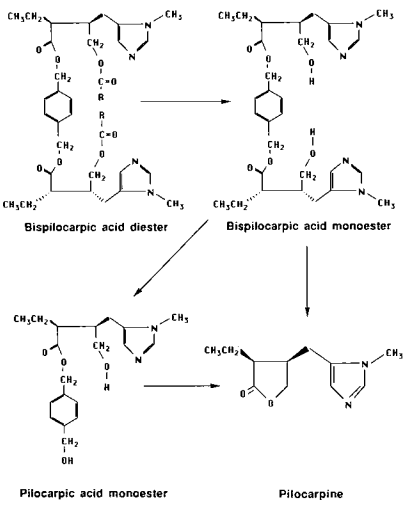
In general, the poor ability of topically applied ophthalmic drugs to penetrate through the cornea is due to their low lipophilicity. One promising way to improve ocular bioavailability is by formulation of prodrugs. For example, dipivefrin, a clinically used pivalic acid diester of epinephrine, improves corneal penetration of epinephrine because of the increased lipophilicity of the compound (8,9). Dipivefrin is cleaved in the cornea by esterases to epinephrine and two pivalic acid molecules (8,9). This prodrug approach was used to improve ocular absorption of several other drugs: carbonic anhydrase inhibitors, steroids, antivirals, and beta blockers (10).

Recent studies with pilocarpic acid diesters, prodrugs of pilocarpine, have indicated improved and prolonged miotic activity in rabbits (11). Thus, it is possible to increase and prolong ocular delivery of pilocarpine by using prodrug derivatives (11). However, irritation of the eye and poor water solubility have been problems with these compounds (12). In order to overcome these problems we synthesized bispilocarpic acid diesters, new prodrugs of pilocarpine (13). Compared with pilocarpic acid diesters with corresponding log PC values, bispilocarpic acid diesters have improved water solubility, and in their structure two molecules of pilocarpine are attached with one pro-moiety. After enzymatic hydrolysis three radicals per two pilocarpine molecules are released in the cornea (Scheme I). Whereas four pro-moieties are released from two pilocarpic acid diesters in the cornea. Earlier Järvinen *et al.* (14) demonstrated that in the presence of human plasma, pilocarpine is formed from bispilocarpic acid diesters quantitatively through enzymatic hydrolysis (Scheme I). The main purpose of this study was to investigate the corneal permeability of a series of bispilocarpic acid diesters. The permeabilities are compared with earlier results on pilocarpic acid diesters and pilocarpine (15).

## MATERIALS AND METHODS

### Materials

Adult male and female albino rabbits (New Zealand strain), weighing between 2.0 and 3.3 kg, were used as the animal model. Lighting was maintained on a 10 hr dark/14 hr light cycle, and the animals were fed a regular diet with no restrictions on the amount of food or water consumed. Pilocarpine hydrochloride was kindly supplied from Huhtamäki OY Leiras (Finland) and isopilocarpine nitrate was purchased from Aldrich-Chemie (West Germany). Bispilocarpic acid diester fumarates were synthesized and identified as described elsewhere (13). Structures of each prodrug are




Scheme I

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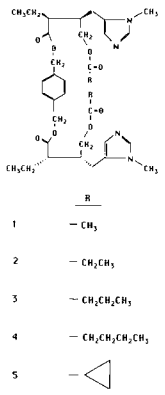

| | R |
|---|---|
| 1 | $-CH_3$ |
| 2 | $-CH_2CH_3$ |
| 3 | $-CH_2CH_2CH_3$ |
| 4 | $-CH_2CH_2CH_2CH_3$ |
| 5 |  |

Fig. 1. Structures of *O,O'*-(1,4-xylylene) bispilocarpic acid esters (i.e., bispilocarpic acid diesters).

shown in Fig. 1. HPLC-grade methanol was from J. T. Baker (Deventer, Holland). All other chemicals used were of analytical grade.

### *In Vitro* Permeability Study

The rabbits were sacrificed by a marginal ear vein injection of a lethal dose of T-61 vet. (Hoechst, Munich, West Germany). The intact eye was proptosed and the exposed cornea of the proptosed eye was carefully placed on a corneal holder (16), which maintained the cornea and held the eye in place. The ocular tissues were dissected leaving the cornea and a scleral ring. Four to eight corneas were used for each permeability determination. The dissection of the eye has been described in detail previously (15). The cornea was placed in a corneal mount (16) between side-by-side glass diffusion cells (Crown Glass Company, Inc., Somerville, NJ).

Glutathione bicarbonated Ringer's (GBR) solution (16) was used in the perfusion studies. Within 20 min from the sacrification the cornea was mounted and clamped between two cylindrical compartments of the perfusion chamber. Immediately thereafter, GBR solution was added to the endothelial side (3.4 ml) and 3.2 ml of this solution containing 21–148 μM of bispilocarpic acid diester in GBR buffer was added to the epithelial side. Otherwise the permeability study was carried out as described earlier (15).

The hydration level of the cornea gives an indication of its condition (16). After each permeability experiment, the cornea was removed from the mounting rings, and the remaining scleral tissue and conjunctiva were cut away. The cornea was weighed and dried at 50°C overnight. After reweighing, the water content of the cornea was calculated. The normal cornea has a hydration level of 76–80% (16), which may rise in the case of corneal damage.

### Drug Assay

High-performance liquid chromatography (HPLC) was performed with a system consisting of a Beckman pump Model 116, a variable-wavelength UV-detector Beckman Type 166 (λ = 215 nm), System Gold data module (Beckman Instruments Inc., San Ramon, CA), Marathon autosampler (Spark Holland, AJ Emmen, The Netherlands) with a column thermostat, and a Rheodyne 7080-080 injection valve equipped with a 20-μl loop.

Pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid were determined with a deactivated LC18-DB Supelcosil column (Supelco, Bellefonte, USA) (250 × 4.6 mm) with 5-μm particles at 40°C or with a μBondapak C18 column (Waters, Milford, USA) (300 × 3.9 mm) packed with 10-μm particles at ambient temperature. The solvent system was 5% $KH_2PO_4$ (pH 2.5)–methanol (97–3%) with a flow rate of 1.5 ml/min.

A deactivated Supelcosil LC8-DB column (Supelco, Bellefonte, USA) (150 × 4.6 mm) with 5-μm particles was used for determination of bispilocarpic acid diesters. The solvent system was 0.02 *M* $KH_2PO_4$ (pH 4.5)–methanol (29–71%) with an effluent flow rate of 1.0 ml/min. pH was measured with an Orion SA 520 pH meter (Boston, MA) at the temperature of study.

## RESULTS

The hydration levels of the corneas after permeability experiments were 78.0 ± 2.8% (mean ± SD; $n$ = 30). Corneal hydration levels for each group are presented in Table I.

Table I. Remaining Pilocarpine or Intact Prodrug on the Epithelial Side, Penetrated Pilocarpine on the Endothelial Side, Corneal Membrane Permeability ($P_{app}$), and Corneal Hydration Levels for Each Compound

| Compound | Remaining pilocarpine or intact prodrug on the epithelial side (%) | | Penetrated pilocarpine in the endothelial side (%) | $P_{app} \times 10^{-6}$ (cm/sec)[a] | Corneal hydration (%) |
|---|---|---|---|---|---|
| | Pilocarpine | Prodrug | | | |
| Pilocarpine[b] | 85.0 (2.5)[c] | — | 4.0 (0.9) | 2.8 (0.6) | 79.5 (0.9) |
| 1 | 21.9 (1.5) | 57.8 (5.9) | 7.9 (1.6) | 6.5 (1.7) | 77.6 (3.0) |
| 2 | 43.4 (5.3) | 27.5 (5.7) | 25.9 (4.2) | 20.2 (3.1) | 79.9 (3.2) |
| 3 | 39.4 (12.2) | 9.5 (3.5) | 18.4 (8.1) | 14.3 (4.8) | 77.5 (3.0) |
| 4 | 38.1 (3.5) | 0.3–29.4[d] | 9.2 (1.2) | 12.1 (4.3) | 76.9 (2.1) |
| 5 | 20.6 (2.2) | 21.2 (10.8) | 10.1 (4.1) | 10.4 (3.9) | 75.6 (1.5) |

[a] Corneal membrane permeability (mean ± SD).
[b] Data from Ref. 15.
[c] Numbers in parentheses are SD with $n$ = 3–8.
[d] Range of variation, $n$ = 4. One value was below the detection limit (corresponds to 0.3% remaining). Mean of the determined values gives 11.9%.

Table II. Permeation Lag Time ($t_L$), Lipophilicity (log PC), and Enzymatic Lability ($t_{1/2}$, $f_{50\%}$) of Pilocarpine and Bispilocarpic Acid Diesters

| Compound | $t_L$ (min)[a] | log PC[b,c] | 80% human plasma $t_{1/2}$ (min)[c] | 80% human plasma $f_{50\%}$ (min)[c,d] |
|---|---|---|---|---|
| Pilocarpine[e] | 18 ± 7 | 0.01 | — | — |
| 1 | 64 ± 11 | 3.04 | 9 | 32 |
| 2 | 55 ± 12 | 4.08 | 6 | 24 |
| 3 | 68 ± 37 | 5.47 | 11 | 35 |
| 4 | 79 ± 25 | 6.87 | 12 | 42 |
| 5 | 100 ± 12 | 4.20 | 14 | 46 |

[a] Lag time before steady-state permeation (mean ± SD).
[b] Apparent partition coefficient between 1-octanol and phosphate buffer of pH 7.40.
[c] Data from Ref. 14.
[d] $f_{50\%}$ = time, when 50% of total pilocarpine is formed.
[e] Data from Ref. 15.

The lag times before the steady-state permeation are shown in Table II. The lag times of bispilocarpic acid diesters are inversely related to the permeability coefficients and degradation rates of prodrugs, and formation rates of pilocarpine (Tables I and II). In other words, rapidly penetrating and cleaving diesters have shorter lag times of permeation. The lag times were longer for the prodrugs than they were in the case of pilocarpine (Table II, Fig. 2).

A typical permeability experiment (Fig. 2) shows permeation of pilocarpine given as pilocarpine and *O,O'*-dipropionyl (1,4-xylylene) bispilocarpate fumarate (compound 2) across excised rabbit corneas. For bispilocarpic acid diesters the penetrated fraction of pilocarpine to the endothelial side during 4 hr of perfusion varied between 7.9 and 25.9% and the permeability coefficient of bispilocarpic acid diesters was 6.5–20.2 × $10^{-6}$ cm/sec (Table I). The permeability coefficient of pilocarpine was 2.8 × $10^{-6}$ cm/sec (Table I).

HPLC analysis showed that the bispilocarpic acid diesters had been completely hydrolyzed in the cornea; no intact prodrug was detected at the receiver side. Further, no other metabolites were seen except pilocarpine and its degradation products: pilocarpic acid, isopilocarpine, and isopilocarpic acid. The molar fraction of these degradation products of pilocarpine was less than 10% of pilocarpine amount at the endothelial side.

Analysis of the epithelial side showed both intact prodrug and free pilocarpine (Table I). At 4 hr the fraction of intact prodrug at the epithelial side varied between 0.3 and 57.8% of the initial amount, and the fraction of pilocarpine at the epithelial side was between 20.6 and 43.4% of the total pilocarpine added as bispilocarpic acid diesters. Control experiments revealed that all the bispilocarpic acid diesters were chemically stable in the perfusion medium without tissue. The corneal uptake determined as the loss of prodrug from the epithelial side at 4 hr is dependent on both the lipophilicity and the enzymatic hydrolysis of the prodrug used (Tables I and II). The largest uptake occurred with the most lipophilic derivatives (compounds 3 and 4) and the smallest with the most hydrophilic derivative (compound 1) (Tables I and II).

## DISCUSSION

The present study shows that *in vitro* all bispilocarpic acid diesters were more extensively taken up by the rabbit cornea than pilocarpine (Table I). The uptake is increased by the increased lipophilicity of the examined substance, suggesting uptake primarily by the lipoidal corneal epithelium (Table I). The correlation between lipophilicity and corneal permeability is not as good and this relationship was parabolic (Fig. 3). It is difficult to estimate the optimal lipophilicity for corneal permeability, in terms of log PC value, because also the rate of chemical pilocarpine formation affects the permeability. Corneal permeability pilocarpine given as bispilocarpic acid diesters was at least as good as that of pilocarpic acid diesters reported earlier (15). This suggests that the effect of increased molecular weight (varied between 638 and 722) on drug penetration is compensated by the fact that each bispilocarpic acid diester carries and releases two pilocarpine molecules to the cornea.

The esterase activity in the corneal epithelium is approximately two times higher than in the stroma–endothelium (17,18) and therefore the residence time of a prodrug in the epithelium has significant impact on ocular bioavailability. All bispilocarpic acid diesters studied are completely hydrolyzed during the penetration through rabbit cornea, and only pilocarpine reached the endothelial side of the perfusion apparatus. The influence of the rate of pilocarpine formation (Table II) on the corneal permeability is evident from Fig. 4. A good correlation existed and the per-

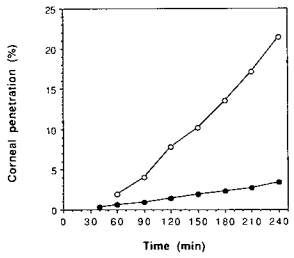


Fig. 2. Penetration of pilocarpine to the endothelial side of rabbit cornea when pilocarpine (●) or *O,O'*-dipropionyl (1,4-xylylene) bispilocarpate fumarate (compound 2) (○) was placed to the epithelial side. Initial concentrations on the epithelial side for pilocarpine and compound 2 were 254 and 117 μ*M*, respectively.

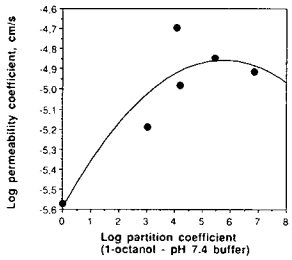


Fig. 3. A log–log plot of permeability coefficient (pH 7.65) through epithelium versus apparent partition coefficient (1-octanol–pH 7.4 phosphate buffer). The regression curve is represented by log $P_{app}$ = −0.02 (log PC)$^2$ + 0.26 (log PC) − 5.60, where $r$ = 0.91.

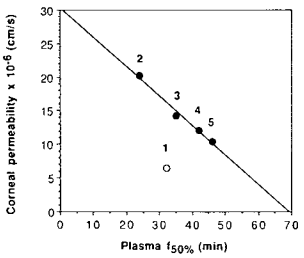


Fig. 4. Corneal permeability coefficient of *O,O'*-(1,4-xylylene) bispilocarpic acid esters versus 80% human plasma $f_{50\%}$. The linear regression line without compound 1 (○) is represented by $P_{app} = -0.44$ (plasma $f_{50\%}$) + 30.38, where $r = 0.99$.

meability was increased with increasing rate of hydrolysis. Although the rank-order of log PC and $t_{1/2}$ values (Table II) was about the same, the correlation between the rate of enzymatic hydrolysis and corneal permeability is not due to lipophilicity and decreased epithelium–stroma transfer rate of bispilocarpic acid diesters, because only formed pilocarpine, and no intact prodrug, penetrated through the cornea. In terms of its high cleavage rate one derivative [*O,O'*-diacetyl (1,4-xylylene) bispilocarpate fumarate; compound 1] had an unexpectedly low permeability (Fig. 4). This may be due to its lower lipid solubility and lower steady-state concentration in the cornea as suggested by the lower corneal uptake (Table I). Permeability of the *O,O'*-diacetyl (1,4-xylylene) bispilocarpate fumarate may be controlled by the corneal uptake rather than the rate of pilocarpine formation.

Our study shows that *O,O'*-(1,4-xylylene) bispilocarpic acid esters are effectively taken up by the cornea due to their lipophilicity and subsequently hydrolyzed by corneal esterases releasing pharmacologically active pilocarpine. Each bispilocarpic acid diester carries two molecules of pilocarpine in the cornea and three pro-moieties, whereas four pro-moieties release from two pilocarpic acid diesters in the cornea. Interestingly, the high molecular weight of these lipophilic prodrugs did not limit their permeation. In conclusion, bispilocarpic acid diesters may be useful prodrugs for improving ocular delivery of pilocarpine.

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