Ocular Absorption and Irritation of Pilocarpine Prodrug Is Modified with Buffer, Polymer, and Cyclodextrin in the Eyedrop

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The influence of buffer, viscosity and cyclodextrin on the ocular absorption and irritation of a pilocarpine prodrug, *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpic acid diester, was studied in albino rabbits. The prodrug solutions, equivalent to 0.5% pilocarpine, were prepared in 0, 10, 20, 50, or 75 mM citrate buffer at pH 5.0. Viscosity of the solutions (20, 50 or 115 cP) was modified with hydroxypropyl methylcellulose. 2-hydroxypropyl-β-cyclodextrin (HPCD) was included at concentrations 5, 10 and 15% (w/v). The formulations were compared to a commercial pilocarpine eyedrop (1.7%). Ocular irritation was graded in a double-masked experiment and miosis was used as a bioassay for pilocarpine delivery to the iris. The prodrug showed decreased peak and prolonged duration of miosis compared to pilocarpine, but it caused ocular irritation. Increasing buffer strength decreased and elevated viscosity intensified the miotic response and irritation by the pilocarpine prodrug. HPCD decreased both the ocular delivery of pilocarpine and the irritation by the prodrug, but the net effect was positive. Thus, administering 1.0% of pilocarpine as a prodrug with 15% (w/v) HPCD, the irritation was at the same level with the commercial pilocarpine eyedrop, but the ocular delivery was substantially improved. In conclusion, the ocular delivery of the pilocarpine prodrug may be enhanced in relation to its local irritation by properly combining buffer, viscosity and HPCD.

KEY WORDS: pilocarpine; prodrug; bispilocarpic acid diester; miotic activity; irritation; ocular absorption; 2-hydroxypropyl-β-cyclodextrin.

INTRODUCTION

Pilocarpine is used topically in the treatment of glaucoma. Peaks of pilocarpine concentrations in the eye cause undesirable intense miosis and myopia (1,2), and pilocarpine eyedrops are applied several times daily due to the short duration of action. Considerable research efforts have been devoted to optimize the activity of pilocarpine (3–9). Controlled release ocular inserts increase the duration of pilocarpine action and decrease the side-effects (8), but sometimes patients have practical problems in their use. Simpler formulations like gels and viscous solutions cause dose dumping and intense miosis and myopia.

The deficiencies of pilocarpine therapy may be overcome by the prodrug approach. Administration of pilocarpine acid diesters increased the duration of action of the drug and decreased the peak levels in rabbits (10). Later, pilocarpine permeability was improved by bispilocarpic acid diesters with greater water-solubility (11,12). In our preliminary experiments, however, these prodrugs were irritating in the eye. Since irritation was immediate, it was considered to be caused by high prodrug concentrations on the ocular surface layers.

Lipophilicity, water solubility and corneal permeability of several bispilocarpic acid diesters are highly dependent on pH (13). Controlling the eyedrop pH and the rate of neutralization on the ocular surface by varying buffer strength or viscosity, should provide a means to control ocular absorption and prodrug concentration in the corneal surface. Cyclodextrins form inclusion complexes with lipophilic compounds (14) and, thus, they should prevent too fast prodrug absorption into the ocular tissues.

In the present study, we explored the possibilities of various formulation factors, such as buffer concentration, viscosity, and the use of 2-hydroxypropyl-β-cyclodextrin (HPCD), to control ocular absorption and irritation of the pilocarpine prodrug, *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpic acid diester in rabbits.

MATERIALS AND METHODS

Preparation of the Solutions

Pilocarpine hydrochloride was from Leiras Corporation (Tampere, Finland). Synthesis and analysis of *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpic acid diester fumarate, has been described previously (15). Hydroxypropyl methylcellulose (HPMC; Methocel E4M) was from Colorcon (England). 2-hydroxypropyl-β-cyclodextrin (HPCD) was from Pharmos Corp. (Alachua, FL, USA). The mean degree of substitution of HPCD was 0.6. All the other chemicals were of reagent grade and distilled water was used. The eyedrop formulations (I–XVI) of this study are presented in Table I.

To study the effect of buffering on the ocular absorption and irritation of the prodrug, unbuffered prodrug solutions equivalent to 0.5% (I) and 1% pilocarpine (XII) were prepared by dissolving 12.2 and 24.4 mg/ml of the prodrug, respectively, in 0.5% sodium chloride. Buffered solutions (V, VI) were prepared by dissolving the prodrug in 20 or 75 mM citrate buffer. Viscosity of the prodrug solutions was modified with HPMC (5.0, 6.5, or 8.5 mg/ml) to give viscosities of 20 cP, 50 cP and 115 cP, respectively (II–IV). To study the effects of buffering and viscosity together, an appropriate amount of HPMC was added to the prodrug solutions in citrate buffer (VII–XI). The viscosities were measured at room temperature using a Brookfield LVDV-III rotary viscometer (Brookfield Engineering Laboratories, Stoughton, MA, USA) at 30 rpm. To study the effect of HPCD on the ocular absorption and irritation of the prodrug, the prodrug

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Table I. Pharmacokinetic Parameters of the Miotic Response and Eye Lid Closure Time After Topical Administration of the Formulations Tested (Each value represents the mean ± S.E. of six to seven experiments, except formulation I represents 12 experiments. Pilocarpine or prodrug was dissolved in sodium chloride solution, unless otherwise mentioned and the pH was adjusted to 5.0.)

Formulation									
	Concentration (%)	Citrate buffer (mM)	Viscosity (cP)	HPCD (%)	T_{max}[a] (min)	I_{max}[b] (%)	$AUC_{0-\infty}$[c] (% · min)	Duration[d] (min)	Eye Lid Closure[e] (min)
Pilocarpine	1.7	83	1	–	35 ± 5	20 ± 3	2091 ± 296	221 ± 29	1.0 ± 0.4
Control	–	75	115	–	90 ± 21	11 ± 1	256 ± 588	129 ± 26	4.2 ± 1.0
I	0.5	–	1	–	112 ± 16¥	15 ± 1	1976 ± 242	244 ± 19	3.6 ± 1.0
II	0.5	–	20	–	105 ± 30	16 ± 1	4352 ± 583*¥	330 ± 11¥	5.8 ± 2.0¥
III	0.5	–	50	–	88 ± 17¥	19 ± 2	4547 ± 981*¥	322 ± 30¥	8.9 ± 2.0*¥
IV	0.5	–	115	–	98 ± 12¥	20 ± 2*	4810 ± 1320*	302 ± 29	26.2 ± 8.8*¥
V	0.5	20	1	–	48 ± 6*	12 ± 2¥	1432 ± 494	202 ± 48	1.1 ± 0.4
VI	0.5	75	1	–	93 ± 20¥	13 ± 1¥	1545 ± 316	184 ± 29	0.8 ± 0.5
VII	0.5	10	50	–	58 ± 14	17 ± 2	4078 ± 767*¥	316 ± 16¥	2.9 ± 0.9
VIII	0.5	20	50	–	125 ± 12¥	15 ± 2	3126 ± 461*	275 ± 28¥	3.4 ± 1.5
IX	0.5	50	50	–	96 ± 23¥	14 ± 1	2745 ± 609	262 ± 38	2.1 ± 0.6
X	0.5	20	115	–	125 ± 14¥	18 ± 1	3898 ± 493*¥	273 ± 34	15.3 ± 8.6¥
XI	0.5	75	115	–	58 ± 17	14 ± 1¥	1725 ± 492	226 ± 36	5.5 ± 2.0
XII	1.0	–	1	–	130 ± 26¥	23 ± 2	5600 ± 1167¥	309 ± 25¥	28.7 ± 13.3¥
XIII	1.0	–	1	5	120 ± 13¥	15 ± 2#	3304 ± 834	267 ± 36	6.3 ± 4.0
XIV	1.0	–	1	10	103 ± 24¥	13 ± 1#¥	3498 ± 935	276 ± 32	1.3 ± 0.4
XV	1.0	–	1	15	120 ± 38	12 ± 2#	3817 ± 1315	279 ± 45	0.1 ± 0.1#¥
XVI	0.5	–	50	5	133 ± 28¥	20 ± 2*	5243 ± 660*¥	346 ± 5*¥	5.2 ± 2.2

[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 at least 3% change over the baseline.
[e] The sum of the time with eyes closed and/or half-closed.
* $p < 0.05$. Mann-Whitney's *U*-test compared to the formulation I.
# $p < 0.05$. Mann-Whitney's *U*-test compared to the formulation XII.
¥ $p < 0.05$. Mann-Whitney's *U*-test compared to pilocarpine.

was dissolved in 5, 10 and 15% (w/v) solutions of HPCD in saline with or without HPMC (XIII–XVI).

The pH of the solutions was adjusted to 5.0 with sodium hydroxide due to stability and solubility reasons (11) and made isotonic with sodium chloride when appropriate. All the test formulations were prepared immediately prior to the experiments. A commercial citrate buffered pilocarpine hydrochloride eyedrop, Oftan Pilocarpin® 2% (Leiras Corp., Finland); (equivalent to 1.7% pilocarpine base with 0.04 mg/ml benzalkonium chloride), was included in the study for comparison. The pH of the commercial product was 5.2.

Miotic Response

Adult male and female New Zealand White albino rabbits (2.8–4.2 kg) were used in two groups of six rabbits. Rabbits were placed in restraining boxes in a quiet room, where the lighting conditions were maintained identical throughout the study. The rabbits were allowed to get accustomed to the environment for 1 hour prior to each experiment. The pupillary diameter before the test was 5.4–7.6 mm.

The test solutions (25 μl) were instilled onto the upper corneoscleral limbus of the right eye of six rabbits in random order using a double-masked experimental design. The eyes were photographed 30 min, 15 min, and immediately before the instillation, and at fixed time intervals after the instillation from a constant distance. The negatives were enlarged with a microfilm reflector and the pupillary diameters were determined as means of horizontal and vertical diameters. At least 48 hours wash-out time was allowed between the experiments.

Following parameters were evaluated: the time of maximum miosis (T_{max}; min), the maximum change in pupillary diameter (I_{max}; %), the area under the miosis vs. time curve ($AUC_{0-\infty}$; % · min), and the duration of the miotic effect (i.e., a change of 3% or greater over the baseline; min). AUC_{0-6h} was determined by a trapezoidal method and $AUC_{6h-\infty}$ was calculated using the value of the last data point at 6 h and the terminal elimination rate. $AUC_{0-\infty}$ was obtained by summing AUC_{0-6h} and $AUC_{6h-\infty}$.

Irritation Test and Statistical Analysis

Discomfort was graded on the basis of duration and severity. Duration of the complete closure and half-closure of the eyelids were determined. Mucoidal discharge was scored from 0 to 2: normal (0), any amount of clear discharge different from normal (1), and milk-like discharge moistening

the lids and hair adjacent to the lids (2). The irritation was always evaluated by the same person.

The statistical significance of the differences in the measured parameters between the formulations were evaluated by Mann-Whitney's *U*-test.

RESULTS

Miotic Response

The miotic responses in rabbits after the administration of pilocarpine prodrug in the formulations are shown in Figures 1 and 2 and in Table I. Pilocarpine caused maximum miosis (20 ± 3%) 35 minutes after administration. The duration of miotic action was about 3.7 hours.

Unbuffered prodrug eyedrop (I) showed a delayed (112 ± 16 min) miosis, but duration of activity, and bioavailability were similar to pilocarpine. Buffering with 20 mM and 75 mM citrate (V, VI) decreased miotic intensity (I_{max}: 12–13%). With viscous eyedrops (II–IV) the $AUC_{0-\infty}$ value of miotic response was doubled compared to that of nonviscous prodrug eyedrop (I). The duration of activity was about 5.0–5.5 hours. Buffering the viscous eyedrops (III–IV, VII–XI) with 10–75 mM citrate decreased the miotic activity of the prodrug. At low buffer concentrations (10–20 mM), however, the bioavailability of viscous buffered eyedrops was still greater than that of I or pilocarpine.

Prodrug dose equivalent to 1% pilocarpine base (XII) showed delayed miosis (130 ± 26 min) and 2.5 times higher $AUC_{0-\infty}$ than 1.7% pilocarpine. The duration of action was more than 5 hours. Addition of HPCD in various concentrations (XIII–XV) did not cause significant changes in miotic response, except for reduced peak miosis. Addition of a viscolyzer to HPCD containing eyedrops (XVI) intensified the miosis to the level of the prodrug in saline (XII).

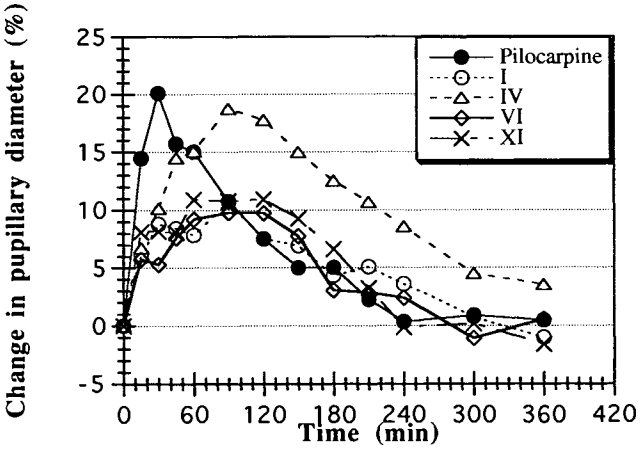

Fig. 1. The average change in pupillary diameter (%) as a function of time following the topical administration (25 μl) of 1.7% pilocarpine base (Pilocarpine), unbuffered 1.2% prodrug (equivalent to 0.5% pilocarpine base) solution (I), viscous (115 cP) 1.2% prodrug solution (IV), 1.2% prodrug solution buffered with 75 mM citrate buffer (VI) and viscous (115 cP) 1.2% prodrug solution buffered with 75 mM citrate buffer (XI) into the rabbit eye. S.E. lines were omitted for clarity. All S.E. values were smaller than ± 4.5, n = 6–12.

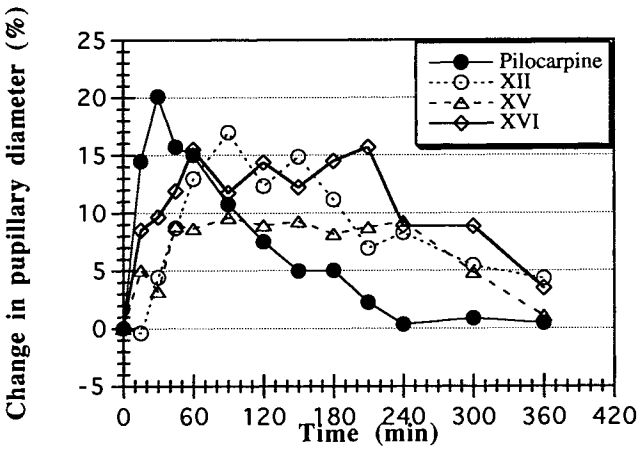

Fig. 2. The average change is pupillary diameter (%) as a function of time following the topical administration (25 μl) of 1.7% pilocarpine base (Pilocarpine), unbuffered 2.4% prodrug (equivalent to 1.0% pilocarpine) solution (XII), unbuffered 2.4% prodrug solution with 15% HPCD (XV) and viscous (50 cP) 1.2% prodrug (equivalent to 0.5% pilocarpine base) solution with 5% HPCD (XVI) into the rabbit eye. S.E. lines were omitted for clarity. All S.E. values were smaller than ± 3.0 n = 6.

Irritation

After instillation of the 1.7% commercial pilocarpine eyedrop the rabbits had their eyes half-closed for 1.0 ± 0.4 min (Table I). Pilocarpine did not induce mucoidal discharge.

Pilocarpine prodrug in saline (I, XII) was more irritating than pilocarpine. The irritation increased with increasing prodrug concentration. Buffering with 20 mM and 75 mM citrate (V, VI) appeared to reduce irritation in a concentration dependent manner.

Increasing the viscosity of the vehicle (II–IV) the irritation of the prodrug was substantially increased and caused mucoidal discharge. The scores ranged from 0.7 ± 0.3 (20 cP) to 1.7 ± 0.2 (115 cP). Adding citrate buffer to the viscous vehicles (VII–XI) decreased their irritation potential substantially.

Cyclodextrin decreased the irritation of the prodrug (1.0% pilocarpine equivalents) in a concentration dependent manner (XIII–XV). With 15% HPCD in the vehicle, the prodrug was better tolerated than the commercial product.

DISCUSSION

In this study, the ocular bioavailability of pilocarpine delivered as the prodrug, *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpic acid diester was assessed indirectly using miosis response. The area under the miosis vs. time curve ($AUC_{0-\infty}$) reflects the biophasic availability of pilocarpine in the iris sphincter. A linear relationship exists between the miotic AUC and aqueous humor concentration of pilocarpine (16).

In rabbits, pilocarpine prodrug showed delayed peak response and lower peak response compared to pilocarpine (Fig. 1). The duration of activity and $AUC_{0-\infty}$, however, were similar to those of pilocarpine, indicating that the prodrug serves as a chemical delivery system that regulates the entry of pilocarpine into the inner eye with the enzymatic

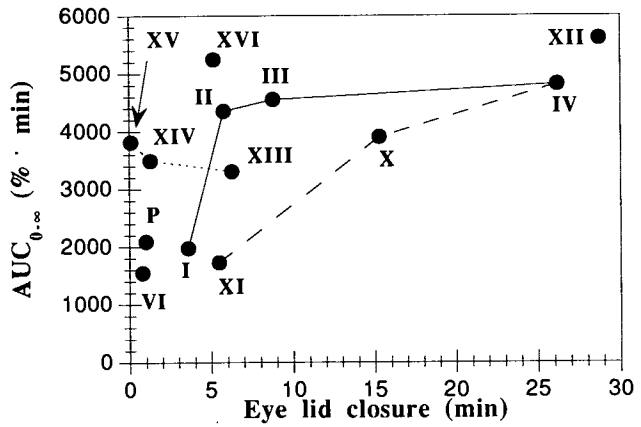

Fig. 3. Relationship between the irritation (the sum of the duration of eye closure*, min) and the area under the curve ($AUC_{0-\infty}$, % · min) for the formulations tested. P represents 1.7% pilocarpine base and all other roman numbers represent the formulations presented in Table I. The solid (viscosity), broken (buffer strength) and dotted (HPCD concentration) lines illustrate the influence of the formulation on irritation and $AUC_{0-\infty}$. *See text for description.

and chemical steps of cleavage leading to the release of pilocarpine. Unfortunately, the prodrug appears to have irritation potential, which may prohibit its clinical use. The irritation is immediate, suggesting that the prodrug itself causes the irritation, not the monoester intermediate that is formed in an enzymatic reaction in the cornea. An isotonic solution of fumaric acid at pH 5 at the same concentration as in the prodrug eyedrop solution is not irritating confirming that only the lipophilic prodrug form, not the fumaric acid released from the fumarate salt form of the prodrug, causes eye irritation.

The rate of neutralization of the instilled solution in the precorneal area affects the ocular absorption of ionizable drugs (17). Increased buffer strength in the eyedrop lowers the rate of eyedrop neutralization and, in the case of basic drugs such as pilocarpine, ocular absorption may be decreased (18). When neutralization is slow, drug escapes from the precorneal area before pH elevates to levels that are favourable for drug partitioning into the corneal epithelium. It appears that the pilocarpine prodrug tested in the present study exhibits pH-sensitive partitioning behavior, since the absorption of pilocarpine decreased with increasing buffer strength in the vehicle (Fig. 3, Table I). At the same time, irritation by the prodrug showed decreasing tendency. The irritation may be caused by the high prodrug concentration on the surface layers of the corneal epithelium, which in turn is lowered with increasing citrate buffer concentration.

Increase in ocular pilocarpine bioavailability has been achieved by the administration of the drug in a vehicle of elevated viscosity (3,16). The viscous solution prolongs the contact time of the drug, resulting in greater drug absorption and, hence, the duration of drug action may be increased. In our study, the $AUC_{0-\infty}$ of miosis was only slightly affected by viscosity increase from 20 cP to 115 cP, but the increase in $AUC_{0-\infty}$ with a viscosity change from 1 to 20 cP was significant (Table I). This is in accordance with earlier reports (3). In the case of prodrug administration, the apparent elimination rate constant of the drug from the eye is low and, thus, absorption enhancement with viscous vehicles may be advantageous. However, this involved concomitant ocular irritation (Fig. 3). By adding citrate buffer to the viscous eyedrops, ocular irritation was decreased to the same level with the commercial pilocarpine eyedrop. Thus, citrate buffer efficiently controls the neutralization of the precorneal fluids, while HPMC prevents too early drainage of the instilled solution from the eye.

The eyedrops used in the present study had a pH of 5.0. Upon neutralization by the precorneal fluid, the solubility of the prodrug decreases substantially (11), but the ability to partition into the corneal epithelium increases rapidly, causing the high peak concentrations and irritation in the cornea. Hydroxypropyl-β-cyclodextrin (HPCD) is known to form inclusion complexes with many lipophilic drugs (14) and it complexes with some bispilocarpic acid diesters as evidenced by phase-solubility test (unpublished results). In our study, HPCD in the formulation may control the concentration of the free drug that partitions into the cornea. Only the uncomplexed drug (together with insignificant amounts of HPCD) is capable of penetrating the cornea (19,20). Upon penetration of the free prodrug into the cornea more prodrug molecules are released from the complex into the lacrimal fluid. This sequence of events seems to control the prodrug delivery into the cornea so that the irritation by the prodrug at concentrations of 1.0% pilocarpine equivalents, is decreased below the level of the commercial pilocarpine product (Table I, Fig. 3). Prodrug in HPCD formulations with or without HPMC resulted in greater bioavailability and similar or less irritation than 1.7% commercial pilocarpine eyedrop or the prodrug in saline.

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