J. Pharm. Pharmacol. 1996, 48: 263–269
Received June 23, 1995
Accepted October 3, 1995

Modified β-Cyclodextrin (SBE7-β-CyD) with Viscous Vehicle Improves the Ocular Delivery and Tolerability of Pilocarpine Prodrug in Rabbits

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Abstract

The complexation of pilocarpine prodrug, *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate, with various β-cyclodextrin (β-CyD) derivatives was studied by the phase solubility method. The effects of coadministered sulphobutyl ether β-CyD (SBE7-β-CyD) with and without poly(vinyl alcohol) (PVA) on the miotic response and eye irritation of the prodrug were investigated in pigmented rabbits. The pilocarpine prodrug formed 1:1 inclusion complexes with variably substituted sulphobutyl ether derivatives of β-CyD (SBE4-β-CyD and SBE7-β-CyD), and 1:1 and 1:2 complexes with hydroxypropyl-β-CyD (HP-β-CyD) at pH 7·4. Coadministered SBE7-β-CyD eliminated the eye irritation due to the pilocarpine prodrug, but also decreased the miotic response. Ocular absorption of the prodrug was improved by increasing the viscosity of prodrug/SBE7-β-CyD solution with PVA without inducing any eye irritation. Eye irritation due to viscous prodrug/SBE7-β-CyD solutions was comparable with isotonic NaCl solution. We conclude that administration of pilocarpine prodrug in viscous SBE7-β-CyD solution decreases substantially eye irritation while ocular absorption is not affected.

Pilocarpine is a widely used drug for controlling the elevated intraocular pressure associated with glaucoma. However, duration of action for ophthalmic pilocarpine is short and its ocular bioavailability is only 0·1–3% of the installed pilocarpine dose (Asseff et al 1973; Chrai & Robinson 1974a; Lazare & Horlington 1975). Various prodrugs such as pilocarpic acid diesters (Bundgaard et al 1986; Mosher et al 1987), bispilocarpic acid diesters (Järvinen et al 1991a, 1992) and quaternary salts of pilocarpine (Druzgala et al 1992) have been developed to improve the ocular delivery of pilocarpine. Bispilocarpic acid diesters are dimeric pilocarpine double prodrugs which release pilocarpine via enzymatic and chemical hydrolysis in-vitro (Järvinen et al 1991a, 1995a). These prodrugs show improved ocular absorption of pilocarpine and prolonged duration of action, but unfortunately also strong eye irritation was observed which may hinder their clinical usefulness (Järvinen et al 1995b; Suhonen et al 1995, 1996). Cyclodextrins (CyDs) are well-known for their ability to form inclusion complexes with a wide variety of hydrophobic drugs and to improve drug solubility or stability as well as bioavailability of the drug (Szejtli 1994). β-CyD derivatives, such as hydroxypropyl-β-CyD (HP-β-CyD) and variably substituted sulphobutyl ether derivatives of β-CyD (SBE-β-CyDs) have been developed to improve the safety, aqueous solubility and subsequent pharmaceutical usefulness of β-CyD (Pitha et al 1986; Stella & Rajewski 1992). The eye irritation due to bispilocarpic acid diesters has been decreased with SBE4-β-CyD (with an average degree of substitution of four) (Järvinen et al 1995b) and HP-β-CyD (Suhonen et al 1995), but the ocular absorption of the studied prodrug decreased with increased CyD concentrations (Järvinen et al 1995b). These results are consistent with the general assumption that only the free drug, not CyD/drug complex, penetrates through biological membranes (Nakanishi et al 1989; Frijlink et al 1990). The purpose of this study was to decrease the eye irritation due to the prodrug with viscous SBE7-β-CyD solution without impairing the ocular absorption of pilocarpine prodrug. SBE7-β-CyD (a sulphobutyl ether with an average degree of substitution of seven) will be most probably the clinical material of SBE-β-CyDs. The complexation of pilocarpine prodrug, *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate, with SBE7-β-CyD was studied by phase-solubility methods and compared with complexation with SBE4-β-CyD and HP-β-CyD.

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Materials and Methods

Chemicals

The pilocarpine prodrug, *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate (Järvinen et al 1991b), SBE7-β-CyD (molecular weight = 2207·5) (Stella & Rajewski 1992) and SBE4-β-CyD (molecular weight = 1725·9) (Stella & Rajewski 1992) were synthesized and identified according to the previously described methods. The chemical structures for *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate and SBE7-β-CyD are shown in Fig. 1. HP-β-CyD (Encapsin, molecular weight = 1297·4) was purchased from Janssen Biotech (Olen, Belgium) and poly(vinyl alcohol) (PVA, molecular weight = 124 000–186 000 g mol^{-1}) from Aldrich Chemicals Company, Inc. (Milwaukee, USA). Sodium chloride and methanol (HPLC grade) were purchased from J. T. Baker

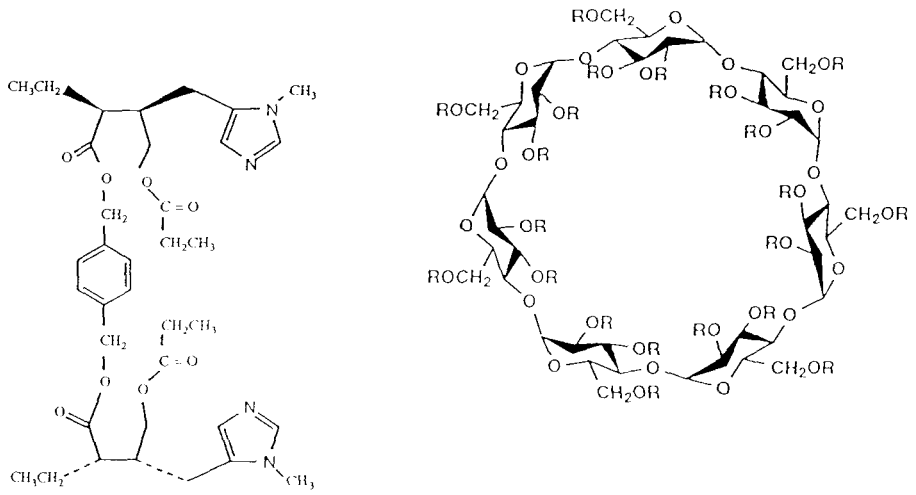

FIG. 1. The chemical structure of *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate (A) and sulphobutyl ether *β*-cyclodextrin with an average degree of substitution of seven (SBE7-*β*-CyD) (B).

(Denventer, The Netherlands) and disodium phosphate dihydrate from Merck (Darmstat, Germany). All other chemicals used were of analytical grade.

Apparatus

High-performance liquid chromatography (HPLC) was performed with a system consisting of the Beckman solvent module 116, a Beckman UV detector (set at 215 nm), the System Gold data module (Beckman Instruments Inc., San Ramon, USA), a Marathon autosampler equipped with column thermostat (Spark Holland, Emmen, The Netherlands) and a Rheodyne loop injector. A deactivated Supelcosil LC8-DB (15 cm × 4·6 mm i.d., 5 *μ*m) reversed-phase column (Supelco, Bellefonte, USA) was used for the separations. The chromatographic conditions were as follows: injection volume, 20 *μ*L; column temperature, 40°C; flow rate, isocratic at 1·0 mL min^{-1}. The mobile phase used consisted of 29% (v/v) monobasic potassium phosphate buffer (0·02 M, pH 4·5) in methanol. An Orion SA 520 pH meter (Boston, USA) equipped with a combination pH electrode, was used for pH determinations.

Solubility studies

The stability constants for inclusion complex formation between the *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate and CyDs were determined at pH 7·4 using the phase-solubility method (Higuchi & Connors 1965). Excess amount of the prodrug was added to phosphate buffer solutions (0·16 M, ionic strength 0·5, pH 7·4) containing various concentrations (3·6 72·5 mM) of SBE7-*β*-CyD, SBE4-*β*-CyD, HP-*β*-CyD or the mixture of SBE7-*β*-CyD and PVA (10 mg mL^{-1}). The suspensions were shaken at 25°C for 72 h and the pH of the suspension was monitored during equilibration. The pH of the suspensions was adjusted to 7·4 with HCl or NaOH, if necessary. After equilibration, the suspensions were filtered through 0·45-*μ*m membrane filters and analysed by HPLC. The intrinsic solubility (S_0) of prodrug in phosphate buffer (0·16 M, pH 7·4) was determined as an average of five determinations. The S_0 of the prodrug in the presence of PVA (10 mg mL^{-1}) was not determined due to analytical problems.

Miotic studies

Preparation of solutions. Pilocarpine prodrug solutions in the presence and absence of SBE7-*β*-CyD were prepared by dissolving the required amount of pilocarpine prodrug and SBE7-*β*-CyD in 5 mL distilled water or distilled water containing 20 or 30 mg mL^{-1} PVA. The pH of the solutions was adjusted to 5·0 or 6·0 with sodium hydroxide and the solutions were made isotonic with sodium chloride.

Animals. The experimental animals used in this study were adult pigmented rabbits, 2·5 3·5 kg. The rabbits were housed singly in cages under standard laboratory conditions: 12 h dark/12 h light cycle. The rabbits had free access to food and water. The experiments conformed to the ARVO Resolution on the use of animals in research.

Miotic response. To perform each miosis evaluation, the rabbit was placed in a plastic restraining box located in a quiet room with constant light. The rabbit was acclimatized in the box for 1 h before eyedrop administration. The test solution (25 *μ*L) was instilled on the upper corneoscleral limbus. During the instillation the upper eyelid was slightly pulled away from the globe. All solutions were administrated into the right eye while the left remained untreated. To measure the pupil diameter, the eyes were photographed from a constant distance 30 min, 15 min and immediately

before administration of solution and 0·25, 0·5, 0·75, 1·0, 1·5, 2·0, 2·5, 3·0, 3·5, 4·0, 5·0 and 6·0 h after. The negatives were enlarged with a microfilm reflector and the pupillary diameters were determined as a mean of the horizontal and vertical diameters. The lighting conditions in the laboratory gave a baseline pupillary diameter in the range of 6·2–8·5 mm. The studies were set up in a 6 × 6 masked cross-over design. At least 72 h wash-out time was allowed for each rabbit between dosings. The magnitude of miotic responses (%) was calculated as $((PD_o - PD_t)/PD_o) \times 100$, where PD_o is the baseline pupillary diameter and PD_t is the pupillary diameter at time t. For each rabbit with each eyedrop solution the pharmacokinetic parameters were calculated. Areas under the miotic response (%) vs time curves (AUC_{0-6}) were calculated using the trapezoidal method. Peak miosis intensity (I_{max}) and its time (t_{max}) were determined from actual data points. The duration of response was determined from miosis vs time profiles by calculating the total period of time when miosis was more than 10% or 20%. All values are expressed as mean ± standard error.

Evaluation of eye irritation. To estimate the discomfort caused by an instilled eyedrop, the extent of eyelid closure (closed or half-closed) after unilateral eyedrop administration (25 μL) was observed and recorded. Once the rabbit opened its eye fully, the eyelid closure behaviour was no longer recorded. The amount of mucoidal discharge at 0·25, 1 and 4 h after eyedrop administration was also recorded. Mucoidal discharge was scored from 0 to 2 as follows: 0 = normal, no lacrimation; 1 = slight discharge (any amount different from normal, clear discharge); and 2 = strong discharge (moisten the lids and hair just adjacent to the lids, milk-like discharge). The irritation was always evaluated by the same person who did not know eyedrop composition.

Statistical analysis of data. A one-factor analysis of variance for repeated measurements was used to test the statistical significance of differences between groups; significance in the differences in the means was tested using Fisher's protected least significant difference (PLSD) at 95% confidence.

Results

Solubility studies

Fig. 2 shows the phase-solubility diagrams of *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate with different CyDs and with a mixture of PVA (10 mg mL^{-1}) and SBE7-β-CyD at pH 7·4. The phase-solubility diagrams of *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate with SBE4-β-CyD and SBE7-β-CyD are A_L-type indicating formation of 1:1 prodrug-CyD complexes at this pH and CyD concentration range (Higuchi & Connors 1965). The stability constants for 1:1 complexes ($K_{1:1}$) were calculated using equation 1:

$$K_{1:1} = \text{Slope}/S_o(1 - \text{Slope}) \quad (1)$$

where $K_{1:1}$ is the stability constant for the complex and S_o is the solubility of the drug in the absence of CyD. The intrinsic solubility (S_o) of prodrug in phosphate buffer solution at 25°C was 15·3 ± 0·7 μg mL^{-1} (mean ± s.e., n = 5). This value was used in all stability constant calculations.

The phase-solubility diagram of *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate with HP-β-CyD is A_p-type indicating formation of 1:1 and 1:2 prodrug/CyD-complexes (Higuchi & Connors 1965). The stability constants for the 1:1 and 1:2 complexes were calculated after constructing a plot by using equation 2:

$$([S_t] - [S_o])/[L_t] = K_{1:1}[S_o] + K_{1:1}K_{1:2}[S_o][L_t] \quad (2)$$

where $[S_t]$ is the total drug concentration at total CyD concentration $[L_t]$, $[S_o]$ is the solubility of prodrug in the absence of CyD and $K_{1:1}$, and $K_{1:2}$ represent the stability constants for 1:1 and 1:2-complexes, respectively. A plot of $([S_t] - [S_o])/[L_t]$ vs $[L_t]$ results in a linear plot with an intercept of $K_{1:1}[S_o]$ and a slope of $K_{1:1}K_{1:2}[S_o]$.

The stability constants for *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate with different CyDs are shown in Table 1. The increase in average degree of substitution with SBE-β-CyDs decreased the complexation of prodrugs ($K_{1:1}$ for SBE4-β-CyD 14 777 M^{-1} and for SBE7-β-CyD 9844 M^{-1}). Coadministered PVA (10 mg mL^{-1}) decreased slightly the complexation of prodrug with SBE7-β-CyD.

Miotic response

The results for miosis studies are summarized in Tables 2 and 3. The control solution (36 mM SBE7-β-CyD + PVA 30 mg mL^{-1}) showed a comparable small area under miotic response curves (AUC_{0-6}) to isotonic NaCl. Increasing the molar ratio of SBE7-β-CyD to prodrug significantly decreased the intensity and duration of the miotic response. The AUC_{0-6} -values for 12 mM prodrug solution in the absence of SBE7-β-CyD and presence of 24 and 36 mM SBE7-β-CyD were 85·5 ± 14·5, 64·1 ± 11·5 and 40·7 ± 13·4% h, respectively. The durations of the miotic responses greater than 10% for the corresponding solutions were 4·1 ± 0·8, 2·7 ± 0·6 and 1·5 ± 0·9 h, respectively. Coadministered PVA (20 30 mg mL^{-1}) increased the apparent ocular absorption of the pilocarpine prodrug when compared

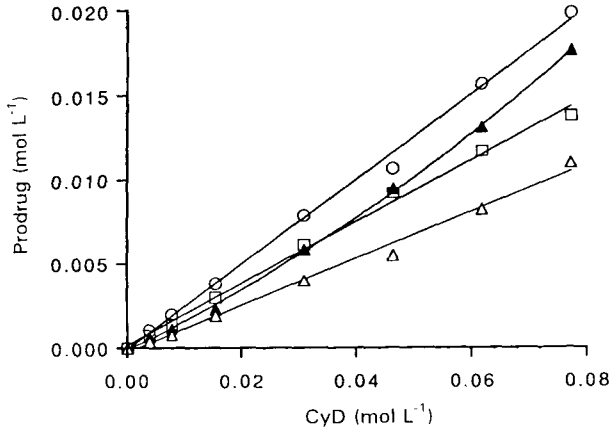

FIG. 2. The phase-solubility diagram of *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate with SBE4-β-CyD (○), SBE7-β-CyD (□), SBE7-β-CyD containing 10 mg mL^{-1} PVA (△) and with HP-β-CyD (▲).

Table 1. The stability constants ($K_{1:1}$ and $K_{1:2}$) for 1:1 and 1:2 inclusion complex formation between the *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate and SBE7-β-CyD (in the presence and absence of 10 mg mL^{-1} poly(vinyl alcohol) (PVA)), SBE4-β-CyD and HP-β-CyD.

Cyclodextrin	$K_{1:1}$ (M^{-1})	$K_{1:2}$ (M^{-1})
SBE7-β-CyD		
Without PVA	9844	–
PVA (10 mg mL^{-1})	7072	–
SBE4-β-CyD	14 777	–
HP-β-CyD	6065	9

with similar eyedrops in the absence of PVA. Prodrug solutions containing SBE7-β-CyD (24–36 mM) and PVA (20–30 mg mL^{-1}) resulted in similar miotic parameters (AUC_{0-6}, I_{max}, t_{max}) to those found for the prodrug in saline. Increasing PVA concentration in eyedrops tends to increase the miotic response of the prodrug (Table 2). Increasing the pH of PVA/SBE7-β-CyD/prodrug eyedrops from 5·0 to 6·0 did not significantly affect the miotic response to the prodrug.

Eye irritation

The data on mucoidal discharge (0 = no discharge, 1 = slight discharge and 2 = strong discharge) and eyelid closure are shown in Table 2. The control solution and 0·9% NaCl did not cause any irritation as judged by eyelid closure or mucoidal discharge. Compared with the control, the prodrug in saline prolonged eyelid closure and increased mucoidal discharge. After administration of 12 mM prodrug in saline the rabbits kept their eyes closed or half-closed for 2·3 ± 1·2 min and ocular mucoidal discharge scores were 1·7 ± 0·2 (1 h).

Coadministered SBE7-β-CyD (24–36 mM) eliminated the eye irritation due to the prodrug. Increasing the viscosity of the prodrug/SBE7-β-CyD eyedrops with PVA did not affect the ocular irritation. The results show that the eye irritation of ophthalmic pilocarpine prodrug is eliminated with viscous SBE7-β-CyD solution without impairing the ocular absorption of prodrug (Fig. 3).

Discussion

Solubility studies

O,O'-Dipropionyl-(1,4-xylylene) bispilocarpate forms inclusion complexes both with SBE-β-CyDs and HP-β-CyD. The prodrug formed only 1:1-complexes with SBE4-β-CyD and SBE7-β-CyD, but both 1:1 and 1:2 complexes with HP-β-CyD. SBE-β-CyDs are negatively charged due to the anionic sulphobutyl group. The repulsion force between SBE-β-CyD molecules may hinder the formation of 1:2-complexes (Johnson et al 1994). An increase in the degree of substitution

Table 2. The area under miotic activity curves (AUC_{0-6}) and eye irritation data after unilateral ocular administration (25 μL) of 12 mM *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate (in the presence or absence of SBE7-β-CyD and poly(vinyl alcohol) (PVA)), the control solution and isotonic NaCl-solution in pigmented rabbits (mean ± s.e., n = 6)

Solution	pH	Eyelid closure[a] (min)	Drainage[b] (0–2)	($AUC_{0\ 6}$)[c] (% h)
12 mM Prodrug	5·0	2·3 ± 1·2*	1·7 ± 0·2*	85·5 ± 14·5*
12 mM Prodrug 24 mM SBE7-β-CyD	5·0	0·0 ± 0·0#	0·0 ± 0·0#	64·1 ± 11·5*
12 mM Prodrug 36 mM SBE7-β-CyD	5·0	0·0 ± 0·0#	0·0 ± 0·0#	40·7± 13·4#
12 mM Prodrug 24 mM SBE7-β-CyD 2% PVA	5·0	0·3 ± 0·3#	0·3 ± 0·2#	84·5 ± 18·9*
12 mM Prodrug 24 mM SBE7-β-CyD 2% PVA	6·0	0·1 ± 0·1#	0·0 ± 0·0#	80·5 ± 7·4*
12 mM Prodrug 24 mM SBE7-β-CyD 3% PVA	5·0	0·0 ± 0·0#	0·0 ± 0·0#	87·3 ± 15·3*
12 mM Prodrug 24 mM SBE7-β-CyD 3%PVA	6·0	0·0 ± 0·0#	0·0 ± 0·0#	97·5 ± 15·5*
12 mM Prodrug 36 mM SBE7-β-CyD 2% PVA	5·0	0·0 ± 0·0#	0·0 ± 0·0#	67·5 ± 13·2*
12 mM Prodrug 36 mM SBE7-β-CyD 3% PVA	5·0	0·0 ± 0·0#	0·2 ± 0·2#	77·3 ± 9·8*
36 mM SBE7-β-CyD 3% PVA, control	5·0	0·0 ± 0·0#	0·0 ± 0·0#	15·6 ± 7·6#
NaCl	5·0	0·0 ± 0·0#	0·0 ± 0·0#	23·2 ± 4·1#

[a]Period of time when eye was closed or half-closed; [b]the amount of mucoidal discharge 1 h after eyedrop administration; [c]area under miosis vs time curve. *Significantly different from value for the control ($P < 0·05$, by Fisher's PLSD test); # significantly different from value for 12 mM prodrug ($P < 0·05$, by Fisher's PLSD test).

Table 3. Miotic activity parameters after unilateral ocular administration (25 μL) of 12 mM *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate solution (in the presence and absence of SBE7-β-CyD and poly(vinyl alcohol) (PVA)), the control solution and isotonic NaCl in pigmented rabbits (mean ± s.e., n = 6).

Solution	pH	Peak time[a] (h)	I_{max}[b] (%)	Duration 10%[c] (h)	20%[d] (h)
12 mM Prodrug	5·0	2·6 ± 0·4	20·7 ± 3·5*	4·1 ± 0·8*	1·2 ± 0·8
12 mM Prodrug 24 mM SBE7-β-CyD	5·0	2·8 ± 0·5	21·5 ± 2·0*	2·7 ± 0·6*	0·8 ± 0·7
12 mM Prodrug 36 mM SBE7-β-CyD	5·0	3·3 ± 0·4	14·4 ± 2·8	1·5 ± 0·9#	0·2 ± 0·2
12 mM Prodrug 24 mM SBE7-β-CyD 2% PVA	5·0	2·6 ± 0·3	22·1 ± 5·0*	3·7 ± 1·0*	1·9 ± 0·9
12 mM Prodrug 24 mM SBE7-β-CyD 2% PVA	6·0	3·0 ± 0·2	20·3 ± 1·8*	4·7 ± 0·4*	0·4 ± 0·2
12 mM Prodrug 24 mM SBE7-β-CyD 3% PVA	5·0	2·6 ± 0·6	22·2 ± 3·8*	4·6 ± 0·5*	1·1 ± 0·8
12 mM Prodrug 24 mM SBE7-β-CyD 3%PVA	6·0	2·7 ± 0·4	25·8 ± 3·1*	4·5 ± 0·7*	2·3 ± 1·0
12 mM Prodrug 36 mM SBE7-β-CyD 2% PVA	5·0	2·5 ± 0·6	20·9 ± 3·6*	3·6 ± 0·9*	0·6 ± 0·5
12 mM Prodrug 36 mM SBE7-β-CyD 3% PVA	5·0	2·7 ± 0·4	23·7 ± 4·3*	4·0 ± 0·8*	1·1 ± 0·5
36 mM SBE7-β-CyD 3% PVA, control	5·0	1·9 ± 0·6	10·5 ± 4·2#	0·4 ± 0·4#	0·1 ± 0·1
NaCl	5·0	2·0 ± 0·5	12·7 ± 2·8#	0·3 ± 0·2#	0·1 ± 0·1

[a]Period of time to reach I_{max}; [b]maximum miotic effect; [c]time period when miosis was ≥ 10%; [d]time period when miosis was ≥ 20%. *Significantly different from value for the control ($P < 0·05$, by Fisher's PLSD test); # significantly different from value for 12 mM prodrug ($P < 0·05$, by Fisher's PLSD test).

of SBE-β-CyD decreased the complexation of prodrug with CyD. This is probably due to steric hindrance on the CyD cavity by the substituents although in SBE-β-CyD the anionic sulphonate group is spaced from the CyD cavity by a butyl chain. Decreased stability constants with increased degree of substitution have also been reported with other β-CyD derivatives (Müller & Brauns 1986; Loftsson & Johannesson 1994).

Coadministered PVA (10 mg mL^{-1}) decreased very slightly the complexation of prodrug with SBE7-β-CyD. Recent reports have shown that some other water-soluble polymers increase the complexation of drugs with CyDs (Loftsson & Sigurdardottir 1994; Loftsson et al 1994a, b). However, the optimum concentration of the polymer for improved complexation varied from 0·5 to 2·5 mg mL^{-1} (Loftsson et al 1994a). At higher concentrations the polymers decreased the complexation and the total solubility of the drug.

The low PVA concentration (10 mg mL^{-1}) and neutral pH (7·4), compared with conditions of eyedrops (in-vivo study), were used in phase-solubility studies for practical reasons. The pH of the solutions with high PVA concentration (20–30 mg mL^{-1}) could not be adjusted precisely during equilibration. The pilocarpine prodrug is a weak base with pK_a of 6·7 (Järvinen et al 1991a) and thus the aqueous solubility of the prodrug is much higher at pH 5·0 than at pH 7·4. The phase-solubility studies were performed at pH 7·4 to limit the required amount of the prodrug. Additionally, the pH on the precorneal area will be readjusted to physiological pH within 2 min of ocular administration of unbuffered acidic eyedrops (Mitra & Mikkelson 1982; Ahmed & Patton 1984).

Miotic response and eye irritation

O,O'-Dipropionyl-(1,4-xylylene) bispilocarpic acid diester is a dimeric double prodrug of pilocarpine which releases pilocarpine via enzymatic and chemical hydrolysis in the eye (Järvinen et al 1991a, 1995a) and increases the ocular absorption and prolongs the duration of action of pilocarpine (Järvinen et al 1995b; Suhonen et al 1996). Due to the linear relationship between miosis and pilocarpine concentration in aqueous humour at low concentrations (Chrai & Robinson 1974b), miosis can be used as a relative indicator of ocular pilocarpine absorption. Ocularly applied SBE7-β-CyD and PVA did not cause significant miosis or mydriasis and thus, the relative effect of SBE7-β-CyD and PVA on the

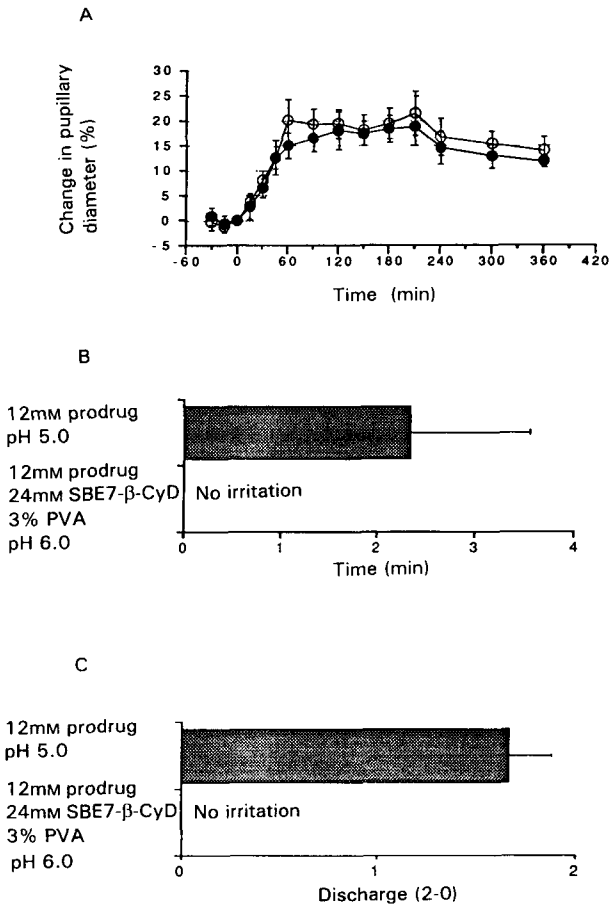

FIG. 3. Change in pupillary diameter (A), time of the eyelid closure (B) and relative amount of mucoidal discharge (1 h) (C) after ocular administration of 25 μL 12 mM prodrug (pH 5·0) (●) and 12 mM prodrug (pH 6·0) containing 24 mM SBE7-β-CyD and 3% PVA (○) in pigmented rabbits. Mean ± s.e., n = 6.

ocular absorption of pilocarpine prodrug was possible to evaluate via quantitation of miosis.

Coadministered SBE7-β-CyD at high concentrations decreased significantly the miotic response of the prodrug, but did not affect significantly the miotic response when the molar ratio of SBE7-β-CyD to prodrug was low. This result is in good agreement with a recent study showing the effects of SBE4-β-CyD on the miotic response of a pilocarpine prodrug (Järvinen et al 1995b). In addition, the apparent permeability coefficient of diclofenac across the cornea in-vitro was greatly reduced in the presence of various CyDs (Reer et al 1994). All these results support the general assumption that drug/CyD complexes do not penetrate across biological membranes (Nakanishi et al 1989; Frijlink et al 1990).

Compared with non-polymeric solution, the ocular absorption of prodrug was increased by increasing the viscosity of prodrug/SBE7-β-CyD eyedrop solution with PVA. The improved absorption of prodrug was most probably due to the prolonged retention of the viscous solution on the precorneal area. In the presence of SBE7-β-CyD, most prodrug molecules were in the complex form and only a small fraction of the molecules were as free prodrug. After topical administration of prodrug/SBE7-β-CyD vehicle, free prodrug is absorbed into the cornea and instantaneously prodrug/SBE7-β-CyD complex will release new prodrug molecules on the precorneal area based on the equilibrium reaction. Due to a slower drainage of the viscous solution, drug/CyD complexes have more time to release the drug from drug/CyD inclusion complexes on the precorneal area.

Increasing the pH of the eyedrop solutions from 5·0 to 6·0 did not significantly affect the ocular absorption of the prodrug. This nonsignificant effect of increased pH on ocular absorption of the prodrug might be explained as the beneficial effect of increased pH being compensated for by the larger stability constant of the inclusion complex between CyD and un-ionized drug (Liu et al 1992), which decreases the concentration of free prodrug on the precorneal area.

Previous studies have shown that coadministered SBE4-β-CyD (Järvinen et al 1995b) and HP-β-CyD (Suhonen et al 1995) decrease the eye irritation caused by bispilocarpic acid diesters. The present study shows that coadministered SBE7-β-CyD also decreases the eye irritation due to *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate. The eye irritation caused by pilocarpine prodrug might be due to fast absorption of lipophilic prodrug molecules into the lipophilic corneal epithelium or to precipitation of prodrug molecules in the precorneal area. Both processes are due to the rapid neutralization of the acidic eyedrop in the tear fluid and rapid increase in lipophilicity as a large fraction of the molecules become un-ionized. Inclusion complex formation with CyDs prevents both processes and increase the tolerability of the prodrug. Prodrug/SBE7-β-CyD complexes can be considered to act as a depot which keep the free prodrug concentration on the precorneal area at a non-irritating level.

In conclusion, the eye irritation due to *O,O'*-dipropionyl-(1,4-xylylene) bispilocarpate could be eliminated with coadministered SBE7-β-CyD in eyedrop solutions in rabbits. The decreased apparent ocular absorption of prodrug in the presence of SBE7-β-CyD was overcome with increased solution viscosity without inducing any eye irritation. Administration of the pilocarpine prodrug in viscous vehicles with CyDs might be a feasible method to increase its clinical acceptability.

Acknowledgements

The authors are grateful to CyDex, L.C. for supplying SBE7-β-CyD and SBE4-β-CyD. The Pharmacal Research Foundation, the Emil Aaltonen Foundation, the Finnish Cultural Foundation, Academy of Finland and Technology development Centre (Finland) are thanked for financial support. The authors would also like to thank Ms Pirjo Hakkarainen for skilful technical assistance.

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