ELSEVIER

International Journal of Pharmaceutics 153 (1997) 225–233

international journal of pharmaceutics

The use of cyclodextrins in ophthalmic formulations of dipivefrin

Pekka Jarho [a,*], Kristiina Järvinen [b], Arto Urtti [b], Valentino J. Stella [c], Tomi Järvinen [a]

[a] *Department of Pharmaceutical Chemistry, University of Kuopio, PO Box 1627, 70211 Kuopio, Finland*
[b] *Department of Pharmaceutics, University of Kuopio, PO Box 1627, 70211 Kuopio, Finland*
[c] *Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, USA*

Received 13 March 1997; accepted 14 April 1997

Abstract

Dipivefrine (dipivalyl epinephrine, DPE) is a dipivalic acid ester prodrug of epinephrine. The present study evaluates the possible use of hydroxypropyl-β-cyclodextrin (HP-β-CD) or sulfobutyl ether β-cyclodextrin ($(SBE)_{7m}$-β-CD) in ophthalmic formulations of DPE in order to increase the aqueous stability of DPE. The solubility of DPE was determined by phase-solubility method at pH 7.4 while the stability of DPE was investigated as a function of temperature (37–70°C) and CD concentrations at pH 5.0 and 7.4. The effect of HP-β-CD and $(SBE)_{7m}$-β-CD on the aqueous phase to organic phase transfer kinetics was studied with an aqueous buffer/*n*-octanol system, while the effect of $(SBE)_{7m}$-β-CD on (in vitro) corneal uptake of DPE was studied with isolated rabbit corneas in order to predict the ophthalmic bioavailability of DPE in the presence of CD. The negatively charged $(SBE)_{7m}$-β-CD formed significantly stronger inclusion complexes with the positively charged DPE ($pK_a = 9.01$) and enhanced the aqueous stability of DPE significantly more compared to the neutral cyclodextrin HP-β-CD. At room temperature and at pH values of 5.0 and 7.4, 9.2 mM $(SBE)_{7m}$-β-CD increased the aqueous stability of DPE about 20- and 100-fold, respectively, while 9.2 mM HP-β-CD increased the stability about four to five times. The phase-transfer and in vitro corneal uptake studies suggested that the complexation of DPE with both CDs may decrease the ophthalmic availability of DPE. © 1997 Elsevier Science B.V.

Keywords: Dipivefrine; Cyclodextrins; Solubility; Stability; Corneal uptake

1. Introduction

The properties of cyclodextrins (CDs) in ophthalmic drug delivery have been poorly under-

* Corresponding author.

PII S0378-5173(97)00111-7

stood until recently. Some recent reviews summarize the factors which affect corneal penetration of a drug after administration of the drug in CD containing ophthalmic formulations (Van Doorne, 1993; Järvinen et al., 1995a; Rajewski and Stella, 1996). Coadministered CDs have increased the corneal penetration, ocular absorption or efficacy of poorly water-soluble drugs like dexamethasone (Kristinsson et al., 1996), dexamethasone acetate (Usayapant et al., 1991), cyclosporin (Kanai et al., 1989), acetazolamide (Loftsson et al., 1994) and prostaglandins (Wheeler, 1991). These positive results are attributed to the higher concentrations of drug that may be administered when aqueous solubility of the drug is substantially increased with CDs. The destructive effects of β-CD and dimethyl β-CD on corneal epithelium may result in enhanced drug absorption but damaging CDs are obviously not suitable for ophthalmic preparations (Jansen et al., 1990).

Still unresolved is whether non-damaging CDs should alter the penetration of water-soluble drugs across the cornea. It is reported that hydroxypropyl-β-CD (HP-β-CD) significantly increases the miotic intensity of pilocarpine solution (Freedman et al., 1993), but in another study coadministration of HP-β-CD did not affect the miotic activity of pilocarpine solution (Järvinen et al., 1994). It is generally assumed that CDs and drug/CD complexes do not penetrate across biological membranes (Nakanishi et al., 1989; Frijlink et al., 1990). Consequently substantial complexation of a relatively water-soluble drug with CDs is expected to decrease ocular absorption due to a significant reduction in the fraction of the free drug in precorneal area. Recently α-CD was reported to increase the miotic effect of pilocarpine, but possible toxic effects of α-CD on the corneal epithelium were not evaluated (Keipert et al., 1996). Non-damaging CDs might be also suitable adjuvants for decreasing local irritation of ophthalmic drugs by controlling drug absorption to the cornea (Järvinen et al., 1995b; Jarho et al., 1996).

Dipivefrine (dipivalyl epinephrine, DPE) is a dipivalic acid ester prodrug of epinephrine which releases epinephrine after corneal absorption (Hussain and Truelove, 1976). DPE penetrates the human cornea 17 times better than epinephrine (Mandell and Stentz, 1978) due to its higher lipophilicity at pH 7.2. Compared to conventional 2% epinephrine hydrochloride eyedrops, a 0.1% DPE results in equivalent efficacy in lowering IOP while side-effects are reduced (Kohn et al., 1979). Therefore, DPE has currently replaced epinephrine in glaucoma treatment. The main pharmaceutical drawback of DPE is its low aqueous stability which was solved by decreasing the pH of eyedrops to 2.5–3.5 (USP DI, 1990). However, acidic eyedrops are irritating and they may result in decreased ocular bioavailability due to induced lacrimation (Lee and Robinson, 1986).

The purpose of the present work was to study if non-damaging CDs, HP-β-CD and a sulfobutyl ether, β-CD ($(SBE)_{7m}$-β-CD), can increase the aqueous solubility and stability of DPE, and to evaluate effect of CDs on corneal bioavailability of DPE by in vitro methods.

2. Materials and methods

2.1. Chemicals

Dipivefrine hydrochloride was obtained from Leiras (Turku, Finland), $(SBE)_{7m}$-β-CD (Captisol™, $M_w = 2160$) from CyDex LC (Overland Park, KS) and HP-β-CD (Encapsin™; $M_w = 1383$, degree of molar substitution 0.61) from Janssen Biotech (Olen, Belgium). Sodium chloride and methanol (HPLC grade) were purchased from J.T. Baker (Denventer, The Netherlands), disodium phosphate dihydrate from Merck (Darmstadt, Germany), hydrochloric acid from Riedel-de Haen (Seelze, Germany), sodium hydroxide from Eka Nobel AB (Bohus, Sweden) and T 61 vet. from Hoechst Veterinair GmbH (Munich, Germany).

2.2. Apparatus

High performance liquid chromatography (HPLC) was performed with a system consisting of a Beckman solvent module 116, Beckman UV detector (set at 215 nm) and System Gold data

module (Beckman Instruments, San Ramon, CA), a Marathon autosampler equipped with column thermostat (Spark Holland, Emmen, The Netherlands) and a Rheodyne loop injector (Rheodyne, Cotati, CA). A deactivated Supelcosil LC8-DB (15 cm × 4.6 mm i.d., 5 μm) reversed-phase column (Supelco, Bellefonte, PA) was used for the separations. The chromatographic conditions were as follows: Injection volume, 20 μl; column temperature, 40°C; flow rate, isocratic at 1.0 ml/min. The mobile phase used consisted of a 40% aqueous monobasic potassium phosphate buffer (0.02 M, pH 5.0) in methanol (for samples at pH 3.0 or 5.0) or a 28% aqueous monobasic potassium phosphate buffer (0.02 M, pH 7.0) in methanol (for samples at pH 7.4). An Orion SA 520 pH meter (Orion Research, Boston, MA) equipped with a combination pH electrode, was used for pH determinations.

2.3. Solubility studies

The complexation of DPE with HP-β-CD and $(SBE)_{7m}$-β-CD was determined by using the phase-solubility method of Higuchi and Connors (1965). An excess amount of the DPE was added to phosphate buffer solutions (0.16 M, pH 7.4, ionic strength of 0.5) containing various concentrations of CDs (0–72.3 mM). The suspensions were shaken at 25°C for 24 h and pH of the suspensions was monitored during equilibration. The pH of suspensions was adjusted to 7.4 with HCl or NaOH, if necessary. After equilibration, the suspensions were filtered through 0.45 μm membrane filters and analysed by HPLC. The intrinsic solubility (S_O) of DPE in CD-free phosphate buffer (0.16 M, pH 7.4) was calculated as an average of three observations.

2.4. Stability studies

The effects of HP-β-CD and $(SBE)_{7m}$-β-CD on aqueous stability of DPE were studied at pH 5.0 and 7.4. The studies were performed at elevated temperatures (37, 50, 60 and 70°C) as a function of CD concentration (0–9.2 mM). In addition, the aqueous stability of DPE in the absence of CD was studied at pH 3.0. The solutions (pH 3.0 and 5.0) were prepared by dissolving 2.0 mg of the DPE in 10.0 ml of CD-containing aqueous phosphate buffer solution (0.16 M, ionic strength 0.5). At pH 7.4, the solutions were similarly prepared, except that the inidal concentration of DPE was 100 μg/ml due to solubility limitations. The solutions were placed in a constant temperature and the samples were taken at the appropriate intervals. The remaining DPE was determined with the HPLC methods described above. The pseudo-first-order rate constants (k_{obs}) and shelf-lives ($t_{90\%}$) for the overall degradation of DPE were determined from the slopes of the linear semilogarithmic plots of remaining DPE versus time.

2.5. Interfacial transfer studies

The interfacial transfer studies were performed by a modification of the method of Dollo et al. (1996). The present studies were performed at room temperature to simplify the method and the volume of the organic phase and aqueous phase were 100 ml and 200 ml, respectively. The two phase device consisted of a beaker equipped with glass-bladed impeller at the upper phase (*n*-octanol) and magnetised agitator at the lower phase (0.16 M phosphate buffer, pH 3.0–7.4, $\mu = 0.5$). The rotation (in opposite directions) speed of both of the agitators was 100 rpm. At the beginning of the experiment, 50 ml of aqueous buffer containing 20 mg of DPE (with or without CD) was added to 150 ml of the aqueous phase equilibrated 2 h with *n*-octanol. The final concentration of CD in the aqueous phase was 2.3 mM. Samples of 300 μl were collected from the aqueous phase at appropriate intervals and the remaining DPE was determined by the HPLC methods described above. The first order rate constant for the transfer of DPE from aqueous phase to organic phase and the half-life of the phenomenon were determined from the slopes of the linear semilogarithmic plots of remaining DPE at aqueous phase versus time.

2.6. In vitro corneal uptake study

The effect of $(SBE)_{7m}$-β-CD on in vitro corneal uptake of DPE was studied using the isolated

corneas of pigmented rabbits and side-by-side diffusion cells. DPE (5.0 mg) with or without $(SBE)_{7m}$-β-CD (250.0 mg) was dissolved in 10.0 ml of glutathione bicarbonated Ringer's (GBR) solution (without NaCl). The pH of solutions was adjusted to 7.65 with NaOH or HCl and the solutions were made isotonic with NaCl. The rabbits (adult pigmented rabbits) were euthanased by a marginal ear-vein injection of lethal dose of T 61 vet. and (within 20 min) the corneas removed and placed between the two cylindrical compartments of the perfusion apparatus. It must be pointed out that the rabbits were not killed with the sole purpose of taking their corneas for the present experiments but the corneas were isolated from the rabbits that should have been sacrificed anyway. The solution containing DPE (3.2 ml) was added to the epithelial side of the cornea and a similar solution (3.4 ml), without DPE and $(SBE)_{7m}$-β-CD, was placed to the endothelial side. Samples (75 μl) were withdrawn from the epithelial side for a period of 90 min and at the same time, an equal volume of GBR solution was withdrawn from the endothelial side of the cornea. After the experiment, the samples were diluted and analysed by HPLC. The method used in the present study is a modification of a corneal penetration method which has been previously described in detail (Suhonen et al., 1991).

2.7. Statistical analysis of the results

For interfacial transfer studies and in vitro corneal uptake studies, a one-factor analysis of variance (ANOVA) was used to test the statistical significance of the results. Significance in differences in means was tested using Fisher's protected least significant difference (PLSD) method at a 95% confidence level.

3. Results and discussion

3.1. Solubility studies

The phase-solubility diagrams (Fig. 1) were classified as A_L-type (Higuchi and Connors, 1965) and the stability constant for a 1:1 inclusion complex with HP-β-CD were calculated according to Eq. (1).

$$K_{1:1} = \frac{\text{Slope}}{(S_O)(1 - \text{Slope})} \quad (1)$$

where $K_{1:1}$ is the stability constant for the complex and S_O is the solubility of DPE in the absence of CD. The solubility (S_O) of DPE in phosphate buffer solution (pH 7.4) at 25°C was 4.44 ± 0.04 mg/ml (mean $\pm$ SE, $n = 3$). The stability constant for the HP-β-CD/DPE complex was 1414 M^{-1}. The 1:1 stability constant for inclusion complex formation between DPE and $(SBE)_{7m}$-β-CD could not be calculated because slope of the phase-solubility diagram was not significantly different from unity (slope = 1.042).

Based on the values of the slopes of the phase-solubility diagrams, DPE forms stronger inclusion complex with $(SBE)_{7m}$-β-CD than with HP-β-CD. DPE is an organic base with a pK_a-value of 9.01 (Hagers, 1993), and therefore, it is positively charged in an aqueous solution at pH 7.4. Electrostatic interactions with negatively charged sulfobutyl groups may increase the complexation of DPE with $(SBE)_{7m}$-β-CD compared to HP-β-CD, which has no ionizable groups in its structure (Okimoto et al., 1996). The difference in

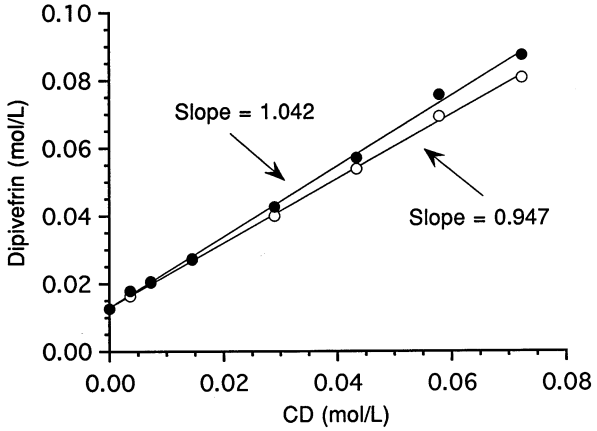

Fig. 1. Phase-solubility diagrams of DPE with $(SBE)_{7m}$-β-CD (●) and HP-β-CD (○) at pH 7.4.

Table 1
The observed shelf-lives ($t_{90\%}$) for degradation of DPE at pH values 3.0, 5.0 and 7.4 at various temperatures and the calculated $t_{90\%}$-values (from Arrhenius plots) at 25 and 4°C

Temperature (°C)	$t_{90\%}$ (days)		
	pH 3.0	pH 5.0	pH 7.4
70	1.13	0.57	0.03
60	1.85	0.93	0.05
50	5.00	3.01	0.10
37	19.93	8.63	0.48
25[a]	62.33	28.39	1.27
4[a]	705.00	286.94	12.33

[a] Calculated value.

magnitude of $K_{1:1}$ between $(SBE)_{7m}$-β-CD and HP-β-CD was later confirmed from the results of the stability studies.

3.2. Stability studies

The degradation of DPE followed the first-order kinetics in the presence and absence of CDs at all pH-values studied. The stability studies were carried out at elevated temperatures (70, 60, 50, 37°C) and the stability of DPE at lower temperatures were estimated using the Arrhenius equation.

As expected the degradation rate of DPE increased with increasing pH (Table 1). The degradation rate of DPE was decreased by increasing HP-β-CD and $(SBE)_{7m}$-β-CD concentration at pH values 5.0 and 7.4 for every temperature studied (Table 2). Fig. 2 shows the typical effect of HP-β-CD and $(SBE)_{7m}$-β-CD concentrations on the aqueous stability of DPE. $(SBE)_{7m}$-β-CD (9.2 mM) increased the aqueous stability of DPE 15–30 times and 20–300 times at pH 5.0 and 7.4, respectively. HP-β-CD (9.2 mM) increased the aqueous stability of DPE only four to five times at both pH values studied. The Arrhenius plots formed parallel lines with different CD concentrations both with HP-β-CD and $(SBE)_{7m}$-β-CD at pH 5.0. However, at pH 7.4, the Arrhenius plots with different $(SBE)_{7m}$-β-CD concentrations formed a group of lines with decreasing slopes with increasing CD concentration.

The degradation rate of the drug, which forms 1:1 inclusion complexes with CDs, depends on the CD concentration according to the following equation (Loftsson et al., 1989)

$$k_{obs} = \frac{k_o + K_{1:1}k_c[CD]}{1 + K_{1:1}[CD]} \quad (2)$$

where, k_{obs} is the observed rate constant for the degradation of drug at known CD concentration, k_o and k_c are the rate constants for degradation of uncomplexed and complexed drug, respectively and $K_{1:1}$ is the stability constant for the 1:1-complex. The values for $K_{1:1}$ and k_c can be determined from stability data by nonlinear regression (Macintosh, KaleidaGraph) fit of data to Eq. (2).

Eq. (2) assumes that $CD_{free} = CD_{total}$ i.e. CD is present in excess of DPE concentration. However, in the present study, the lowest CD concentration in each experiment was 0.58 mM and the initial DPE concentrations at pH 5.0 and pH 7.4 were 0.52 mM and 0.26 mM, respectively. In the case of $(SBE)_{7m}$-β-CD, the strong complexation made this assumption invalid. Therefore for the $(SBE)_{7m}$-β-CD study, the initial DPE concentration was decreased.

The results from curve fitting studies (Table 3) show that DPE forms significantly more stable inclusion complexes with $(SBE)_{7m}$-β-CD than with HP-β-CD. As discussed earlier, the electrostatic interactions with the negatively charged sulfobutyl groups of $(SBE)_{7m}$-β-CD and positively charged DPE may increase the complexation of DPE with $(SBE)_{7m}$-β-CD (Okimoto et al., 1996). At both pH values, DPE molecules were mostly in their ionized form, thus similar complexation kinetics were observed at pH 5.0 and 7.4. With $(SBE)_{7m}$-β-CD, the complexation of DPE decreased at higher temperatures suggesting a strong enthalpic contribution to the interaction while with HP-β-CD the complexation of DPE was found to be relative independent of temperature. The relative stability of DPE in the complexed state as indicated by the k_c values was also greater for $(SBE)_{7m}$-β-CD compared to HP-β-CD.

Table 2
The observed and calculated shelf-lives ($t_{90\%}$) for overall degradation of DPE (initial DPE concentrations 0.52 and 0.26 mM at pH 5.0 and 7.4, respectively) at various temperatures in aqueous solutions containing HP-β-CD and $(SBE)_{7m}$-β-CD at pH values 5.0 and 7.4

pH	Temperature	$t_{90\%}$ (days) relative stabilization[b]			
		2.3 mM HP-β-CD	9.2 mM HP-β-CD	2.3 mM $(SBE)_{7m}$-β-CD	9.2 mM $(SBE)_{7m}$-β-CD
5.0	70	1.3/2.3	1.5/2.7	7.2/12.6	9.5/16.6
	60	2.5/2.7	3.3/3.6	14.5/15.6	20.6/22.1
	50	7.1/2.3	9.6/3.2	40.2/13.4	50.5/16.8
	37	25.6/3.0	31.9/3.7	159.5/18.5	213.7/24.8
	25[a]	88.9/3.1	117.0/4.1	569.3/20.1	729.0/25.7
	4[a]	1074.8/3.8	1447.4/5.0	7468.0/26.0	9172.0/32.0
7.4	70	0.1/3.0	0.1/3.7	0.4/12.0	0.7/18.7
	60	0.1/2.2	0.2/3.2	0.9/18.2	1.2/24.4
	50	0.3/2.7	0.4/3.9	4.2/42.3	5.8/57.8
	37	1.1/2.3	1.6/3.3	19.7/41.0	27.6/57.4
	25[a]	2.7/2.1	4.4/3.4	114.2/89.9	148.1/116.6
	4[a]	22.3/1.8	41.0/3.3	3310.5/268.5	3971.4/322.1

[a] Calculated value.
[b] $t_{90\%}$ with CD/$t_{90\%}$ without CD.

Therefore the greater stability effect of $(SBE)_{7m}$-β-CD derive both from stronger complexation (as indicated by the $K_{1:1}$ values) and greater intrinsic stability in the complexed state.

3.3. Interfacial transfer studies

Interfacial transfer studies were used as a preliminary method to predict the likely effect of HP-β-CD and $(SBE)_{7m}$-β-CD on partitioning of DPE to lipoidal membranes of the eye. The interfacial transfer method is based on the fact that polar CD molecules are not expected to partitioning into the organic phase. However, drug molecules will transfer due to their relative lipophilic character. In two-phase models, it is assumed that the transfer of drug from an aqueous phase to an organic phase may reflect the ability of the drug to be absorbed into the corneal tissue. At the beginning of the experiment, both drug and CD molecules were added to the aqueous phase and, depending on the extent of complexation of the drug with the CDs, the transfer rate of drug to organic phase changed.

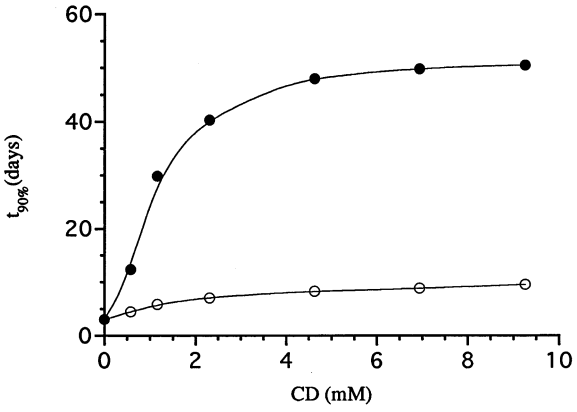

Fig. 2. Effect of increasing $(SBE)_{7m}$-β-CD (●) and HP-β-CD (○) concentration on shelf-life ($t_{90\%}$) of DPE at pH 5.0 (50°C, $\mu = 0.5$).

The ability of DPE to diffuse from an aqueous phase (0.16 M phosphate buffer) into an organic phase (*n*-octanol) was studied at pH 3.0, 5.0 and 7.4. In the presence of CDs, the studies were performed only at pH 5.0. At all pH-values and regardless of the presence and absence of CDs, the diffusion of DPE from aqueous phase into organic phase followed apparent first-order kinetics. Table 4 shows determined first-order rate constants (with or without CDs) at the pH-values studied. In the absence of CDs the increase in pH

Table 3
Pseudo-first-order rate constants for degradation of complexed (k_c) and uncomplexed (k_o) DPE in aqueous solution (pH 5.0 and 7.4) containing HP-β-CD or $(SBE)_{7m}$-β-CD at temperatures studied and calculated stability constants ($K_{1:1}$) for inclusion complexes

pH	Temperature (°C)	$k_o \times 10^{-3}(h^{-1})$	HP-β-CD			$(SBE)_{7m}$-β-CD		
			$k_c \times 10^{-3}$ (h^{-1})	k_o/k_c	$K_{1:1}$ (M^{-1})	$k_c \times 10^{-3}$ (h^{-1})	k_o/k_c	$K_{1:1}$
5.0	70	7.66	2.07	3.7	1171	0.38	20.2	14238
	60	4.71	1.12	4.2	1991	0.20	23.4	20336
	50	1.46	0.39	3.7	1534	0.07	19.7	28716
	37	0.51	0.12	4.3	2406	0.02	28.2	30384
7.4	70	141.91	33.12	4.3	2284	6.74	21.1	9802
	60	94.42	21.71	4.4	1492	2.07	45.6	14499
	50	44.27	7.82	5.7	1261	0.58	76.7	31033
	37	9.18	2.40	3.8	1642	0.15	60.8	64415

of the aqueous phase had no effect on measured first-order interfacial transfer constants for DPE. However, in the presence of $(SBE)_{7m}$-β-CD (2.3 mM) or HP-β-CD (2.3 mM) the diffusion rate of DPE from aqueous phase to organic phase decreased significantly (Fig. 3). As expected from the relative binding constants $(SBE)_{7m}$-β-CD ($k = 2.52\ min^{-1}$) had a greater negative effect on the interfacial transfer of DPE compared to HP-β-CD ($k = 16.03\ min^{-1}$).

3.4. In vitro corneal uptake study

The effect of $(SBE)_{7m}$-β-CD on the corneal uptake of DPE was studied in vitro by using the isolated cornea of pigmented rabbits. $(SBE)_{7m}$-β-CD (11.5 mM) significantly decreased the in vitro corneal uptake of DPE (Fig. 4). The calculated first-order rate constants for the corneal uptake of DPE with or without $(SBE)_{7m}$-β-CD were $3.50 \times 10^{-4}\ min^{-1}$ and $2.96 \times 10^{-3}\ min^{-1}$, respectively.

The concentrations of DPE and $(SBE)_{7m}$-β-CD for the in vitro uptake studies were five-times larger than the concentrations of compounds at interfacial transfer studies, due to lack of sensitivity in the analytical method. However, the molar ratio (drug:CD, 1:9) of DPE and $(SBE)_{7m}$-β-CD was the same as in the interfacial transfer studies.

The results show clearly that the complexation of DPE with $(SBE)_{7m}$-β-CD decreases signifi-

Table 4
Pseudo-first-order rate constants (k) for interfacial transfer of 0.26 mM DPE from aqueous phase (with or without CD) into organic phase at pH-values studied (mean ± SD, $n = 3$)

pH	CD	$k \times 10^{-3}$ (min^{-1})
3.0	—	25.42 ± 1.64
5.0	—	25.87 ± 1.33
	2.3 mM HP-β-CD	16.03 ± 0.63
	2.3 mM $(SBE)_{7m}$-β-CD	2.52 ± 0.23
7.4	—	28.45 ± 4.87

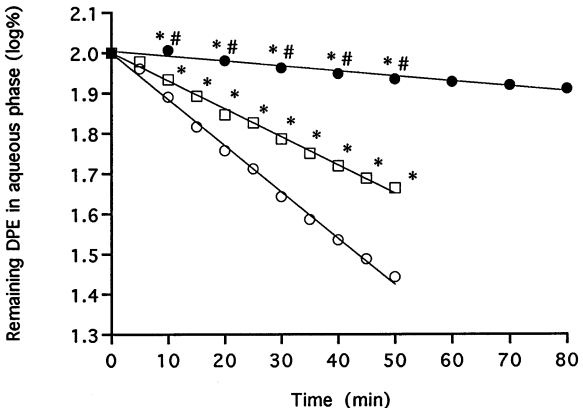

Fig. 3. First-order plots (interfacial transfer study) for the decrease of DPE concentration in aqueous phase containing no CDs (○), 2.3 mM HP-β-CD (□) and 2.3 mM $(SBE)_{7m}$-β-CD (●) at pH 5.0. Each point represents the mean ($n = 3$) with SD smaller than the symbol size. *, Significantly different from the value of DPE without CDs ($P < 0.05$, by Fisher's PLSD test); #, significantly different from the value with HP-β-CD ($P < 0.05$, by Fisher's PLSD test).

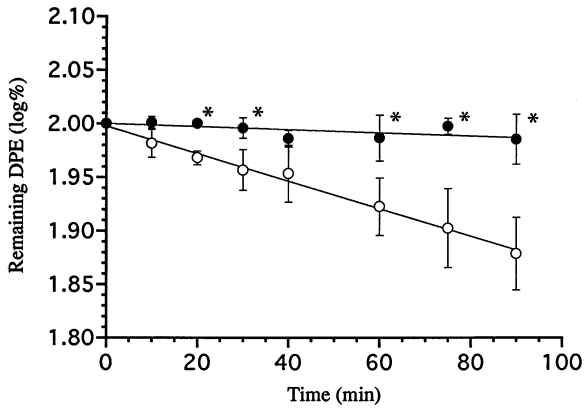

Fig. 4. First-order plots (in vitro uptake study) for the decrease of DPE concentration at the epithelial side of the cornea in the presence (●) and absence (○) of 11.6 mM $(SBE)_{7m}$-β-CD (mean ± SD, $n = 3–4$). *, Significantly different from the value of DPE without $(SBE)_{7m}$-β-CD ($P < 0.05$, by Fisher's PLSD test).

cantly the in vitro corneal uptake of DPE. $(SBE)_{7m}$-β-CD (11.6 mM) decreased the first-order rate constants for the corneal uptake of DPE (1.29 mM) 8.5 times compared to the DPE solution (1.29 mM) without $(SBE)_{7m}$-β-CD. The result is in good agreement with the result from the interfacial transfer study.

However, it must pointed out that the in vitro methods used here have limitations in prediction the in vivo behaviour. In vivo, drug uptake by the cornea, displacement by endogenous material and protein binding etc. can compete with the CD complexation.

4. Conclusions

The negatively charged cyclodextrin $(SBE)_{7m}$-β-CD, forms significantly stronger inclusion complexes with the positively charged DPE and had a greater stabilizing effect when compared to the neutral HP-β-CD. However, the interfacial transfer and in vitro corneal uptake studies suggested that the complexation of DPE with CDs, especially with $(SBE)_{7m}$-β-CD, may adversely affect the ocular availability of the DPE. This study demonstrates the possible difficulties associated with the use of CDs in ophthalmic preparations in order to increase the aqueous stability of water soluble drugs. Complexation of a drug with CDs may improve the pharmaceutical properties of the drug but simultaneously may decrease ocular absorption. By increasing the viscosity of the eye-drop solutions and therefore increasing the residence time of solution on the precorneal area it may be possible to overcome this problem.

Acknowledgements

We wish to thank the Academy of Finland and the Technology Development Centre (Finland) for financial support. The authors would also like to thank Mrs Helly Rissanen and Ms Pirjo Hakkarainen for their skillful technical assistance. CyDex, LC is gratefully acknowledged for providing $(SBE)_{7m}$-β-CD material.

References

Dollo, G., Le Corre, P., Chevanne, F., Le Verge, R., 1996. Inclusion complexation of amide-typed local anaesthetic with β-cyclodextrin and its derivatives. II. Evaluation of affinity constants and in vitro transfer rate constants. Int. J. Pharm. 136, 165–174.

Freedman, K.A., Klein, J.W., Crosson, C.E., 1993. β-Cyclodextrins enhance bioavailability of pilocarpine. Curr. Eye Res. 12, 641–647.

Frijlink, H.W., Eissens, A.C., Schoonen, A.J.M., Lerk, C.F., 1990. The effects of cyclodextrins on the drug absorption II. In vivo observation.. Int. J. Pharm. 64, 195–205.

Handbuch der Pharmaceutichen Praxis, 1993. Hagers Handbuch der Pharmaceutichen Praxis, 5. Auflage, Bd. 7 Stoffe A-D, s.1391 dipivefrindihydrochlorid, Spinger-Verlag, Berlin.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Instr. 4, 117–212.

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

Jansen, T., Xhonneux, B., Mesens, J., Borgers, M., 1990. β-Cyclodextrins as vehicles in eyedrop formulations: An evaluation of their effects on rabbit corneal epithelium. Lens Eye Toxic. Res. 7, 459–468.

Jarho, P., Järvinen, K., Urtti, A., Stella, V.J., Jarvinen, T., 1996. Modified β-cyclodextrin (SBE7-β-CyD) with viscous vehicle improves the ocular delivery and tolerability of pilocarpine prodrug in rabbits. J. Pharm. Pharmacol. 48, 263–269.

Järvinen, K., Järvinen, T., Thompson, D.O., Stella, V.J., 1994. The effect of a modified β-cyclodextrin, SBE4-β-CD, on the aqueous stability and ocular absorption of pilocarpine. Curr. Eye Res. 13, 891–905.

Järvinen, K., Järvinen, T., Urtti, A., 1995a. Ocular absorption following topical delivery. Adv. Drug Deliv. Rev. 16, 3–19.

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

Kanai, A., Alba, R.M., Takano, T., Kobayashi, C., Nakajima, A., Kurihara, K., Yokoyama, T., Fukami, M., 1989. The effect of the cornea of α cyclodextrin vehicle for cyclosporin eyedrops. Transplant. Proc. 21, 3150–3152.

Keipert, S., Fedder, J., Böhm, A., Hanke, B., 1996. Interactions between cyclodextrins and pilocarpine—as an example of a hydrophilic drug. Int. J. Pharm. 142, 153–162.

Kohn, A.N., Moss, A.P., Hargett, N.A., Ritch, R., Smith, H., Podos, S.M., 1979. Clinical comparison of dipivalyl epinephrine and epinephrine in the treatment of glaucoma. Am. J. Ophthalmol. 87, 196–201.

Kristinsson, J.K., Fridriksdottir, H., Thorisdottir, S., Sigurdardottir, A.M., Stefansson, E., Loftsson, T., 1996. Dexamethasone-cyclodextrin-polymer co-complexes in aqueous eye drops. Aqueous humor pharmacokinetics in humans. Invest. Ophthalmol. Vis. Sci. 37, 1199–1203.

Lee, V.H.L., Robinson, J.R., 1986. Review: Topical ocular drug delivery recent developments and future challenges. J. Ocular Pharmacol. 2, 67–108.

Loftsson, T., Björnsdottir, S., Palsdottir, G., Bodor, N., 1989. The effect of 2-hydroxypropyl-β-cyclodextrin on the solubility and stability of chlorambucil and melphalan in aqueous solution. Int. J. Pharm. 57, 63–72.

Loftsson, T., Fridriksdottir, H., Thorisdottir, S., Stefansson, E., Sigurdardottir, A.M., Gudmundsson, Ö., Sigthorsson, T., 1994. 2-Hydroxypropyl-β-cyclodextrin in topical carbonic anhydrase inhibitor formulations. Eur. J. Pharm. Sci. 1, 175–180.

Mandell, A.I., Stentz, F., 1978. Dipivalyl epinefrine: A new pro-drug in the treatment of glaucoma. Ophthalmology 85, 268–275.

Nakanishi, K., Masada, M., Nadai, T., Miyajima, K., 1989. Effect of the interaction of drug-β-cyclodextrin complex with bile salts on the drug absorption from rat small intestinal lumen. Chem. Pharm. Bull. 37, 211–214.

Okimoto, K., Rajewski, R.A., Uekama, K., Jona, J.A., Stella, V.J., 1996. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins. Pharm. Res. 13, 256–264.

Rajewski, R., Stella, V.J., 1996. Pharmaceutical application of cyclodextrin. 2. In vivo drug delivery. J. Pharm. Sci. 85, 1142–1169.

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

Usayapant, A., Karara, A.H., Narurkar, M.M., 1991. Effect of 2-hydroxypropyl-β-cyclodextrin on the ocular absorption of dexamethasone and dexamethasone acetate. Pharm. Res. 8, 1495–1499.

USP DI, Drug Information for the Health Care Professional. 1990 10th ed. United States Pharmacopeial Convention, IA, pp. 1198–1199.

Van Doorne, H., 1993. Interactions between cyclodextrins and ophthalmic drugs. Eur. J. Pharm. Biopharm. 39, 133–139.

Wheeler, L.A., 1991. The use of inclusion complexes of prostaglandins with cyclodextrins in the treatment of ocular hypertension. European Patent O 435 682 A3.