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DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 29, No. 2, pp. 223–230, 2003

RESEARCH PAPER

Influence of Hydroxypropyl β-Cyclodextrin on the Corneal Permeation of Pilocarpine

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ABSTRACT

The influence of hydroxypropyl β-cyclodextrin (HPβCD) on the corneal permeation of pilocarpine nitrate was investigated by an in vitro permeability study using isolated rabbit cornea. Pupillary-response pattern to pilocarpine nitrate with and without HPβCD was examined in rabbit eye. Corneal permeation of pilocarpine nitrate was found to be four times higher after adding HPβCD into the formulation. The reduction of pupil diameter (miosis) by pilocarpine nitrate was significantly increased as a result of HPβCD addition into the simple aqueous solution of the active substance. The highest miotic response was obtained with the formulation prepared in a vehicle of Carbopol® 940. It is suggested that ocular bioavailability of pilocarpine nitrate could be improved by the addition of HPβCD.

Key Words: Pilocarpine; Ocular bioavailability; Cyclodextrin; Polyacrylic acid.

INTRODUCTION

Pilocarpine is a direct-acting parasympathomimetic agent that lowers intraocular pressure by increasing the outflow of aqueous humor.[1] It has been used the longest as a mainstay drug for glaucoma therapy, and is one of the least-expensive and the most readily available medications. Despite its good pharmacodynamic effect on decreasing intraocular pressure, ocular bioavailability of aqueous pilocarpine eye drops is limited because of the barrier properties of the cornea and the precorneal loss factors.[2,3] The poor ocular absorption of pilocarpine nitrate is known to be from the extensive solution drainage via the nasolacrimal duct and tear turnover.[4,5] To improve corneal

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DOI: 10.1081/DDC-120016730
0363-9045 (Print); 1520-5762 (Online)

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bioavailability of the ocular drugs, several approaches—including the use of viscosity enhancers[6–9]—alternative delivery systems[10–14] have been attempted. Acrylic acid polymers, such as Carbopol® 940, has been investigated in ophthalmic delivery systems because of their mucoadhesive properties. Drug levels in the aqueous humor have been substantially prolonged as a result of adhesion of these polymers to ocular surfaces.[15–18]

The lipophilic nature of corneal epithelium prevents hydrophilic drugs from penetrating throughly into the inner tissues of the eye. Penetration enhancers are also useful to facilitate the transport of drugs, which are poorly absorbed through epithelial membranes.[19–22] An approach to improve ocular drug absorption is the use of cyclodextrins (CDs).[23–29] These compounds have been used to improve the solubility and stability, as well as the bioavailability of drugs. Cyclodextrins are known to be able to form inclusion complexes with mainly the lipophilic drugs. Cyclodextrin inclusion complexes with rather hydrophilic drugs, like pilocarpine hydrochloride, has also been investigated.[30–32] Miosis tests on rabbit eyes performed with α-CD and β-CD containing pilocarpine hydrochloride solutions have yielded a relatively improved bioavailability in comparison with the CD-free solutions.[31,32]

The aim of the present study was to investigate the influence of hydroxypropyl β-CD (HPβCD) on the corneal permeation of pilocarpine nitrate. We also aimed to prepare a suitable polymeric vehicle for ocular delivery of the pilocarpine–CD complex.

EXPERIMENTAL

Materials

Pilocarpine nitrate was purchased from Sigma Chemical (St. Louis, MO), and HPβCD was obtained from Cyclolab (Budapest, Hungary). Carbopol 940 was a gift sample by Goodrich Chemical Co. (Cleveland, OH). A commercially available product of pilocarpine, Pilokarsol™, was provided from Sanovel (Turkey). All other chemicals used were of analytical grade.

Male albino rabbits (New Zealand), weighing 3–4 kg, were used in the study. No particular pretreatment regimens regarding water, diet, or environment were followed.

Preparation of Ophthalmic Formulations

Two different concentrations (1% and 2%) of pilocarpine nitrate in bicarbonated Ringer's solution (BRS) were prepared as simple aqueous solutions. To prepare pilocarpine–CD complex, 5% of HPβCD was added to the solutions. To prepare the gel forms, HPβCD (5%) and a given amount of pilocarpine nitrate were added to the dispersion obtained by the hydration of Carbopol 940 at a concentration of 0.2%. Gel form occurred after neutralization of the dispersion using a 10% solution of sodium hydroxide. Mannitol (5%) was added into the gel formulations as the osmotic agent. pH and molarity of the formulations were measured by a pH-meter Sesa model 1400 (Turkey) and by a cryoscopic osmometer (Osmomat 050, Advanced Instruments, Inc., Needham Heights, MA). Formulations were sterilized by autoclave before the in vivo experiments. Table 1 demonstrates the pH and osmolarity values of the formulations prepared.

In Vitro Permeability Studies

Side-by-side diffusion cells with 1.08 cm^2 surface area were used as described earlier for in vitro permeability studies.[33] Rabbits were killed by injection of an overdose of succinylcholine into the marginal ear vein. The corneas were excised from globes with an approximately 2 mm scleral ring and mounted onto the ring of the perfusion apparatus. The corneas were gently rinsed with saline, and extreme care was taken not to produce any wrinkles or folding of the membrane before mounting. Five milliliters of BRS preadjusted to a temperature of 37°C was placed into the receptor chambers of the apparatus. The same volume of BRS containing pilocarpine nitrate in given strengths was placed into the donor chamber. The concentration of pilocarpine nitrate in the donor chamber was 1,000 μg/mL for CD-free solutions. A 100 μg/mL concentration of pilocarpine nitrate, providing perfusion sink conditions, was choosen for HPβCD combinations. HPβCD concentration was 5% (w/v) of the total volume for all the test solutions. A mixture of O_2:CO_2 (95:5) was bubbled through the chambers to provide a good mixing. Samples were taken at 0.25, 0.5, 1, 2, 3, 4, and 5 hr. The amount of the drug permeated across the cornea was assayed with UV spectroscopy at $\lambda = 215$ nm, and permeability coefficient was calculated.

Table 1. Test formulations of pilocarpine nitrate.

Formulation	Osmolarity (Osm/kg)	pH	Viscosity (Pa · sec)**
BRS	0.284	7.72	ND
1,000 μg/mL PN (AS in BRS)*	0.280	7.04	ND
20 μg/mL PN + 5% HPβCD (AS in BRS)*	0.300	7.03	ND
1% PN (AS in BRS)	0.291	7.00	4
2% PN (AS in BRS)	0.295	6.98	4
1% PN + 5% HPβCD (AS in BRS)	0.308	7.00	4
2% PN + 5% HPβCD (AS in BRS)	0.313	7.00	4
1% PN + 5% HPβCD + 0.2% Carbopol 940 (AG)	ND	7.21	242
2% PN + 5% HPβCD + 0.2% Carbopol 940 (AG)	ND	7.32	77
Pilokarsol (2% PN + PVA)	0.306	5.52	ND

BRS, bicarbonated Ringer's solution; PN, pilocarpine nitrate; AS, aqueous solution; AG, aqueous gel; PVA, polyvinyl alcohol; ND, not determined.
*Solutions tested only in vitro.
**Solutions tested at 12 rpm, at room temperature.

Corneal permeability coefficients for pilocarpine nitrate were calculated using the equation below as it is referred to by Grass and Robinson.[33]

$$\text{P.C.} = \frac{\Delta C \cdot 5}{\Delta t A \cdot Co} \quad (1)$$

In Eq. (1), $\Delta C/\Delta t$ is the change in concentration (μg/mL) of sample with time represented by the slope of the linear part of the permeability curve; 5 is the volume of the receiver chamber in mL; A is the surface area of the mounted membrane, taken to be 1.08 cm^2; and Co is the initial concentration of the drug in the donor chamber. The steady-state flux was calculated as the slopes by linear regression in the range from 30 to 300 min for all curves.

Viscosity Measurements

Viscosity measurements were performed by a rotating cylinder viscometer (Lab-Line Instruments, Inc., Melrose Park, IL). Flow properties of the aqueous gels were examined in different rotation rates, ranging as 0.3–0.6, 1.5–3, 6–12, and 30–60 rpm. Viscosity of the formulations was recorded at 12 rpm.

Pharmacodynamic Studies

Formulations given in Table 1 were used for this part of the study. Male albino rabbits (New Zeland) weighing 3–4 kg were kept in restraining boxes in the normal upright position. Rabbits were acclimatized to the test room for approximately 30 min before the experiments.

Fifty microliters of a topical anesthetic (Alcaine™) was applied into the eyes. After a 5-min resting period, 25 μL of the test formulations were instilled into the lower conjunctival sac of the right eyes, and the left eyes served as controls. Pupillary diameter measurements were taken before administration and at 0.25, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hr after instillation. Pupillary diameter measurements were done manually using a scale. Response parameters (RP) were calculated by the formula below [Eq. (2)] and plotted vs. time.[34] Response parameter is proportional to the drug concentration in the sphincter muscles. From the response parameter-time curves, area under the curves ($AUC_{0\to\infty}$), maximum miotic response (R_{max}), and time to reach maximum miotic response (t_{max}) were calculated by the following equation.

$$RP = \frac{Do - D}{D - 1} \quad (2)$$

Where, Do and D (mm) are the pupil diameters before and after administration of drug, respectively.

RESULTS AND DISCUSSION

pH and Osmolarity

pH values of the formulations ranked from 6.98 to 7.72 and the osmolarity values of the solutions

were between 0.280 and 0.313 Osm/kg (Table 1). Osmolarity of the gels could not be measured because of their high viscosity, and they were assumed to be isoosmotic because of the mannitol content. No change was observed in the initial pH and viscosity values of the solutions and the gels after sterilization.

In Vitro Permeability Studies

As shown in Fig. 1, an increase in the corneal permeation of pilocarpine nitrate was observed by HPβCD. The permeability coefficient for pilocarpine nitrate was found to be $1.67 \times 10^{-5} \pm 2.12 \times 10^{-7}$ cm/sec. Permeability coefficients were increased to $6.38 \times 10^{-5} \pm 2.82 \times 10^{-7}$ cm/sec in the presence of HPβCD.

Viscosity Measurements

Viscosity of the solutions and the aqueous gels are given in Table 1. Viscosity values of the aqueous solutions were the same for 1% and 2% concentrations of pilocarpine nitrate with/without HPβCD. The increase of pilocarpine nitrate concentration from 1% to 2% caused a decrease in the initial viscosity of Carbopol 940 aqueous gels. Flow property of the gels was found to be non-Newtonian in behavior.

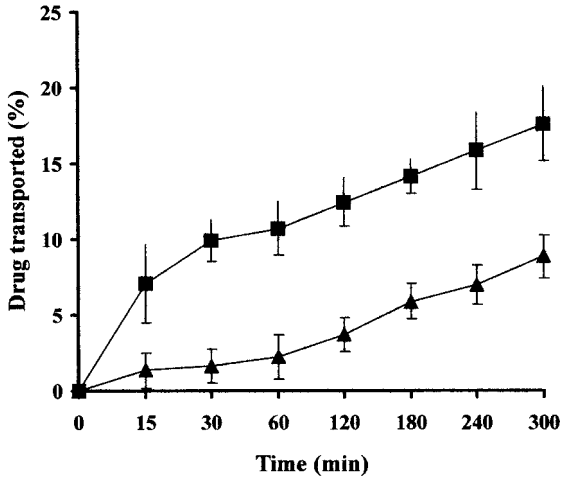

Figure 1. Corneal permeation curves of 1,000 μg/mL pilocarpine nitrate (▲) and 100 μg/mL pilocarpine nitrate + Δ5% HPβCD (■). Error bars represent the means ± SD of 10 determinations.

Pharmacodynamic Studies

The curves of miotic response vs. time plotted for 1% and 2% pilocarpine nitrate-containing formulations are given in Fig. 2. The reduction of pupil diameter (miosis) by pilocarpine nitrate was significantly increased as a result of HPβCD addition into the simple aqueous solution of the active substance. The pharmacokinetic parameters including $AUC_{0\to\infty}$, t_{max}, R_{max}, and the rate coefficients representing the absorption and elimination phases of the miosis time curves (k_{abs}, K_{el}) are listed in Table 2. The highest area under the curve was observed with the gel forms containing pilocarpine nitrate and HPβCD together.

There was no significant difference between the miotic responses of 1% and 2% concentrations of pilocarpine nitrate. Carbopol 940 gel was not applied into the rabbits' eye alone, so we could not evaluate individual contribution of the polymer to the pilocarpine's pharmacological activity. After addition of HPβCD, $AUC_{0\to\infty}$ was augmented three times for the aqueous solutions and 5.5 times for the gel forms containing HPβCD, compared with HPβCD-free solutions.

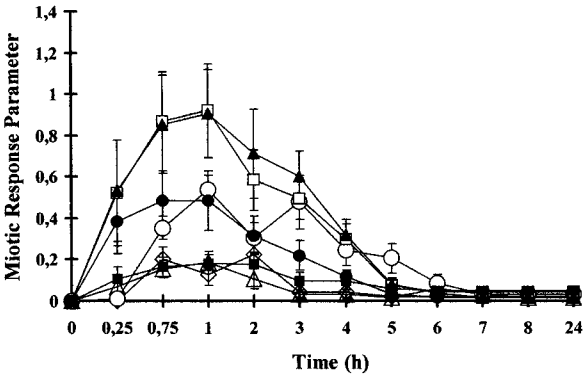

Figure 2. Miotic response parameters after the application of pilocarpine-containing formulations. (△) 1% PN (AS in PBS); (◇) 2% PN (AS in PBS); (○) 1% PN + 5% HPβCD (AS in PBS); (●) 2% PN + 5% HPβCD (AS in PBS); (□) 1% PN + 5% HPβCD + 0.2% Carbopol 940 (AG); (▲) 2% PN + 5% HPβCD + 0.2% Carbopol 940 (AG); (■) Pilokarsol (2% PN + PVA). Error bars represent the means ± SD of 10 determinations. PN, pilocarpine nitrate; AS, aqueous solution; PBS, phosphate-buffered saline; AG, aqueous gel; PVA, polyvinyl alcohol.

Table 2. Pharmacokinetic parameters (±SD) of test formulations.

Formulation	$AUC_{(0\rightarrow\infty)}$	t_{max} (hr)	k_{el} (hr^{-1})	K_{abs} (hr^{-1})	R_{max}
1	0.71 (0.56)	2.11 (0.05)	0.0457 (0.04)	1.7765 (0.83)	0.20 (0.02)
2	0.73 (0.69)	2.07 (0.03)	0.0617 (0.05)	1.6237 (0.87)	0.19 (0.03)
3	2.302 (0.98)	1.12 (0.04)	0.0366 (0.03)	0.5290 (0.14)	0.43 (0.08)
4	2.090 (0.87)	0.78 (0.07)	0.0866 (0.08)	0.1756 (0.08)	0.39 (0.09)
5	3.90 (1.17)	1.22 (0.06)	0.0810 (0.07)	0.3112 (0.11)	0.92 (0.13)
6	4.08 (1.19)	1.10 (0.08)	0.0814 (0.07)	0.8640 (0.23)	0.91 (0.11)
7	1.039 (0.73)	1.12 (0.07)	0.025 (0.02)	0.6550 (0.33)	0.18 (0.04)

1, 1% PN (AS in BRS); 2, 2% PN (AS in BRS); 3, 1% PN + 5% HPβCD (AS in BRS); 4, 2% PN + 5% HPβCD (AS in BRS); 5, 1% PN + 5% HPβCD + 0.2% Carbopol 940 (AG); 6, 2% PN + 5% HPβCD + 0.2% Carbopol 940 (AG); **7**, Pilokarsol (2% PN + PVA). PN, pilocarpine nitrate; AS, aqueous solution; BRS, bicarbonated Ringer's solution; AG, aqueous gel; PVA, polyvinyl alcohol.

CONCLUSIONS

The general mechanism of action for CDs is the formation of inclusion complexes with lipophilic substances because of their special molecular structure.[35,36] Ocular absorption enhancement of different CD derivatives has been reported.[24,30–32] Because they are not able to permeate biological membranes easily, CDs, in aqueous eye drops, act as carriers for the drugs complexed and delivers them to the absorptive surfaces of the eye. Only the free drug can penetrate membranes, and the drug must be released from the inclusion complex before absorption.[37]

In the current study, an approximately 4-fold increase in the permeability coefficient of pilocarpine nitrate was obtained in presence of HPβCD, compared with CD-free drug solution. This permeation enhancing of CD was supported by pharmacodynamic results. Siefert and Keipert[38] have compared the diffusion behavior of pilocarpine hydrochloride in the presence of α-CD and HPβCD. They found permeation of pilocarpine to be significantly increased by α-CD, but slightly decreased by HPβCD. The authors assumed that an interaction between HPβCD and the drug might be the cause of the decrease in corneal permeability.

The maximum response (R_{max}) appeared to be increased by 5% HPβCD to the simple solution of pilocarpine nitrate. This increase was found to be more significant for the aqueous gels. In the case of aqueous solutions, a rapid absorption and elimination process were found. By the addition of HPβCD, area under the time-response curve ($AUC_{0\rightarrow\infty}$) was increased three times for the solution and 5.5 times for the gel, in comparison with HPβCD-free solutions. A similar observation has been reported by Freedman et al.[31] They found a significant increase in the miotic response for 0.1% pilocarpine hydrochloride in a 5% HPβCD solution when compared with pilocarpine alone. Earlier reports related to CD's role in ocular absorption of water-soluble drugs are conflicting. Contrary to our finding, it has been reported that HPβCD reduced the efficacy of water-soluble pilocarpine on intraocular pressure in rabbits.[30,38] The pilocarpine–HPβCD complex has not caused an improvement in corneal uptake of pilocarpine, according to data from the study by Keipert et al.[32]

As the miotic response data demonstrates, there was no significant difference in the pharmacological response between two concentrations (1% and 2%) of pilocarpine nitrate in the aqueous solution. These data show that an increase in the concentration of the drug does not correspond to an improvement in absorption, because the drainage and precorneal loss factors are more prominent in the absorption process.

It is known that the more the concentration of the drug is increased, it is not possible for the pupil diameter to become decreased under a threshold.[34]

Despite their different pH values, two simple solutions of pilocarpine nitrate and the brand-name product demonstrated the response-time curves differently. The absorption of pilocarpine through the cornea is known to be increased when the drug solution is applied at neutral or alkaline pHs. Pilocarpine, in an eye drop that possesses relatively acidic pH (5.5 in the case of Pilokarsol®) is not expected to be absorbed as well as from the neutral solutions. Polyvinyl alcohol content of the commercial product, despite its lower pH, might be the reason for a slight increase in the miotic response of pilocarpine, compared with the simple aqueous solutions in neutral pH.

It has been reported that CDs increased intraocular bioavailability of pilocarpine by shifting the equilibrium of ionized pilocarpine toward the nonionized form.[31] Siefert and Keipert[38] announced that the interaction between epithelium and CD could decrease the lipophilicity of the membrane by interacting with some lipophilic compounds. Supporting in this regard, a decrease in the bioavailability has been observed with higher lipophilic prodrugs of pilocarpine after the addition of CDs.[27,37] This decreased transport of the prodrug through the cornea may be from the changed lipophilicity of the membrane.

It is known that topical application of HPβCD is well-tolerated by the corneal epithelium. The ocular tolerance of HPβCD has been proved by earlier reports.[23,26,28,31] Some authors state that CDs enhance drug permeation by disrupting the membrane. Freedman et al.[31] have announced that HPβCD did not disrupt the normal ion transport and the barrier properties or surface features of the corneal epithelium.

Pilocarpine may exist as the nonionized form in the CD–drug complex, as mentioned before.[31] Because formulations of the current study were buffered to physiological pH, the free drug, before its absorption, may remain as the nonionized form after dissociation of the CD–pilocarpine complex. Then, diffusion by partitioning could be facilitated. HPβCD did not appear to increase the residence time in the precorneal area; therefore, interaction of the CD–drug complex with the tear film may not be a possible explanation for increased AUC. Although HPβCD is well-tolerated by the corneal epithelium, alteration in the membrane structure can be considered. In this case, the ionized form of the drug can penetrate faster through the increased aqueous pores of the membrane.

The dissociation rate of the CD–drug complex on the precorneal area should be faster than the rate of their clearance from the precorneal area. This has been achieved by increasing solution viscosity. To enhance the solubilization effect of CD, the addition of water-soluble polymer to aqueous drug solutions has been attempted.[39,40] It can be considered that the water-soluble polymers interact with the CD–drug complexes by complex formation between several CD–drug complexes and a polymer chain. Significantly higher $AUC_{0\rightarrow\infty}$ and the R_{max} values for the formulations containing Carbopol 940 reflected an additional augmentation in the pharmacological activity of drug. This could be from both the mucoadhesive property and the viscolyzing effect of the polymer. It was suggested that Carbopol 940, with an alternative mechanism, potentialized the penetration-enhancing effect of HPβCD.

Viscosity of the gel containing 2% pilocarpine was lower than that of the gel containing 1% pilocarpine (Table 1). The amount of pilocarpine could affect the structure of the gel and cause a reduction in viscosity. The lower response parameter of the 2% pilocarpine nitrate compared with that of 1%, which supported the viscosity data.

An enhancing effect of HPβCD in combination with a viscolyzing and adhesive effect of Carbopol 940 can be considered notable for improvement of pilocarpine bioavailability through the cornea.

ACKNOWLEDGMENT

This study was supported by the Hacettepe University Research Foundation.

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