this generalization, however, at least one and preferably several other units of this instrument should be tested to determine how much inter-unit variability exists. Presumably, there will be little. The exercise is worthwhile because the TP is such a convenient instrument to use.

Key Words

cynomolgus monkey, glaucoma, intraocular pressure, tonometry, Tono-Pen.

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Dexamethasone–Cyclodextrin–Polymer Co-complexes in Aqueous Eye Drops

Aqueous Humor Pharmacokinetics in Humans

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Purpose. To test an aqueous eye drop solution containing a high concentration of dexamethasone in a cyclodextrin-based drug delivery system. This system increases both drug solubility in aqueous eye drops and drug permeability into the eye, through drug-cyclodextrin-polymer co-complexes.

Methods. 2-hydroxypropyl-β-cyclodextrin is a water-soluble oligosaccharide that can be used to dissolve lipophilic drugs, such as dexamethasone, in aqueous solutions. Co-complexation with a polymer further increases the solubility and increases drug permeability through biologic membranes. Eye drops containing dexamethasone (0.32% and 0.67%), 2-hydroxypropyl-β-cyclodextrin, and polymer were given to patients before cataract surgery, and the resultant dexamethasone concentration was measured from aqueous humor samples.

Results. The dexamethasone–cyclodextrin drops give a significantly higher concentration of dexamethasone in aqueous humor than dexamethasone alcohol 0.1% (Maxidex). Heating of the dexamethasone-cyclodextrin-polymer co-complexes appears to enhance the permeability of the drug into the eye.

Conclusions. The cyclodextrin-based drug delivery system enhances both the solubility of dexamethasone in aqueous eye drops and the permeability of the drug into the human eye. Dexamethasone concentration levels in the human aqueous humor exceed those reported with currently available steroid eye drops. Invest Ophthalmol Vis Sci. 1996;37:1199–1203.

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Dexamethasone has been one of the most frequently used topical ocular corticosteroids.[1] Topical corticosteroids are used for diseases of the outer eye and anterior segment of the eye. Inflammatory diseases in the posterior segment of the eye usually require systemic corticosteroids. Topical corticosteroids are lipophilic water-insoluble compounds that are only soluble to a limited extent in aqueous eye drop formulations and, thus, frequently are formulated as suspensions, such as 0.1% dexamethasone alcohol, or as hydrophilic water-soluble prodrugs, such as 0.1% dexamethasone sodium phosphate.

To increase the bioavailability of topical ocular steroids, it is desirable to increase the concentration of the steroid drug in aqueous eye drops and the permeability through the cornea or sclera. Our hypothesis is that both these aims can be accomplished with the use of the cyclodextrin-based drug delivery system for eye drops.

2-hydroxypropyl-β-cyclodextrin (HPβCD) is a hydroxypropyl substituted β-cyclodextrin.[2,3] It is a cyclic oligosaccharide with a hydrophilic outer surface. Although soluble in water, it has a lipophilic cavity in the center. HPβCD forms inclusion complexes with many lipophilic drugs by taking up a drug molecule, or part of it, into the cavity. In this way, it is possible to form aqueous drug–HPβCD complexes of lipophilic drugs.[4] The complexes are readily dissociated because no covalent bonds are formed. As a rule, HPβCD molecules do not penetrate biologic membranes but act as penetration enhancers by assuring constant high concentration of dissolved drug at the membrane surface. In topical drug formulations, HPβCD keeps water-insoluble drug molecules in solution, delivering them to the surface of the barrier where they partition into the barrier.[5,6] In doing so, HPβCD improves ocular bioavailability of drugs by increasing their rate of absorption through the corneal barrier. Complexation of dexamethasone with HPβCD increases the absorption of dexamethasone 0.1% through the rabbit cornea by 40%.[7]

We have discovered that in aqueous solutions, water-soluble polymers (hydroxypropyl methylcellulose [HPMC]) increase the solubilizing effect of cyclodextrins on lipophilic drugs by increasing the stability constants of the drug–cyclodextrin complexes. The polymers form co-complexes with the drug and cyclodextrin molecules. At the cornea, the polymers may adhere to the surface. This promotes the release of drug molecules from the cyclodextrin inclusion complexes into the solution leading to a high concentration of drug molecules at the corneal surface, resulting in permeability enhancement. However, the cyclodextrin-based drug delivery system has not been found to alter the barrier function of the cornea.[8,9]

We have formed eye drops containing a novel aqueous dexamethasone–HPβCD–HPMC co-complex with a dexamethasone concentration up to 1.28%. In a previous study,[10] we compared the administration of 1.28% dexamethasone eye drop solution in rabbits to 0.1% dexamethasone alcohol suspension (Maxidex; Alcon Laboratories (Fort Worth, TX). Dexamethasone concentration in aqueous humor measured, on average, 4.7 times greater with 1.28% dexamethasone than with 0.1% dexamethasone. The purpose of this study was to determine the ocular bioavailability of 0.32% and 0.67% dexamethasone–HPβCD–HPMC by administrating the eye drops to patients undergoing cataract surgery and comparing it to the bioavailability of the commercially available dexamethasone 0.1% suspension (Maxidex). Furthermore, we tested the effect of heating (i.e., forming the co-complex) on the ocular bioavailability of 0.67% dexamethasone.

MATERIALS AND METHODS. Patients scheduled to undergo cataract surgery were recruited to the study. Informed consent was obtained. Patients with corneal disease, inflammatory ocular disease, glaucoma, or with the only potentially seeing eye undergoing surgery or receiving systemic or topical steroid treatment were excluded.

Dexamethasone was obtained from Sigma Chemical (St. Louis, MO), HPβCD of molar substitution 0.6 was obtained from Wacker–Chemie (Munich, Germany), hydroxypropyl methylcellulose (HPMC) 4000 was obtained from Mecobenzon (Copenhagen, Denmark), and Maxidex eye drops were obtained from Alcon Laboratories. The dexamethasone–HPβCD–HPMC co-complex was produced by heating the dexamethasone–HPβCD with 0.10% hydroxypropyl methylcellulose in an autoclave (120°) for 20 minutes.

Four types of preparations were tested:

Preparation 1
- 0.32% dexamethasone
- 0.1% HPMC
- 5% HPβCD
- 0.05% sodium edetate
- 0.7% sodium chloride
- 0.01% benzalconium chloride

Preparation 2
- 0.67% dexamethasone
- 0.1% HPMC
- 10% HPβCD
- 0.05% sodium edetate
- 0.55% sodium chloride
- 0.01% benzalconium chloride
- Heated in an autoclave (120°) for 20 minutes

Preparation 3
0.67% dexamethasone
0.1% HPMC
11.5% HPβCD
0.05% sodium edetate
0.55% sodium chloride
0.01% benzalconium chloride
Unheated
Preparation 4
0.1% dexamethasone alcohol (Maxidex)

Other preoperative procedures were routine and included the administration of 1 drop of eye drop solution containing 0.1% indomethacine 60 and 30 minutes before surgery and 1% cyclopentolate and 10% phenylephrine, each 60, 45, and 30 minutes before surgery.

One drop (50 μl) of 0.32% dexamethasone–HPβCD–HPMC or 0.67% dexamethasone–HPβCD–HPMC (heated or unheated) or 0.1% dexamethasone alcohol (Maxidex) was administered into the lower conjunctival fornix of the eye prepared for cataract surgery at predetermined time points before surgery. Twelve patients acted as controls and received no dexamethasone preparation. The selection of preparations to patients was randomized.

Before opening the anterior chamber, 0.1 ml of aqueous humor was withdrawn with a small needle and syringe. The time interval between the topical administration of the dexamethasone eye drops and aspiration of the aqueous humor was recorded. Quantitative determination of dexamethasone concentration was performed by liquid chromatography on a high-performance liquid chromatography component system (ConstaMetric [Bie & Bernsten a/s, Denmark] 3200 solvent delivery system, Rheodyne [Cotati, CA] 7125 injector, a Beckman Ultrasphere [Caltech, Pasadena, CA] ODS 5 μm [4.6 × 150 mm] column and a Spectro Monitor [Bie & Bernsten] 3200 UV, Milton Roy [Spectronic Instruments, Rochester, NY], variable-wavelength detector operated at 242 nm). The mobile phase consisted of acetonitrite, tetrahydrofurane, and water (30:1:69). The flow rate was 1.8 ml/minute, and the retention time was 4.4 minutes. Aqueous humor samples were injected directly into the column without pretreatment. The drug recovery from aqueous humor samples that had been spiked with dexamethasone was estimated to be approximately 100%.

The area under the curve (AUC) for each preparation was generated as a linear combination of means from 0 through the last observation time. Statistical methods for demonstrating bioequivalence by comparing the drug preparations were adopted from Schoenwald et al.[11] These included calculating the variance estimates for each AUC and using them to construct 95% confidence limits on each area and calculating a *t*-test comparing two areas.

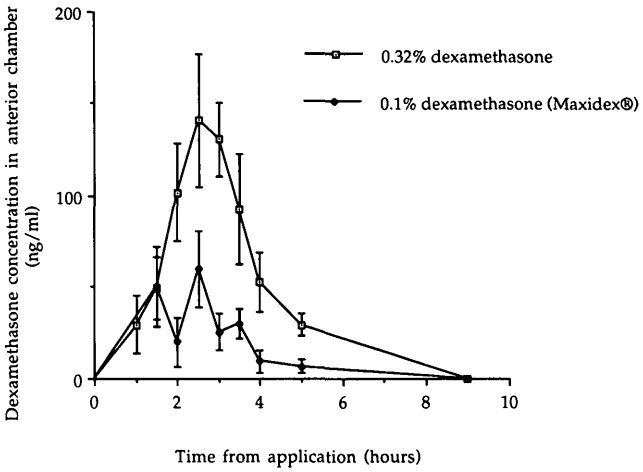

FIGURE 1. Dexamethasone concentration in aqueous humor after the administration of 0.32% dexamethasone–2-hydroxypropyl-β-cyclodextrin–hydroxypropyl methylcellulose and 0.1% dexamethasone alcohol (Maxidex). Mean concentration ± SEM is shown at appropriate time points after administration of the eye drops.

This research followed the tenets of the Declaration of Helsinki. Approval was obtained from the ethics committee of Landakotsspítali and the Icelandic Drug Administration (Ministry of Health).

RESULTS. One hundred twenty-five patients participated in the study. Of those, 47 received 0.32% dexamethasone–HPβCD–HPMC, 26 patients received heated 0.67% dexamethasone—HPβCD–HPMC, 18 patients received unheated dexamethasone 0.67%–HPβCD, and 34 patients received Maxidex. Two additional patients were excluded because of abnormally high values of dexamethasone in the anterior chamber, one after receiving heated 0.67% dexamethasone–HPβCD–HPMC (655 ng/ml after 4 hours, mean concentration$_{4\ \text{hours}}$ ± SEM = 50.5 ± 33.5 ng/ml) and the other after receiving unheated 0.67% dexamethasone–HPβCD–HPMC (116 ng/ml after 1.9 hours, mean concentration$_{1.9\ \text{hours}}$ ± SEM = 6.4 ± 6.4 ng/ml).

Dexamethasone concentration in the anterior chamber was higher after the administration of 0.32% dexamethasone–HPβCD–HPMC solution compared with Maxidex suspension (Fig. 1) or 2.6 times higher levels comparing AUC curves of the preparations (Table 1), which was a statistically significant difference ($P < 0.001$).

Administering dexamethasone solution containing an approximately 2-fold higher concentration of dexamethasone, or 0.67% compared to 0.32%, did not result in a much higher dexamethasone concentration in the aqueous humor, with a 0.67% dexamethasone–0.32% dexamethasone concentration ratio of 1.13. Conversely, the duration of activity seemed to be longer with 0.67% dexamethasone because dexameth-

TABLE 1. Statistical Comparison of Dexamethasone Concentration Curves After Administration of 0.1% Dexamethasone Alcohol Suspension (Maxidex) and 0.32% Dexamethasone-HPβCD-HPMC (0.32%) Solution

	AUC	*SE*	*95% CI*	*df*
Maxidex	124.11	26.42	97.69–150.52	9.13
0.32%	327.57	34.53	293.03–362.10	26.64

AUC = area under the curve; SE = standard error of the estimate of AUC; 95% CI = 95 percent confidence interval for AUC; *df* = approximate degrees of freedom for the standard error of the estimate of the AUC.
0.32% vs. Maxidex (*t*-test): $AUC_{difference}$ = 203.46; $SEM_{difference}$ = 43.38; $df_{difference}$ = 32.86; *t* value = 4.68; $P < 0.001$.

TABLE 2. Statistical Comparison of Dexamethasone Concentration Curves After Administration of Unheated 0.67% Dexamethasone-HPβCD-HPMC (Unheated) and Heated 0.67% Dexamethasone-HPβCD-HPMC (0.32%) Solution (Heated)

	Group AUC	*Group SEM*	*95% CI*	*df*
Unheated	90.70	19.88	70.83–110.6	1.61
Heated	260.08	59.84	200.24–319.91	3.84

AUC = area under the curve; SE = standard error of the estimate of AUC; 95% CI = 95 percent confidence interval for AUC; *df* = approximate degrees of freedom for the standard error of the estimate of the AUC
Heated vs. unheated (*t*-test): $AUC_{difference}$ = 169.38; $SEM_{difference}$ = 63.05; $df_{difference}$ = 4.67; *t* value = 2.69; $P < 0.05$.

asone concentration was measurable in the aqueous humor 9 hours after instillation with 0.67% dexamethasone, but not with 0.32% dexamethasone or Maxidex.

By heating the 0.67% dexamethasone–HPβCD–HPMC (i.e., formation of the cyclodextrin–polymer co-complex), a higher concentration was obtained in the anterior chamber compared to unheated 0.67% dexamethasone–HPβCD–HPMC (Fig. 2) or 2.9 times higher levels comparing AUC curves of the preparations (Table 2), which was statistically significant ($P < 0.05$).

DISCUSSION. This study shows that it is possible to achieve effective trans-ocular delivery of lipophilic corticosteroids, such as dexamethasone, by producing a three-way co-complex consisting of the drug, 2-hydroxypropyl-β-cyclodextrin, and hydroxypropyl methylcellulose. The drug delivery system produces a higher concentration of dexamethasone in aqueous eye drops than would otherwise be possible. Heating the dexamethasone–cyclodextrin–polymer co-complexes seems to enhance permeability into the eye regardless of the drug concentration.

The cyclodextrin-based drug delivery system is associated with a large increase in the intraocular availability of dexamethasone compared with the commercially available 0.1% dexamethasone, Maxidex. No toxic effects were observed.

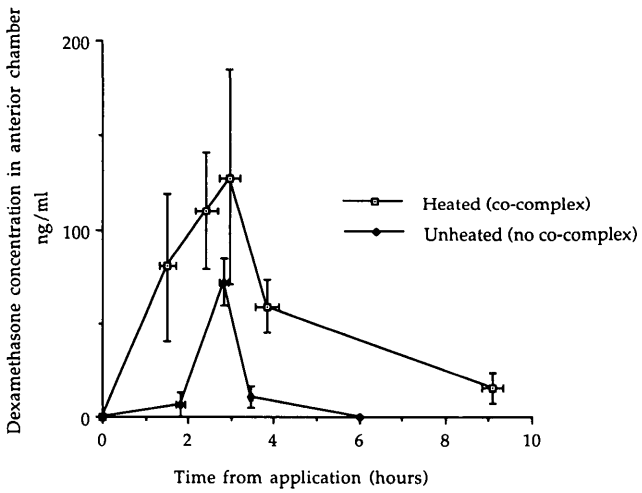

FIGURE 2. Dexamethasone concentration in aqueous humor after the administration of heated 0.67% dexamethasone–2-hydroxypropyl-β-cyclodextrin–hydroxypropyl methylcellulose and unheated 0.67% dexamethasone–2-hydroxypropyl-β-cyclodextrin. Mean concentration ± SEM is shown at appropriate time points after administration of the eye drops.

TABLE 3. Adjusted Mean Peak Concentrations (± SEM) of Corticosteroids in Aqueous Humor of Human Patients After Topical Administration

Corticosteroids	*Mean Concentration (ng/ml)*
0.32% dexamethasone-HPβCD-HPMC	140.5 ± 36.4
Dexamethasone alcohol 0.1%, Maxidex	59.5 ± 20.8
Prednisolone acetate 1%[10]	95.7 ± 19.4*

*Prednisolone is a sevenfold weaker steroid than dexamethasone.[11,12] The intraocular concentrations reported by McGhee et al[10] are adjusted accordingly.

Watson and associates[12] measured the dexamethasone in human aqueous humor after the administration of Maxidex with results that were similar to ours. Prednisolone concentration in the anterior chamber after the administration of prednisolone acetate 1% is 20-fold higher than dexamethasone concentration after the administration of Maxidex 0.1%.[13] However, prednisolone is a 7-fold less potent steroid than dexamethasone,[14,15] calling for adjustment of the concentration in anterior chamber when compared with dexamethasone preparations (Table 3). Results also show that heating the dexamethasone–HPβCD complex with 0.10% hydroxypropyl methylcellulose increases the delivery of dexamethasone into the human eye.

Our results indicate that by using a cyclodextrin-based drug delivery system, it is possible to raise the intraocular corticosteroid concentration from what is possible with currently available steroid eye drops, and presumably to exert a greater anti-inflammatory effect inside the eye. Further study is needed to establish the clinical efficacy of these drug formulations.

Key Words

aqueous humor, corticosteroids, cyclodextrin, dexamethasone, drug penetration

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