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ORIGINAL ARTICLE

Dexamethasone delivery to posterior segment of the eye

Thorsteinn Loftsson · Hákon H. Sigurdsson · Dagný Hreinsdóttir · Fífa Konrádsdóttir · Einar Stefánsson

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Abstract Due to anatomic barriers and lacrimal drainage it is difficult to obtain therapeutic drug concentrations in the posterior part of the eye after topical drug administrations. Lipophilic cyclodextrins, such as randomly methylated β-cyclodextrin (RMβCD), are known to act both as solubilizers of water-insoluble drugs in aqueous solutions and as penetration enhancers that reduce the barrier function of lipophilic membranes. The purpose of this study was to investigate the effects of RMβCD on dexamethasone delivery from aqueous eye drop solution into rabbit eyes. Dexamethasone (0.5 and 1.5% w/v) drops (50 µl) were administered to the left eye of rabbits (n = 6) and the drug levels measured in different eye tissues 2 h after administration. In aqueous humor dexamethasone levels were 1,190 ± 110 and 1,670 ± 630 ng/g (mean ± SD) after administration of the 0.5 and 1.5% dexamethasone eye drops, respectively. In the retina the levels were 33 ± 7 and 66 ± 49 ng/g, and in optic nerve 41 ± 12 and 130 ± 50 ng/g, respectively. In a previous study the dexamethasone concentration in aqueous humor after topical administration of 1.3% (w/v) dexamethasone eye drops in aqueous 2-hydroxypropyl-β-cyclodextrin (HPβCD) solution was determined to be 320 ± 230 ng/g and 66 ± 20 ng/g after administration of Maxidex® eye drops. Both the hydrophilic HPβCD and the lipophilic RMβCD enhance topical dexamethasone delivery into the eye, but of the two, the lipophilic RMβCD results in higher dexamethasone concentrations.

Keywords Ophthalmic drug delivery · Cyclodextrin · Steroid · Eye drops · Topical

T. Loftsson (✉) · H. H. Sigurdsson · D. Hreinsdóttir · F. Konrádsdóttir
Faculty of Pharmacy, University of Iceland, Hofsvallagata 53, 107 Reykjavik, Iceland
e-mail: thorstlo@hi.is

E. Stefánsson
Department of Ophthalmology, University Hospital-Landspitalinn, 101 Reykjavik, Iceland

Introduction

Drug delivery to the posterior part of the eye (e.g. to retina, choroid, vitreous and optic nerve) is vital for treatment of some of the most common blinding disorders such as age-related macular degeneration, diabetic retinopathy, retinal vessel occlusion and glaucoma. Due to anatomic barriers (i.e. cornea, conjunctiva and sclera) and lacrimal drainage it is challenging to obtain therapeutic drug concentrations in the posterior part of the eye after topical drug administration. Topically applied drugs must be, at least to some degree, soluble in the aqueous tear fluid and at the same time they must be somewhat lipid-soluble to be able to penetrate through the lipophilic membrane barriers [1, 2]. In other words, for successful formulation in aqueous eye drop solution the drug must both be water-soluble (i.e. hydrophilic) and at the same time lipid-soluble (i.e. hydrophobic) [3, 4]. The continuous secretion of tear fluid adds to this difficulty by limiting the contact time of topically applied drugs with the eye surface which again reduces their ocular bioavailability, especially from low viscosity aqueous eye drop solutions [5]. Consequently, less than 5% of a topically applied drug is absorbed through cornea into the eye [6–8], or more specifically, into the anterior

part of the eye. Drug delivery to the posterior part of the eye is even more challenging due to the anatomical and physiological barriers that separate the anterior and posterior parts of the eye. Consequently, it is generally thought that eye drops cannot deliver therapeutic levels of drugs to the posterior segment of the eye [9–11]. Therefore various approaches have been developed where drugs are injected into the vitreous cavity [12] or under the conjunctiva and various devices have been invented that may be implanted into the eye [11]. All of these approaches are based on the premise that non-invasive topical methods to effectively deliver drugs, such as corticosteroids, to the posterior segment of the eye are not available, and invasive methods are the only alternative [9–12].

The chemical structure of hydrophilic cyclodextrins (i.e. the large number of hydrogen donors and acceptors), their molecular weight (i.e. greater than 970 Dalton) and their very low octanol/water partition coefficient (log $K_{o/w}$ less than –3) are all characteristics of compounds that do not readily permeate biological membranes [13, 14]. Based on these observations it has been suggested that hydrophilic cyclodextrin enhance drug delivery through biological membranes by increasing the availability of dissolved drug molecules in an aqueous layer immediate to the lipophilic membrane surface [15–17]. According to this model cyclodextrins solubilize the lipophilic water-insoluble drug molecules in the aqueous vehicle and enhance their permeation through an aqueous diffusion layer at the membrane surface. Cyclodextrins can only act as penetration enhancers if permeation through an unstirred water layer at the membrane surface, such as the aqueous tear film, contributes to the overall barrier function of the biological membrane [17]. Furthermore, the physicochemical properties of the drug (e.g. its solubility in water), the drug cyclodextrin concentration ratio and the composition of the drug formulation (e.g. aqueous or non-aqueous) will also determine whether cyclodextrins will enhance or hamper drug delivery through a biological membrane. Cyclodextrins are in most cases unable to enhance drug permeation through a lipophilic membrane barrier, and excess cyclodextrin (more than is needed to dissolve the drug) will hamper drug permeation through the membrane [18]. However, there is one exception. The somewhat lipophilic and surface active methylated cyclodextrins, such as randomly methylated β-cyclodextrin (RMβCD), are known, under certain conditions, to act as conventional penetration enhancers, increasing drug permeability through biological membranes by decreasing their barrier function [19, 20]. Thus, since the somewhat lipophilic and surface active RMβCD is both able to enhance drug delivery through the aqueous diffusion layer as well as reducing the barrier function of the membrane, this cyclodextrin derivative frequently exhibits greater penetration-enhancing properties than the more hydrophilic derivatives such as 2-hydroxypropyl-β-cyclodextrin (HPβCD). Since cyclodextrins can both enhance and hamper drug delivery through biological membranes it is of uttermost importance to optimize cyclodextrin containing drug formulations with regard to drug delivery from the formulations [15]. Too much or too little cyclodextrin can result in less than optimum drug bioavailability.

The purpose of this study was to investigate the effects of RMβCD on delivery of dexamethasone from aqueous eye drop solution into the eye and to compare the effects of this cyclodextrin with previous results obtained with the more hydrophilic HPβCD [21].

Experimental

Materials

[1,2,4,6,7–^{3}H]-dexamethasone in ethanol solution with specific activity of 88 Ci/mmol was purchased from Amersham Biosciences. Dexamethasone was purchased form Sigma (Germany) and Bufa (Netherlands). Randomly methylated β-cyclodextrin with degree of substitution 1.8 (RMβCD) was purchased from Wacker–Chemie GmbH (Germany). Analytical grade of disodium edetate dihydrate (EDTA) was purchased from Merck (Germany). Hydroxypropyl methylcellulose (HPMC) and benzalkonium chloride were purchased from Sigma (USA). Soluene®-350 solubilizer, and liquid scintillation cocktails Hionic Fluor™ and Ultima Gold™ were purchased from Perkin Elmer (UK). All other chemicals used in this study were commercially available compounds of special reagent or analytical grade.

Methods

Solubility studies

A phase-solubility study was performed to determine the exact amount of RMβCD needed to solubilize dexamethasone in the eye drop medium. An excess amount of dexamethasone was added to aqueous solution containing from 0 to 25% (w/v) RMβCD, benzalkonium chloride (0.02% w/v), EDTA (0.1% w/v), sodium chloride (0.00–0.72% w/v) and HPMC (0.1%). The suspensions formed were heated in an autoclave (Midmark M7 SpeedClave, USA) in sealed containers

to 121°C for 20 min. The suspensions were allowed to cool to room temperature (22–23°C) and equilibrate under constant agitation for 7 days. After equilibrium was attained, the suspension was filtered through a 0.45 μm membrane filter, diluted and analyzed by HPLC [22]. The phase-solubility diagram obtained in the aqueous eye drop formulation was determined to be of A_L-type [23].

Formulation of the eye drops

Cold solution: Aqueous 0.5 and 1.5% (w/v) dexamethasone eye drop solution was prepared by dissolving 250 or 750 mg of dexamethasone in 45 ml of aqueous solution containing benzalkonium chloride (10 mg), EDTA (50 mg), HPMC (50 mg), sodium chloride (360 or 0 mg) and RMβCD (2.65 or 9.0 g) and then filled up to 50 ml. The solution was heated in an autoclave in sealed container to 121°C for 20 min. The solution was allowed to cool to room temperature (22–23°C) and equilibrate for 7 days. To prevent drug precipitation during storage 10% excess RMβCD was included in the aqueous eye drop formulation. The osmolarity of the solutions was measured by the freezing point depression method using a Knauer Osmometer Automatic (Netherlands). The viscosity was determined by a Brookfield digital viscometer model DV-1+ (U.S.A.) operated at room temperature. The osmolarity of the eye drops was determined to be 284 mOsm/kg. The viscosity was about 2.5 cps.

Dosing solution (labeled): The required volume of radioactive dexamethasone ethanolic solution was pipetted in a vial and ethanol allowed to evaporate almost to dryness without precipitating the dexamethasone. Then, a required amount of dexamethasone eye drop solution was added and that solution was shaken for at least 2 h.

In vivo studies

Young, unanaesthetized female albino rabbits (HB Lidköbings Kaninfarm, Sweden) were used. The rabbits were fed on a regular diet and weighed about 3 kg. The study adhered to the ARVO declaration for the use of laboratory animals in research. Eye drops (50 μl) were instilled using a micropipette inside the center of the lower cul-de sac of the left eye in six rabbits. During instillation, the lower eyelid was pulled slightly away from the globe and was returned to its normal position immediately after instillation. Great care was taken not to irritate the eye or to touch the corneal surface. Blood samples were taken at 30 min intervals. After two hours, the rabbits were sacrificed by intravenous injection of sodium pentobarbital and the left eye was proptosed and enucleated immediately and rinsed with an isotonic saline solution. All solutions were well tolerated by the rabbits and no macroscopic signs of irritation, redness or other toxic effect were observed.

Sample preparation

The aqueous humor was removed from the eye using 1 ml syringe attached to a 26-gauge needle and placed in a polyethylene (scintillation) vial. One lateral incision was performed in the sclera (center of the eyeball) and the eye was totally opened (anterior part and posterior part). From the anterior part, the lens and the iris-ciliary body were removed and the cornea was separated from the remaining anterior sclera. From the posterior part, the vitreous humor was emptied into a vial, the retina was gently scraped away and the optic nerve (about 2 mm) was cut off. While dissecting the eyes, all the samples were immediately put in a dry scintillation vials and weighed. Great care was taken to prevent cross-contamination between individual tissue samples and eye fluids. The entire procedure took less than 15 min per eye so that any errors due to redistribution of drug were minimized.

Quantitative determination of dexamethasone

Aqueous humor: About 10 ml of Ultima gold was added to aqueous humor samples (about 0.2 g), the vials were stoppered, shaken and kept in the dark for at least 12 h prior to counting in a liquid scintillation counter.

Blood samples: Blood samples were prepared by adding 1 ml mixture of Soluene®-350:isopropanol to 0.1–0.2 ml of the blood and incubated at 50°C for 60 min. The vials were then allowed to cool down to room temperature and 0.5 ml of 30% hydrogen peroxide solution was then added drop wise with swirling to each vial for decolorization. The solutions were allowed to stand at room temperature for 10 min. The vials were then incubated again at 50°C for 30 min to remove excess hydrogen peroxide. After cooling down to room temperature, 10 ml of Hionic fluor™ was added to each vial which were stoppered, shaken and kept in the dark for at least 12 h prior to counting in a liquid scintillation counter.

Other ocular tissue samples: Other tissue samples were handled in the same way as the blood samples except 0.5–2.0 ml of Soluene®-350 was added to the samples depending on the size of the tissue sample. No isopropanol or hydrogen peroxide was used and the

samples were incubated for 240 min at 50°C before adding the Hionic Fluor™ (5–20 ml depending on sample size). Dexamethasone was detected in all blood samples and in all tissue samples from the eyes after all three different routes of administration. Blank tissue samples were spiked with various amounts of labeled dexamethasone from the dosing solution and used as a standard.

Results and discussion

During formulation of the dexamethasone eye drop solution the exact amount of cyclodextrin needed to solubilize 0.5 and 1.5% (w/v) dexamethasone in the aqueous eye drop vehicle was determined from a phase-solubility profile obtained in the same vehicle containing the preservative, polymer and all other additives. Then about 10% excess cyclodextrin was included in the vehicle to prevent drug precipitation during storage and handling. Thus the 0.5 and 1.5% (w/v) dexamethasone eye drop solutions contained 5.3 and 18% (w/v) RMβCD and 0.72 and 0.0% (w/v) sodium chloride, respectively. The eye drops were nonirritating isotonic clear solutions. The viscosity of the solutions was almost the same as water or about 2.5 cps (the viscosity of water is 1.0 cps at room temperature). Table 1 lists the concentration of dexamethasone after topical application of 0.5 and 1.5% (w/v) dexamethasone eye drop solution containing RMβCD. Significant amounts of dexamethasone reached aqueous humour after topical administration of the aqueous eye drop solutions resulting in dexamethasone concentrations that were 2.5- to almost 9-times higher than after administration of the commercially available product Maxidex® that contains 0.1% dexamethasone as an alcoholic suspension and 0.01% (w/v) benzalkonium chloride (Table 2). Notable are also the relatively high dexamethasone concentrations in the posterior segment of the eye, i.e. vitreous, retina and optic nerve, where the obtained concentrations after topical administration of the 1.5% dexamethasone solution are close to the estimated therapeutic dexamethasone concentrations obtained after subconjunctival injection or ocular implants [24, 25].

Table 1 Concentration (ng/g) of dexamethasone in blood and various ocular tissues 2.0 h after administration of 0.5 or 1.5% (w/v) dexamethasone eye drop solutions in the rabbit (mean ± standard deviation; $n = 6$)

Tissue	0.5% Dexamethasone	1.5% Dexamethasone
Cornea	1,190 ± 110	1,670 ± 630
Sclera	450 ± 140	230 ± 120
Aqueous humour	170 ± 80	580 ± 230
Iris-ciliary body	140 ± 40	550 ± 290
Lens	11 ± 3	19 ± 9
Vitreous	11 ± 3	22 ± 9
Retina	33 ± 7	66 ± 49
Optic nerve	41 ± 12	130 ± 50
Blood	26 ± 5	45 ± 24

Table 2 Concentration (ng/g) of dexamethasone in aqueous humor 2 h after administration of aqueous dexamethasone eye drop solutions containing either randomly methylated β-cyclodextrin (RMβCD) or 2-hydroxypropyl-β-cyclodextrin (HPβCD) in rabbits (mean ± standard deviation; $n = 4$–6). The values for 1.3% dexamethasone/HPβCD eye drop solution and Maxidex® were obtained from [21]

Aqueous eye drop solution	Dexamethasone conc. Mean ± standard deviation (ng/ml)
0.5% Dexamethasone in RMβCD	170 ± 76
1.5% Dexamethasone in RMβCD	580 ± 230
1.3% Dexamethasone in HPβCD	320 ± 230
Maxidex® (0.1% Dexamethasone)	66 ± 20

The dexamethasone concentration in aqueous humour was somewhat higher after topical administration of the drug in a RMβCD containing vehicle compared to a HPβCD containing vehicle and significantly higher than after administration of the commercial eye drops, Maxidex®, that contains 0.1% (w/v) dexamethasone as an alcoholic suspension (Table 2). This was expected since the lipophilic RMβCD does not only enhance drug delivery through the aqueous tear film to the lipophilic cornea and sclera but also reduces their barrier function by penetrating into the membranes. As a continuation of this project we are currently developing aqueous eye drop solutions, containing hydrophilic cyclodextrins that yield higher dexamethasone concentrations in vitreous, retina and optic nerve than the RMβCD containing aqueous eye drop formulations.

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References

1. Ahmed, I., Gokhale, R.D., Shah, M.V., Patton, T.F.: Physicochemical determinants of drug diffusion across the conjunctiva, sclera, and cornea. J. Pharm. Sci. **76**, 583–586 (1987)
2. Wang, W., Sasaki, H., Chien, D.-S., Lee, V.H.L.: Lipophilicity influence on conjunctival drug penetration in the pigmented rabbit: a comparison with corneal penetration. Curr. Eye Res. **10**, 571–579 (1991)
3. Loftsson, T., Stefánsson, E.: Effect of cyclodextrins on topical drug delivery to the eye. Drug Devel. Ind. Pharm. **23**, 473–481 (1997)

4. Loftsson, T., Stefánsson, E.: Cyclodextrins in eye drop formulations: enhanced topical delivery of corticosteroids to the eye. Acta Ophthalmol. Scand. **80**, 144–150 (2002)
5. Chrai, S.S., Patton, T.F., Mehta, A., Robinson, J.R.: Lacrimal and instilled fluid dynamics in rabbit eye. J. Pharm. Sci. **62**, 1112–1121 (1973)
6. Gangrade, N.K., Gaddipati, N.B., Ganesan, M.G., Reddy, I.K.: Topical ophthalmic formulations: basic considerations. In: Reddy, I.K. (ed.) Ocular Therapeutics and Drug Delivery, pp. 377–403. Technomic Publications, Lancaster (1996)
7. Loftsson, T., Järvinen, T.: Cyclodextrins in ophthalmic drug delivery. Adv. Drug Deliv. Rev. **36**, 59–79 (1999)
8. Washington, N., Washington, C., Wilson, C.G.: Ocular drug delivery. Physiological pharmaceutics: barriers to drug absorption, pp. 249–270. Taylor and Francis, London (2001)
9. Myles, M.E., Neumann, D.M., Hill, J.M.: Recent progress in ocular drug delivery for posterior segment disease: emphasis on transsclera iontophoresis. Adv. Drug Deliv. Rev. **57**, 2063–2079 (2005)
10. Raghava, S., Hammond, M., Kompella, U.B.: Periocular routes for retinal drug delivery. Exp. Opin. Drug Deliv. **1**, 99–114 (2004)
11. Yasukawa, T., Ogura, Y., Sakurai, E., Tabata, Y., Kimura, H.: Intraocular sustained drug delivery using implantable polymer devices. Adv. Drug Deliv. Rev. **57**, 2033–2047 (2005)
12. Jonas, J.B.: Intravitreal triamcinolone acetonide for treatment of intraocular oedematous and neovascular diseases. Acta Ophthalmol. Scand. **83**, 645–663 (2005)
13. Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J.: Experimental and computatorial approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. **46**, 3–26 (2001)
14. Amidon, G.L., Lennernäs, H., Shah, V.P., Crison, J.R.: A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm. Res. **12**, 413–420 (1995)
15. Loftsson, T., Masson, M.: Cyclodextrins in topical drug formulations: theory and practice. Int. J. Pharm. **225**, 15–30 (2001)
16. Sinha, V.R., Bindra, S., Kumria, R., Nanda, A.: Cyclodextrin as skin-penetration enhancers. Pharm. Technol. **27**(3), 120–138 (2003)
17. Loftsson, T., Konráðsdóttir, F., Másson, M.: Influence of aqueous diffusion layer on passive drug diffusion from aqueous cyclodextrin solutions through biological membranes. Pharmazie **61**, 83–89 (2006)
18. Loftsson, T., Sigfússon, S.D., Sigurðsson, H.H., Másson, M.: The effects of cyclodextrins on topical delivery of hydrocortisone: the aqueous diffusion layer. S.T.P. Pharma Sci. **13**, 125–131 (2003)
19. Merkus, F.W.H.M., Verhoef, J.C., Marttin, E., Romeijn, S.G., van der Kuy, P.H.M., Hermens, W.A.J.J., Schipper, N.G.M.: Cyclodextrin in nasal drug delivery. Adv. Drug Deliv. Rev. **36**, 41–57 (1999)
20. Yang, T., Hussain, A., Paulson, J., Abbruscato, T.J., Ahsan, F.: Cyclodextrins in nasal delivery of low-molecular-weight heparins: in vivo and in vitro studies. Pharm. Res. **21**, 1127–1136 (2004)
21. Loftsson, T., Fridriksdottir, H., Thorisdottir, S., Stefansson, E.: The effect of hydroxypropyl methylcellulose on release of dexamethasone from aqueous 2-hydroxypropyl-β-cyclodextrin formulations. Int. J. Pharm. 104:181–184 (1994)
22. Loftsson, T., Hreinsdóttir, D., Másson, M.: Evaluation of cyclodextrin solubilization of drugs. Int. J. Pharm. **302**, 18–28 (2005)
23. Higuchi, T., Connors, K.A.: Phase-solubility techniques. Adv. Anal. Chem. Instrum. **4**, 117–212 (1965)
24. Weijtens, O., Feron, E.J., Schoemaker, R.C., Cohen, A.F., Lentjes, E.G.W.M., Romijn, F.P.H.T.M., van Meurs, J.C.: High concentration of dexamethasone in aqueous and vitreous after subconjunctival injection. Am. J. Ophthalmol. **128**, 192–197 (1999)
25. Shiah, J.-G., Bhagat, R., Nivaggioli, T., Peng, L., Chou, D., Weber, D.A.: Ocular implant made by a double extrusion process. US Patent Application No.: 2005/0048099, Allergan Inc. 3 March 2005, USA