Cyclodextrins in eye drop formulations: enhanced topical delivery of corticosteroids to the eye

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ABSTRACT.
Cyclodextrins are cylindrical oligosaccharides with a lipophilic central cavity and hydrophilic outer surface. They can form water-soluble complexes with lipophilic drugs, which 'hide' in the cavity. Cyclodextrins can be used to form aqueous eye drop solutions with lipophilic drugs, such as steroids and some carbonic anhydrase inhibitors. The cyclodextrins increase the water solubility of the drug, enhance drug absorption into the eye, improve aqueous stability and reduce local irritation.
Cyclodextrins are useful excipients in eye drop formulations of various drugs, including steroids of any kind, carbonic anhydrase inhibitors, pilocarpine, cyclosporins, etc. Their use in ophthalmology has already begun and is likely to expand the selection of drugs available as eye drops.
In this paper we review the properties of cyclodextrins and their application in eye drop formulations, of which their use in the formulation of dexamethasone eye drops is an example. Cyclodextrins have been used to formulate eye drops containing corticosteroids, such as dexamethasone, with levels of concentration and ocular absorption which, according to human and animal studies, are many times those seen with presently available formulations. Cyclodextrin-based dexamethasone eye drops are well tolerated in the eye and seem to provide a higher degree of bioavailability and clinical efficiency than the steroid eye drop formulations presently available. Such formulations offer the possibility of once per day application of corticosteroid eye drops after eye surgery, and more intensive topical steroid treatment in severe inflammation.
While cyclodextrins have been known for more than a century, their use in ophthalmology is just starting. Cyclodextrins are useful excipients in eye drop formulations for a variety of lipophilic drugs. They will facilitate eye drop formulations for drugs that otherwise might not be available for topical use, while improving absorption and stability and decreasing local irritation.

Keywords: drug delivery – eye drops – cyclodextrin – steroids – solubility.

Acta Ophthalmol. Scand. 2002: 80: 144–150

A new group of pharmaceutical excipients called cyclodextrins has recently been introduced into ophthalmology (Table 1). This group of excipients is able to solubilize many lipophilic water-insoluble drugs which previously were impossible to formulate in aqueous eye drop solutions. Aqueous cyclodextrin containing eye drop solutions have already been registered in Europe. A chloramphenicol eye drop solution (Clorocil®) was recently registered in Portugal; 2001 saw the registration of a diclofenac eye drop solution (Voltaren Ophthalmic®) in France. Previous reviews on this subject include those by van Dorne (1993); Rajewski & Stella (1996); Loftsson & Stefánsson (1997); and Loftsson & Järvinen (1999). The object of this short review is to describe how cyclodextrins enhance aqueous solubility and bioavailability of lipophilic water-insoluble drugs in aqueous eye drop formulations. The formulation and *in vivo* testing of dexamethasone eye drops as performed within our own research group are used as examples.

Corticosteroids in ophthalmology

The most common use of corticosteroids in eye drops is for inflammation following eye surgery, such as cataract surgery and corneal operations. In mild cases it is usually adequate to apply the eye drops one to four times per day and in some cases topically applied nonsteroidal anti-inflammatory drugs may be sufficient. However, in cases of severe inflammation, such as after complicated eye surgery, corneal transplant rejection or severe uveitis, applications as frequently as once every hour may not be adequate. In these severe cases the eye drops have to be supplemented with systemic steroids, such as prednisolone, or with subconjunctival or subtenon injection of steroids. Rather than using commercially available eye drops, it would then be

more advantageous to use corticosteroid containing eye drops of greater bioavailability. Furthermore, topically applied corticosteroids are generally not effective in the posterior segment of the eye and, therefore, systemic corticosteroids are needed to fight inflammatory disease in this area.

Corticosteroids are generally lipophilic and dissolve very poorly in water. The commercially available eye drop formulations solve this dilemma by forming prodrugs, usually acetate or phosphate esters such as prednisolone acetate (Pred forte ®, Pred mild ®) and dexamethazone phosphate or suspensions, such as dexamethazone alcohol suspension (Maxidex®).

Various researchers have studied the penetration of topically applied ocular steroids into the anterior chamber of the human eye (Watson et al. 1988; McGhee et al. 1990). They found that of the commercially available formulations, those containing 1% prednisolone acetate *(Pred forte®)* gave the highest concentration in the aqueous humour per average 96 ng/mL. Eye drops containing 0.1% dexamethasone alcohol suspension *(Maxidex®)* gave a considerably lower concentration. However, if we take into account the fact that dexamethasone is seven times more potent than prednisolone, then the dexamethasone concentration obtained in the aqueous humour corresponded to about 60 ng/mL of prednisolone. The most effective corticosteroid eye drops available today give aqueous humour concentration of less than 100 ng/mL (prednisolone equivalents). This bioavailability can be improved through the use of cyclodextrin formulation, where a single drop topical application gives aqueous humour concentration of about 140 ng/mL (prednisolone equivalents) and also extends its duration in the eye, as will be discussed later.

The corticosteroid concentrations achieved in the aqueous humour from application of Maxidex ® or Pred Forte ® is usually sufficient for mild to moderate ocular inflammation. More potent formulations may allow topical treatment of more severe intraocular inflammation and also less frequent applications for mild to moderate inflammation.

Table 1. Cyclodextrins in topical formulations for ocular drug delivery.

Drug	Cyclodextrin	References
Acetazolamide	HPβCD	(Loftsson et al. 1994; Loftsson et al. 1996)
Anandamides	HPβCD	(Jarho et al. 1996;Pate et al. 1996)
Cannabinoids (various)	HPβCD	(Pate et al. 1998)
Cyclosporin	αCD	(Kanai et al. 1989; Sasamoto et al. 1991; Cheeks et al. 1992
Dehydroepiandrosterone	HPβCD	(Kearse et al. 2001)
Dexamethasone	HPβCD	(Usayapant et al. 1991; Loftsson et al. 1994; Kristinsson et al. 1996; Gavrilin et al. 1999)
Diclofenac	HPβCD, RMβCD	(Reer et al. 1994)
Dipivefrine	SBEβCD	(Jarho et al. 1997)
Fluorometholone	HPγCD	(Morita et al. 1996)
Hydrocortisone	HPβCD	(Davies et al. 1997; Bary et al. 2000
Loteprednol etabonate	HPβCD, DMβCD	(Reddy et al. 1996)
Pilocarpine	αCD, βCD, HEβCD, HPβCD	(Freedman et al. 1993; Järvinen et al. 1994;
	SBEβCD	Keipert et al. 1996; Siefert & Keipert 1997)
Prostaglandins	HPβCD	(Wheeler 1991)
Talidomide	HPβCD	(Siefert et al. 1999)
Tropicamide	HPβCD	(Cappello et al. 2001)
Δ9-Tetrahydrocannobinol	αCD, βCD, HPβCD, γCD	(Green & Kearse 2000; Kearse & Green 2000)

HPβCD = 2-hydroxypropyl-β-cyclodextrin
αCD = α-cyclodextrin
RMβCD = randomly methylated β-cyclodextrin
SBEβCD = sulfobutylether β-cyclodextrin
HPγCD = 2-hydroxypropyl-γ-cyclodextrin
DMβCD = heptakis (2,6-di-O-methyl)-β-cyclodextrin
HEβCD = hydroxyethyl-β-cyclodextrin
βCD = β-cyclodextrin
γCD = γ-cyclodextrin

Physiological considerations

In ophthalmology, local drug administration in the form of topically applied low viscosity aqueous eye drop solutions is generally preferred. Topically applied drugs must be, at least to some degree, soluble in the aqueous tear fluid. However, they must also be somewhat lipid-soluble in order to penetrate the lipophilic corneal epithelium, through the corneal stroma and the lipophilic endothelium into the aqueous humour (Ahmed et al. 1987; Wang et al. 1991). In other words, for successful formulation in an aqueous eye drop solution. a drug must be both water-soluble (i.e. hydrophilic) and lipid-soluble (i.e. hydrophobic) (Loftsson & Stefánsson 1997). 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 after application in low viscosity aqueous eye drop solutions (Chrai et al. 1973). Consequently, less than 5% of a topically applied drug is absorbed through the cornea into the eye (Gangrade et al. 1996; Loftsson & Järvinen 1999; Washington et al. 2001). Steroids used to treat ocular inflammation are lipophilic water-insoluble compounds that have to be introduced into aqueous eye drop formulations as suspensions or as water-soluble prodrugs. In both cases, ocular bioavailability is seriously hampered by the low aqueous solubility or the hydrophilic properties of the penetrating molecules, respectively. In addition, insufficient chemical stability of the steroid prodrugs in aqueous solution, as well as poor *in vivo* conversion to parent steroid, has limited their use in ophthalmology (Tammara & Crider 1996).

Common adjuvants to aqueous eye drop formulations can enhance ocular bioavailability of steroids by reducing the barrier function of, for example, the cornea (e.g. benzalkonium chloride and other surfactants (Lang & Stiemke 1996) or by increasing the contact time of the drug with the eye surface (e.g. viscosity enhancers such as water-soluble poly-

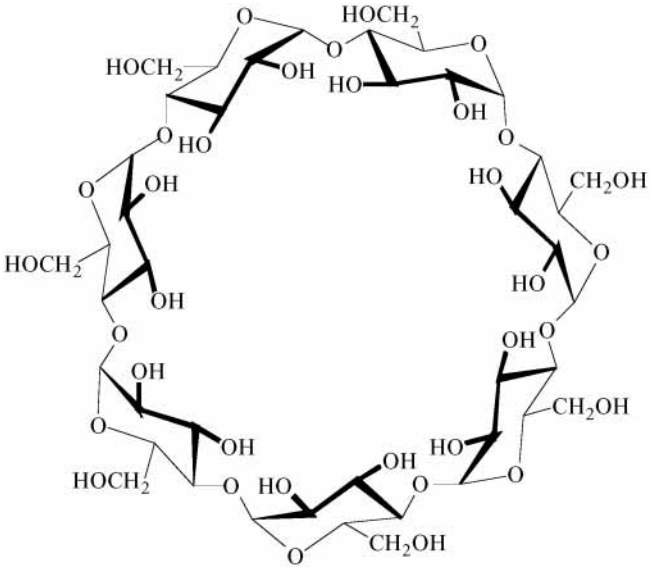

Fig. 1. β-Cyclodextrin.

mers). Specialized ocular delivery systems such as hydrogels, microemulsions, solid inserts and liposomes have also been designed in order to enhance bioavailability of topically applied ophthalmic drugs (Reddy 1996). However, these have never gained much popularity, due to both their side-effects (such as blurred vision and local irritation) and their instability (i.e. limited shelf-life).

Cyclodextrins are novel, chemically stable adjuvants that enhance ocular bioavailability of ophthalmic drugs without affecting the barrier function of the eye or increasing the viscosity of the aqueous eye drop formulation (Loftsson & Masson 2001).

Cyclodextrins

Cyclodextrins are a group of structurally related natural products formed during bacterial digestion of cellulose. These cyclic oligosaccharides consist of (α-1,4)-linked α-D-glucopyranose units with a hydrophilic outer surface and a lipophilic central cavity. The natural α-, β- and γ-cyclodextrins consist of six, seven and eight glucopyranose units (Fig. 1), respectively. The aqueous solubility of these natural cyclodextrins is somewhat limited and thus several different water-soluble derivatives have been synthesized. Cyclodextrin derivatives which have been applied in ophthalmology include the hydroxypropyl derivatives of β- and γ-cyclodextrin, the randomly methylated β-cyclodextrin and sulfobutylether β-cyclodextrin (Table 1).

In an aqueous environment, cyclodextrins form inclusion complexes with many lipophilic molecules through a process in which water molecules located inside the central cavity are replaced by either a whole molecule, or, more frequently, by some lipophilic structure of the molecule. Cyclodextrin complexation of a drug molecule changes the physicochemical properties of the drug, such as its aqueous solubility and chemical stability (Loftsson & Brewster 1996). Since the cyclodextrin molecule is hydrophilic on the outside, the complex formation usually increases the water-solubility of lipophilic water-insoluble drugs. Thus, it has been possible through cyclodextrin complexation to formulate lipophilic water-insoluble steroids as aqueous eye drop solutions (Usayapant et al. 1991; Loftsson et al. 1994; Kristinsson et al. 1996; Morita et al. 1996; Reddy et al. 1996; Davies et al. 1997; Gavrilin et al. 1999; Bary et al. 2000; Kearse et al. 2001). Furthermore, the chemical stability of the drug molecule is enhanced by the inclusion complexation (Loftsson & Brewster 1996). This increases the shelf-life of the aqueous eye drop formulation.

Once included in the cyclodextrin cavity, the drug molecules may be dissociated from the cyclodextrin molecules through complex dilution in the aqueous tear fluid. The included drug may also be replaced by some other suitable molecule (such as lipids), or, if the complex is located in close approximation to a lipophilic biological membrane (such as the eye cornea), the guest may be transferred to the matrix for which it has the highest affinity. Importantly, since no covalent bonds are formed or broken during the guest-host complex formation, the complexes are in dynamic equilibrium with free drug and cyclodextrin molecules.

The effects of cyclodextrins on drug solubility, permeability, chemical stability and delivery through biological membranes have been investigated by a number of research groups (Rajewski & Stella 1996; Uekama et al. 1998; Loftsson & Järvinen 1999; Masson et al. 1999; Stella et al. 1999; Uekama 1999; Loftsson & Masson 2001). Their studies show that hydrophilic cyclodextrins act as true carriers by keeping the lipophilic water-insoluble drug molecules in solution and delivering them to the membrane surface where they

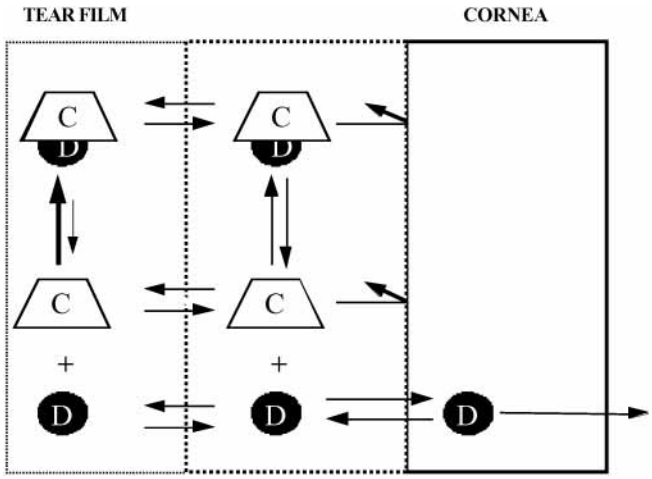

Fig. 2. The mechanism of drug (D) penetration into the eye from an aqueous cyclodextrin (CD) containing eye drop solution in the tear film. Modified from Loftsson & Järvinen (1999) with permission from Advanced Drug Delivery Reviews.

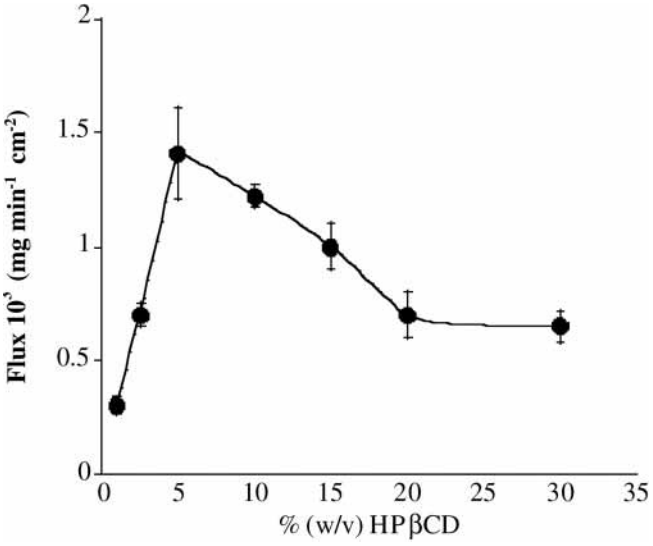

Fig. 3. The effect of 2-hydroxypropyl-β-cyclodextrin (HPβCD) concentration on the flux of dexamethasone from an aqueous HPβCD solution containing 0.5% (w/v) dexamethasone through a semipermeable cellophane membrane (mean ± SEM, $n = 4$). The dexamethasone was in suspension at HPβCD concentration below 5% but in solution at higher HPβCD concentrations. Modified from Loftsson et al. (1994) with permission from the International Journal of Pharmaceutics.

partition from the cyclodextrin cavity into the lipophilic membrane. The relatively lipophilic membrane has low affinity for the large hydrophilic cyclodextrin molecules or the hydrophilic drug/cyclodextrin complexes, which thus remain in the aqueous skin exterior, e.g. the aqueous tear fluid. Conventional penetration enhancers, such as benzalkonium chloride, disrupt the ophthalmic barrier, whereas hydrophilic cyclodextrins enhance drug penetration into the eye by carrying the lipophilic water-insoluble drug molecules through the aqueous mucin layer and thereby increasing drug availability at the lipophilic eye surface (Fig. 2) (Loftsson & Masson 2001).

Formulation with cyclodextrin

Since neither cyclodextrins nor their complexes are absorbed into lipophilic barriers, cyclodextrins can both increase and decrease drug availability at the eye surface. For example, the effect of cyclodextrin concentration on the permeability of the lipophilic water-insoluble drug dexamethasone through semipermeable membrane is shown in Fig. 3. At low cyclodextrin concentrations, when the drug is in suspension, the flux of the drug increases with increasing cyclodextrin concentration. At higher cyclodextrin concentrations, when the entire drug is in solution, the flux decreases with increasing cyclodextrin concentration. Maximum permeability is observed when just enough cyclodextrin is added to the vehicle to solubilize the entire drug. Figure 3 shows that it is very important to optimize the dexamethasone release from an aqueous eye drop formulation by adjusting the cyclodextrin concentration in the aqueous eye drop formulation. Too much or too little cyclodextrin will result in less than optimum drug availability. Some of the ingredients of the eye drop formulation will compete with dexamethasone for a space in the cyclodextrin cavity, thereby reducing the solubilizing effect of the cyclodextrin. At the same time, some other ingredients may have a solubilizing effect on the drug, thereby reducing the amount of cyclodextrin needed to solubilize the drug. Consequently, the amount of cyclodextrin included in the aqueous eye drop formulation has to be based on availability studies performed on the actual eye drop formulation which must contain all necessary excipients (e.g. preservatives, polymers and buffer salts).

It is possible to increase drug availability in aqueous cyclodextrin formulations by including small amounts of water-soluble polymer. Polymers enhance the cyclodextrin complexation of the drug, thereby reducing the amount of cyclodextrin needed in the formulation, while simultaneously enhancing the absorption of the drug/cyclodextrin complex to the eye surface through the formation of ternary complexes or co-complexes (Kristinsson et al. 1996). This increases the drug availability at the eye surface (Loftsson 1998; Loftsson & Järvinen 1999). The addition of 0.10% hydroxypropyl methylcellulose increases the apparent stability constant of dexamethasone/2-hydroxypropyl-β-cyclodextrin complex from 1200 M^{-1} to 1600 M^{-1} (Loftsson & Stefánsson 1997). At the same time, the polymer increases the availability of dexamethasone in the aqueous eye drop formulation (Kristinsson et al. 1996). Using the described op-

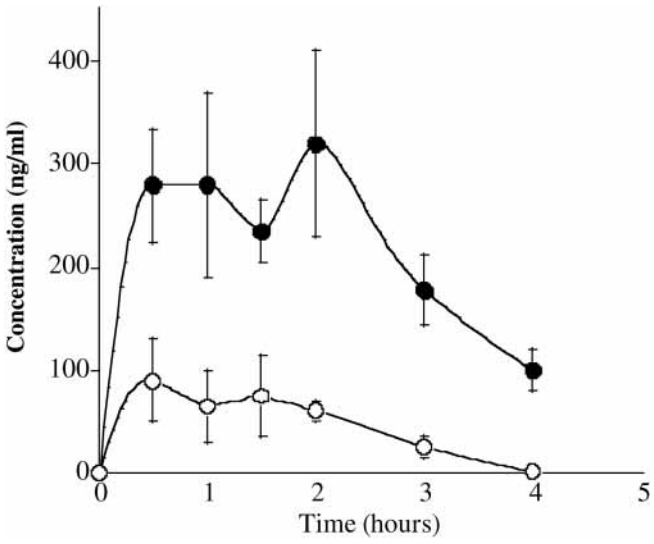

Fig. 4. Dexamethasone concentration in aqueous humour of rabbits after administration of 50 µL of 1.3% dexamethasone in an aqueous cyclodextrin solution or a 0.1% dexamethasone alcoholic suspension (Maxidex®) (O) (mean ± SEM, $n = 3$). Modified from Loftsson et al. (1994) with permission from the International Journal of Pharmaceutics.

timization technologies, aqueous eye drops containing 0.32, 0.67 and 1.3% (w/v) dexamethasone were prepared and tested both in animals and humans.

***In vivo* observations**

Dexamethasone (1.3% w/v) was tested in English brown rabbits in an aqueous eye drop solution containing 2-hydroxypropyl-β-cyclodextrin and Maxidex® (Loftsson et al. 1994). A single drop of the solution was applied in the rabbit's eye and aqueous humour samples withdrawn at specified times following the administration. Dexamethasone (0.1% w/v) alcohol suspension (Maxidex®, Alcon Inc, Texas, USA) was used for control. The 1.3% dexamethasone/2-hydroxypropyl-β-cyclodextrin eye drops gave a significantly higher concentration of dexamethasone in the aqueous humour than did Maxidex, even though the difference in concentration in the aqueous humour was less than the 13-fold difference in the concentration of dexamethasone in the eye drop. Four hours after the application of Maxidex®, the concentration of dexamethasone in the aqueous humour was essentially zero, whereas the cyclodextrin-dexamethasone solution gave about 100 ng/mL (Fig. 4). The cyclodextrin-dexamethasone eye drop solution was well tolerated and no irritation was seen on clinical examination of the rabbits.

The ocular absorption of dexamethasone eye drops containing 2-hydroxypropyl-β-cyclodextrin was also tested in human patients and compared with Maxidex® (0.1% dexamethasone alcohol suspension). The patients received the eye drops at a certain time prior to cataract surgery and, at the time of cataract surgery, an aqueous humour sample was withdrawn and dexamethasone levels determined. Figure 5 shows the dexamethasone concentration in the aqueous humour after administration of 0.32% dexamethasone/2-hydroxypropyl-β-cyclodextrin and Maxidex® (Kristinsson et al. 1996). The concentration of dexamethasone in the aqueous humour was significantly higher ($P < 0.001$) and the area under the curve was 2.6 times higher with the 0.32% cyclodextrin-dexamethasone eye drop solution than with Maxidex®. The peak concentration of dexamethasone did not increase when the dexamethasone concentration in the aqueous cyclodextrin containing eye drops was increased from 0.32 to 0.67% (w/v) (Fig. 6). However, as can be seen by concentration values obtained 9 hr after administration, the duration of activity was increased (Table 2). It is interesting to compare these results with the measurements of Watson and associates and McGhee and associates (see Table 2) (Watson et al. 1988; McGhee et al. 1990). The 0.32% (w/v) dexamethasone solution gives a considerably higher effective concentration in the aqueous humour than does prednisolone acetate, which is the most potent corticosteroid eye drop on the market.

Figure 6 shows the effect of the co-complexation involving the water-soluble polymer, hydroxypropyl methylcellulose, on the dexamethasone bioavailability *in vivo*. The two eye drop solutions were

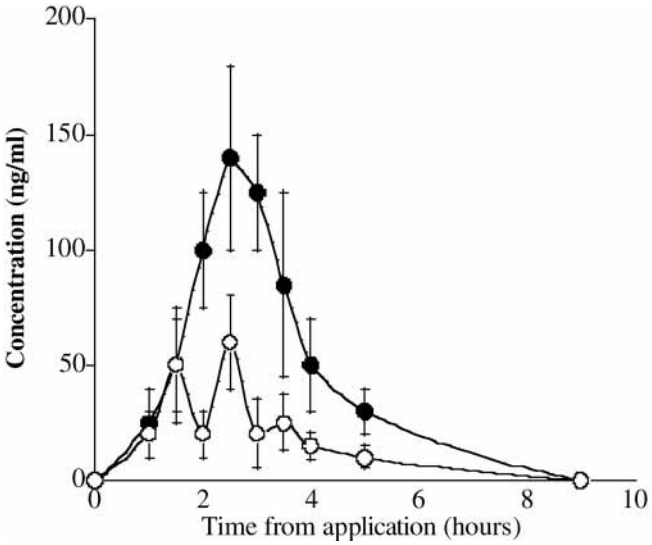

Fig. 5. Dexamethasone concentration in aqueous humour after administration of 1 drop (50 μL) of 0.32% dexamethasone in an aqueous cyclodextrin solution (?)or a 0.1% dexamethasone alcoholic suspension (Maxidex®) (O). The concentration (mean ± SEM, $n = 3$) is shown at appropriate time points after administration of the eye drops to human volunteers. Reprinted from Kristinsson et al. (1996) with permission from Investigative Ophthalmology and Visual Science.

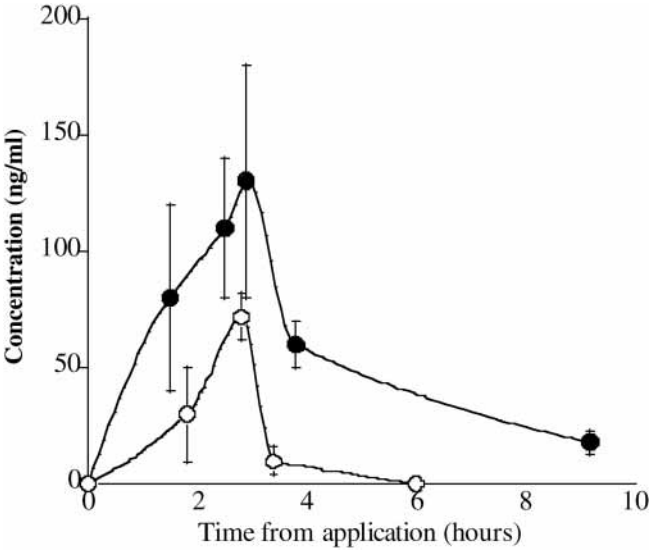

Fig. 6. Dexamethasone concentration in aqueous humour after administration of 1 drop (50 μL) of 0.67% dexamethasone in an aqueous cyclodextrin solution; the dexamethasone/cyclodextrin/polymer co-complex (?), the simple dexamethasone/cyclodextrin complex (O). The concentration (mean ± SEM, $n = 3$) is shown at appropriate time points after administration of the eye drops to human volunteers. Reprinted from Kristinsson et al. (1996) with permission from Investigative Ophthalmology and Visual Science.

Table 2. Adjusted mean peak concentration (± SEM) of dexamethasone and prednisolone acetate, and the concentration at +9 hrs, in aqueous humour of human volunteers after topical administration. Concentrations are adjusted for potency of prednisolone, which is a seven-fold weaker steroid than dexamethasone.

Eye drop solution	Mean peak concentration (ng/ml)	Concentration at +9 hrs (ng/mL
Dexamethasone 0.32%	141 ± 36	0
Dexamethasone 0.67%	130 ± 50	18 ± 5
Maxidex*	60 ± 21	0
Prednisolone acetate 1%	96 ± 19	–

*Maxidex® contains 0.1% dexamethasone alcoholic suspension
Modified from Kristinsson et al. 1996; McGhee et al. 1990; Schoenwald et al. 1987).

identical except for the study formulation's co-complex formation (induced through heating) between the drug/cyclodextrin complex and hydroxypropyl methylcellulose. The control formulation contained a simple drug/cyclodextrin complex. Formation of the co-complex resulted in significant enhancement of the bioavailability of the drug (Kristinsson et al. 1996).

Clinical studies

Saari et al. (1998) studied the use of dexamethazone-cyclodextrin eye drops following cataract surgery. Eye drops containing 0.67% dexamethazone-cyclodextrin and used once per day were compared with a 0.1% dexamethazone used three times per day. Cell flare measurements of the aqueous humour and clinical evaluation indicated that the two treatment regimens were equally clinically efficient. Once per day application of the cyclodextrin dexamethazone formulation was quite effective in controlling postoperative inflammation following cataract surgery.

Conclusions

Cyclodextrins make it possible to formulate lipophilic drugs in aqueous eye drop solutions. This may be useful for the formulation of a variety of lipophilic drugs that hitherto have not been available as eye drops or in suboptimal formulations. Steroid drugs, including corticosteroids, are a good example of such drugs. They are lipophilic and have only been available in eye drops as prodrugs or suspensions with limited concentration and bioavailability. With cyclodextrins, it is possible to increase the drug concentration and bioavailability and create formulations that offer more effective and less frequent treatment schedules for patients with ocular inflammation.

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Received on August 13th, 2001.
Accepted on January 3rd, 2002.

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Journal of Controlled Release xx (2004) xxx–xxx
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Cyclodextrin formulation of dorzolamide and its distribution in the eye after topical administration

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Received 24 August 2004; accepted 7 October 2004

Abstract

Due to limited aqueous solubility of dorzolamide at physiologic pH, the pH of Trusopt® eye drops (cont. 2% dorzolamide) has to be kept at about 5.65, and to increase the topical bioavailability of the drug from Trusopt® the contact time of the drug with the eye surface is increased by increasing the viscosity of the eye drops to 100 cps. This low pH and high viscosity can lead to local irritation. In this study, dorzolamide hydrochloride was formulated as 2% and 4% low viscosity solutions (viscosity 3 to 5 cps) containing randomly methylated β-cyclodextrin at pH 7.45. These formulations were evaluated in rabbits. The animals were sacrificed at various time points after topical administration of the drug and the dorzolamide concentration determined in the different parts of the eye. Trusopt® was used as a reference standard. The topical availability of dorzolamide from the cyclodextrin-containing eye drops appeared to be comparable to that from Trusopt® and the drug reached retina and optic nerve to give measurable concentrations for at least 8 h after administration of the eye drops.

Keywords: Dorzolamide; Cyclodextrin; Ocular drug delivery; Rabbits; Tissue distribution

1. Introduction

Dorzolamide is a carbonic anhydrase inhibitor (CAI) used in the treatment of glaucoma. Carbonic anhydrase (CA) is responsible for generation of bicarbonate anions secreted by the ciliary process into the posterior chamber of the eye. Inhibition of CA results reduction in intraocular pressure (IOP) [1,2]. Orally administered CAIs, such as acetazolamide, are very effective ocular hypotensive agents but their oral administration also results in myriad of systemic side effects including general malaise, depression, loss of

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0168-3659/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.jconrel.2004.10.004

appetite, fatigue, weight loss, gastrointestinal disturbances, paresthesias and renal calculi [3]. Studies in the 1960s showed that, when applied topically, acetazolamide did not have any IOP lowering effect and therefore topical administration of CAIs was considered impossible [4]. There are at least seven different isoenzymes of CA and two of them, CA-I and CA-II, are relevant to the human eye. Isoenzyme II is thought to play a major role in aqueous humor secretion [5]. However, this enzyme has to be almost 100% inhibited to obtain IOP lowering and topically applied acetazolamide, or other CAIs synthesized before 1980, were not topically active due to limited bioavailability. Dorzolamide hydrochloride ((4-*S*-*trans*)-4-ethylamino-5,6-dihydro-6-methyl-4*H*-thieno-[2,3-*b*]thiopyran-2-sulfonamide-7,7-dioxide monohydrochloride) (Fig. 1) was synthesized in the 1980s [6]. Dorzolamide was shown to be an about 20 times more potent CAI, with regard to isoenzyme II, than acetazolamide, and topically active [7]. Topically effective aqueous dorzolamide eye drop solution (Trusopt®) became available in 1995. The concentration of dorzolamide HCl in Trusopt is 2.2% (w/v), corresponding to 2.0% of the free base, at pH 5.65. Hydroxyethyl cellulose is used to increase the viscosity of the eye drops. Increased viscosity leads to increased corneal contact time and, consequently, to increased bioavailability. However, the relatively low pH and high viscosity have been shown to generate local irritation after topical administration of the eye drops [8]. The eye presents unique challenges when it comes to delivery of drug molecules. In general, less than 5% of an applied dose is absorbed into the eye and more typically, less than 1% is absorbed [9,10]. The cornea consists of five layers, i.e., the epithelium, Bowman's membrane, stroma, Descement's membrane and the endothelium. Studies have shown that the outermost layer, the epithelium, is generally the rate-limiting barrier to transcorneal drug transport and that drug molecules must possess sufficient lipophilicity to be able to penetrate this barrier [11]. The task of formulating hydrophilic dorzolamide hydrochloride as a lipophilic base, at pH around 7.4, is therefore especially interesting. The corneal contact time of eye drops can be increased by increasing the viscosity of the aqueous eye drop vehicle in the lower viscosity region (5–25 cps) [12,13].

The study of dorzolamide and its effect on ocular blood flow and oxygenation of the retina has gained much attention in recent years. Dorzolamide has been shown to raise the oxygen tension in optic nerve in pigs when administered intravenously [14]. However, the large amounts of data yielded about dorzolamide and its effect on ocular blood flow has not been consistent [15]. Some reports have indicated that dorzolamide eye drops used for treatment of glaucoma have direct pharmacological effect on the blood flow of the retina and optic nerve [14,16,17]. Other reports indicate that dorzolamide has no measurable vascular effect in both glaucoma patients and healthy individuals when given topically [18,19]. Data from experimental studies have not been in agreement either. Some studies have shown that dorzolamide has effect on retinal arteries [20] but other studies do not show this effect [21]. Measuring the effect of dorzolamide on the blood flow in retina and optic nerve is rather difficult and therefore it is easy to miss this effect. One aspect of this study is to show that it is possible that dorzolamide has a direct effect on the human CA isoenzyme II in the back of the eye.

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic cavity in the center. They are able form water-soluble drug/cyclodextrin inclusion complexes of lipophilic water-insoluble drugs. No covalent bounds are formed or broken during the complex formation, and in aqueous solution the complexes are readily dissociated upon dilution. In general, cyclodextrin molecules do not penetrate biological membranes but act as penetration enhancers by assuring high concentration of dissolved drug at the membrane surface. Cyclodextrins increase the aqueous solubility of

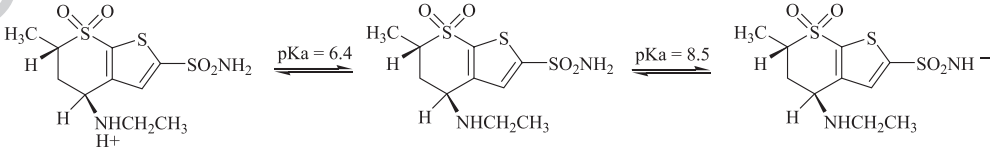

Fig. 1. The chemical structure and ionization of dorzolamide.

lipophilic water-insoluble drugs without decreasing the intrinsic ability of the lipophilic drug molecules to penetrate lipophilic biological membranes. Cyclodextrin can act as a drug carrier that delivers the drug molecule through the aqueous exterior of the membrane, i.e., the mucin layer, and releases it to the relatively lipophilic biological membrane such as the cornea [22]. Cyclodextrins do not disrupt the ophthalmic barrier like conventional penetration enhancers do (like, for example, benzalkonium chloride) [23]. Care must be taken to use the right amount of cyclodextrin since too much cyclodextrin can decrease topical bioavailability of drugs.

The purpose of this study was to formulate low viscosity aqueous dorzolamide eye drop solution containing the unionized drug at pH 7.45 using randomly methylated β-cyclodextrin (RMβCD) as a solubilizer, and to evaluate the formulations in rabbits. In a previous study we have used RMβCD to solubilize dexamethasone in aqueous eye drop solutions and shown that eye drops containing RMβCD are well tolerated in humans [24].

2. Materials and methods

2.1. Materials

Dorzolamide HCl, internal standard (L-662614-002J005) and Trusopt® eye drops were obtained from Merck (USA). Randomly methylated β-cyclodextrin with some degree of substitution 1.8 (RMβCD) was purchased from Wacker-Chemie GmbH (Germany). Analytical grades of disodium phosphate dihydrate, monosodium phosphate monohydrate and disodium edetate dihydrate (EDTA) were purchased from Merck (Germany). Hydroxypropyl methylcellulose (HPMC) was obtained from Mecobenzon (Denmark). Benzalkonium chloride was purchased from Sigma (USA). All other chemicals used in this study were commercially available compounds of special reagent or analytical grade.

2.2. Solubility studies

Phase-solubility study was performed to determine the exact mount of RMβCD needed to solubilize dorzolamide at neutral pH. An excess amount of dorzolamide HCl was added to aqueous phosphate buffer pH 8.0 (0.05 M). The buffer contained 0% to 20% (w/v) RMβCD, benzalkonium chloride (0.02% w/v), EDTA (0.1% w/v) and HPMC (0.1%). The pH of the solution was adjusted to pH 7.5 with 10 M NaOH. The suspensions formed were heated in an autoclave (Midmark M7 SpeedClave) in sealed containers to 121 °C for 20 min. The suspensions were allowed to cool to room temperature (22–23 °C) and equilibrate for 7 days. After equilibrium was attained, the suspension was filtered through a 0.45-μm membrane filter, diluted and analyzed by HPLC. The pH was also determined at room temperature at the end of the equilibration period. To prevent drug precipitation during storage, 10% excess RMβCD was included in the aqueous eye drop formulation.

2.3. Formulation of the eye drops

Initial evaluations showed that in aqueous solutions, RMβCD solubilized dorzolamide much better than either 2-hydroxypropyl-β-cyclodextrin or sulfobutylether β-cyclodextrin. The optimum amount of RMβCD needed to solubilize dorzolamide in the aqueous eye drop formulation was determined from a phase-solubility diagram of dorzolamide in the eye drop formulation. Ten percent excess RMβCD (i.e., 10% more RMβCD than needed to solubilized given amount of dorzolamide) was used to prevent precipitation during storage [23]. The aqueous 2% (w/v) dorzolamide eye drop solution was prepared by dissolving 2.25 g of dorzolamide HCl in 95 ml of aqueous 0.05 M pH 8.0 phosphate buffer solution containing benzalkonium chloride (20 mg) (preservative), EDTA (100 mg) (preservative), HPMC (100 mg) (viscosity enhancing agent) and RMβCD (7.70 g). The pH of the solution was then adjusted to 7.5 with 10 M NaOH and water was added to 100 ml. The solution was heated in an autoclave (Midmark M7 SpeedClave) in sealed containers to 121 °C for 20 min. The solution was allowed to cool to room temperature (22–23 °C) and equilibrate for 7 days. The aqueous 4% (w/v) dorzolamide eye drop solution was prepared the same way except it contained 18.7 g of RMβCD and 4.45 g of dorzolamide HCl. 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+ (USA) operated at room temperature.

2.4. In vivo studies

Unanaesthetized pigmented rabbits, fed on a regular diet, were placed in restraint boxes. The study adhered to the ARVO declaration for the use of laboratory animals in research. One drop (50 μl) of each eye drop solution was administered to both eyes. Six rabbits were sacrificed at each time point, at 1, 2, 4 and 8 h. Samples were taken from both eyes, in all 72 rabbits (144 eyes). The rabbits were sacrificed by intravenous injection of sodium pentobarbital and the eyes were proposed and enucleated immediately and rinsed with an isotonic saline solution. Six rabbits (control group) received saline eye drops devoid of dorzolamide and their eyes were enucleated 2 h after application.

2.5. Sample preparation

The aqueous humor was removed from the eye using 1-ml syringe attached to a 26-G needle. The cornea was cut from the limbus with scissors and placed in a sampling bottle, and the iris into another bottle. The lens was removed and placed in a separate sampling bottle. The vitreous humor was emptied into a sampling bottle by turning the eye backside up. Four incisions (anterior to posterior) were performed in the sclera to open the eye totally and remove the ciliary body. The retina was gently scraped away and placed in a sampling bottle. The optic nerve was removed and placed in a sampling bottle. Great care was taken to prevent cross-contamination between individual tissue samples and eye fluids. While dissecting the eyes, all the samples were put immediately into small, dry polypropylene bottles, which were then immersed in liquid nitrogen. Following the finishing dissection of each rabbit, the samples were moved from the liquid nitrogen and stored at −70 °C. Identical tissues from each pair of eyes were pooled for the dorzolamide concentration measurement and the number of samples for each time point was therefore six.

After weighing or pipetting into culture tubes, the samples were spiked with an internal standard solution. The samples were buffered to pH 8 with 0.2 M Tris buffer, followed by extraction into ethyl acetate. After centrifugation, the organic phase was transferred into culture tubes. For samples of cornea, aqueous humor, and iris and corpus ciliare, the organic solvent was evaporated to dryness under a stream of dry nitrogen. The residue was then dissolved in 250 μl of 0.025 M HCl and 100 μl was injected into the HPLC system for quantification. For samples of vitreous humor, retina, and optic nerve, back-extraction into 300 μl of 0.025 M HCl was performed. After centrifugation, the organic phase was aspirated off and 100 μl of the remaining aqueous phase was injected into the HPLC system for quantification. All stocks and standard solutions were prepared and diluted in 0.025 M HCl. A test to evaluate the extraction ratio of four different rabbit eye tissue was performed. Blank tissue samples were spiked with dorzolamide and internal standard. The samples were put through the sample work-up procedure and compared to blank samples of each tissue spiked with dorzolamide and internal standard after sample work-up. Extraction efficiency was found to be 100%, 83.6%, 87.5% and 69.9% for aqueous humor, iris and ciliary body, cornea, and optic nerve, respectively. The standard solutions and extracted samples were stable for at least 24 h at ambient temperature.

2.6. Quantitative determination of dorzolamide

The HPLC apparatus, Hewlett Packard Series 1100, consisted of a G1312A binary pump with a G1322A solvent degasser, a G1314A variable wavelength detector, a G1313A auto-injector, and a G1316A column oven, set to 40 °C. The separation was accomplished with a HyPurity Elite C18, 5 μm, 4.6×150 mm column with a matching 4.0×10-mm precolumn, using a gradient program. The gradient program was as follows: 100% mobile phase A (0.01 M sodium phosphate, pH 6.0, acetonitrile (85:15)) for 2 min, then linearly changing to 100% mobile phase B (0.01 M sodium phosphate, pH 6.0, acetonitrile (50:50)) over 9 min. At 11 min, the mobile phase was linearly changed to 100% mobile phase C (HPLC grade water, acetonitrile (50:50)) over 10 min and then to 100% mobile phase A over 1 min. The column was equilibrated for 10 min before the next injection. The flow rate was 1.50 ml/min and dorzolamide was detected at 250 nm.

The HPLC method was validated with respect to sensitivity, linearity, accuracy and precision before the start of study. The lower limit of quantification was set at 0.10 μg/ml (precision 0.84%, accuracy 11.1%). Linearity was confirmed over the concentration range of 0.1 to 10 μg/ml (r^2 0.9999–1.000). Intra-assay accuracy (−9.2% to −0.6%), intra-assay precision (0.3% to 5.5%), inter-assay accuracy (−4.3% to 1.1%), and inter-assay precision (2.1–7.9%) were all within the set requirements for the analysts.

3. Results

Dorzolamide has two pK_a values of 6.35 (pK_{a1}) and 8.5 (pK_{a2}) corresponding to the protonized secondary amino group and the sulfonamide group, respectively (Fig. 1). It is mainly in its hydrophilic cationic form at pH below 6.4 and mainly in its hydrophilic anionic form at pH above 8.5. The largest fraction of the lipophilic unionized form exists at pH right between the two pK_a values or at pH 7.45. Dorzolamide exists in two polymorphic forms: form I which is the more thermodynamically stable form and form II which is slightly more soluble in water. Solubility in water at room temperature (~23 °C) was determined to be about 40 mg/ml at pH 4.0–5.5. The commercial product contains 2.25% (w/v) of the hydrophilic hydrochloride salt in an aqueous pH

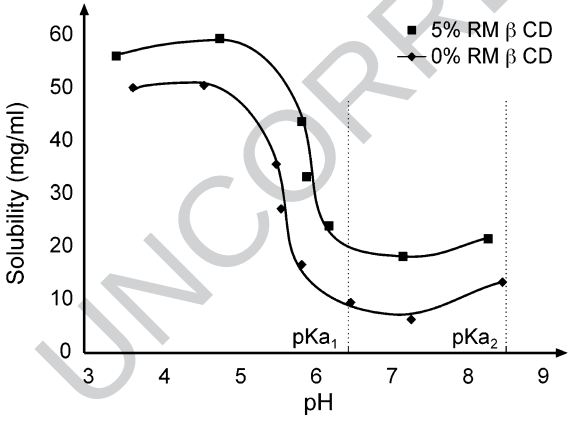

Fig. 2. The pH-solubility profile of dorzolamide in aqueous solution and in aqueous 5% (w/v) RMβCD solution at room temperature (approximately 23 °C). The pH of the aqueous solution was adjusted with 10 M hydrochloric acid or 10 M sodium hydroxide solution.

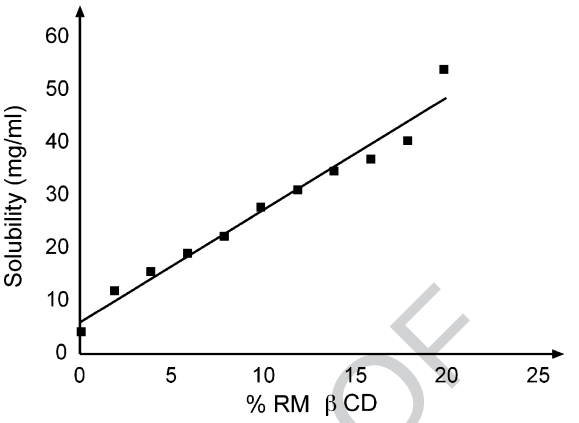

Fig. 3. The phase-solubility diagram of dorzolamide in the aqueous eye drop formulation at room temperature (approximately 23 °C).

5.65 citrate buffer solution, and to prevent precipitation the pH has to be maintained below 5.8.

3.1. Formulation of the eye drop solution

The aqueous solubility of dorzolamide is a function of the ionization constants of the drug molecule. The pH solubility profile for dorzolamide with and without 5% RMβCD is shown in Fig. 2. The solubility of the drug molecule is lowest just right between the two ionization constants or at pH about 7.4. Fig. 3 shows the phase-solubility diagram of dorzolamide in aqueous RMβCD eye drop solutions (0–20% w/v) at pH 7.52±0.17 (mean±standard deviation). The phase-solubility is of A_L-type and thus dorzolamide appears to form a 1:1 dorzolamide/RMβCD complex in the aqueous eye drop formulation.

The final concentration of dorzolamide free base in the 2% and 4% dorzolamide eye drop solutions was determined from the phase-solubility diagram to be 19.72 and 38.84 mg/ml, respectively. The pH was determined to be 7.45 for the 2% eye drop solution and 7.51 for the 4% eye drop solution. The osmolarity of the 2% and 4% dorzolamide/RMβCD eye drops was determined to be 358 and 714 mosM/kg, respectively. Thus, the 2% dorzolamide eye drop solution was close to isotonic but the 4% solution was hypertonic. The osmolarity of Trusopt® (20 mg/ml) is about 290 mosM/kg, which is isotonic with the tear fluid. In this study, dorzolamide HCl was used and the pH of the solution had to be adjusted with 10 M solution of sodium hydroxide. It is possible to prepare

aqueous isotonic 4% dorzolamide/RMβCD eye drop solution at pH 7.4 by using the free dorzolamide base. However, the free base was not available.

Trusopt® is relatively a viscous solution; the viscosity was determined to be about 100 cps. The viscosity of 2% and 4% eye drops is very low or about 3 and 5 cps, respectively. For comparison, the viscosity of water is 1 cps.

3.2. In vivo evaluation

All solutions were well tolerated by the rabbits and no macroscopic signs of irritation, redness or other toxic effects were observed. Dorzolamide was absorbed from all three test and control formulations into the anterior part of the eye. The results are summarized in Table 1. Dorzolamide was detected in most samples from retina and optic nerve in measurable concentrations but in very few samples from vitreous humor. Differences in concentration between the three formulations did not reach statistical significance due to the variability and relatively low number of data points. The results indicated that after 1 and 2 h, the 4% (w/v) dorzolamide RMβCD solution was superior in the back of the eye (i.e., retina and optic nerve), while Trusopt® was superior in the front of the eye (i.e., cornea, aqueous humor, iris and corpus ciliare). In retina and optic nerve, the 4% dorzolamide cyclodextrin formulation gave mean (±standard deviation) concentration/tissue weight (μg/g) of 1.04 (±0.59) and 2.8 (±1.4) after 2 h, while Trusopt® gave 0.52 (±0.34) and 1.78 (±1.53), respectively. In cornea and iris-ciliary body the 4% dorzolamide cyclodextrin formulation gave mean (±S.D.) concentration/tissue weight (μg/g) of 8.02 (±4.50) and 10.79 (±3.82) after 2 h, while Trusopt® gave 15.81 (±8.61) and 30.9 (±19.75), respectively. There were no notable differences between the test and control formulations at 8 h. Fig. 4A shows the

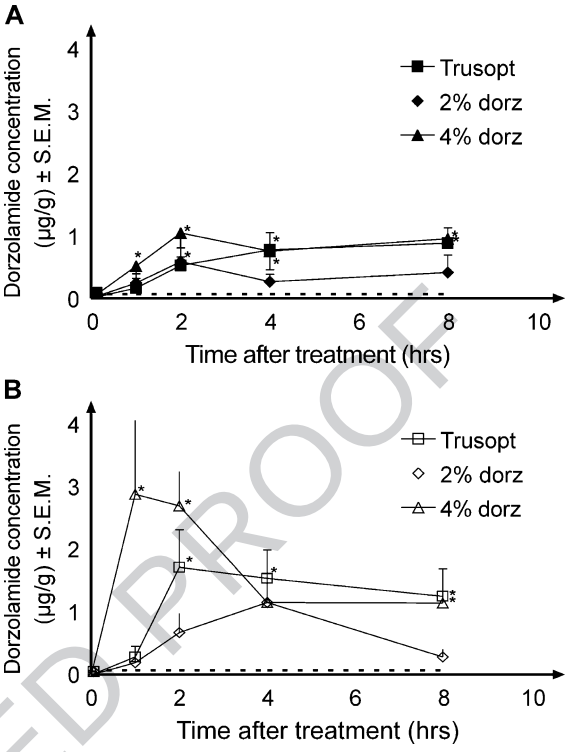

Fig. 4. Dorzolamide concentration (μg/g) in retina (A) and optic nerve (B) (mean±standard deviation; n=6). The dotted line shows the (IC_{50}) value for human CA isoenzyme II.

Table 1
Concentration of dorzolamide (in μg/g) in various parts of rabbit eye (mean±standard deviation; n=6)

Time (h)	Eye drops	Aqueous humor	Vitreous humor	Cornea	Retina	Optic nerve	Iris-ciliary body
1	2% dorz.	1.4±0.6	0.1±0.1	9.5±3.5	0.2±0.4	0.2±0.4	8.0±3.4
1	4% dorz.	1.3±0.4	0.1±0.1	11.0±3.6	0.5±0.5	3.0±3.0	6.8±3.5
1	Trusopt®	2.0±1.0	0.1±0.1	16.5±6.4	0.2±0.2	0.3±0.4	7.7±6.2
2	2% dorz.	0.7±0.3	0.1±0.1	5.3±2.0	0.6±1.0	0.7±0.8	6.9±4.4
2	4% dorz.	0.7±0.4	0.2±0.1	8.0±4.5	1.0±0.6	2.8±1.4	10.8±3.8
2	Trusopt®	2.2±1.5	0.2±0.2	15.8±8.6	0.5±0.3	1.8±1.5	30.9±19.8
4	2% dorz.	0.2±0.2	0.1±0.1	4.0±3.5	0.3±0.3	1.2±1.6	8.5±3.1
4	4% dorz.	0.3±0.2	<0.1	3.5±1.3	0.8±0.7	1.2±1.6	8.1±3.4
4	Trusopt®	0.4±0.2	<0.1	5.1±2.2	0.8±0.7	1.6±1.2	16.2±11.2
8	2% dorz.	0.1±0.1	<0.1	2.7±3.3	0.4±0.7	0.3±0.3	8.5±7.7
8	4% dorz.	0.1±0.1	0.1±0.2	6.5±7.0	1.0±0.2	1.2±1.2	11.4±4.5
8	Trusopt®	0.1±0.1	<0.1	4.6±3.3	0.9±0.6	1.3±1.1	15.8±13.4

concentration of dorzolamide in the optic nerve after topical application of the three formulations and Fig. 4B shows the concentration of dorzolamide in retina. The concentrations of dorzolamide in samples, which were significantly greater than zero, are marked in Fig. 4. The dotted line shows the in vitro inhibition value (IC_{50}) for human CA isoenzyme II [5]. Fig. 4 indicates that dorzolamide concentrations in the back of rabbit eyes (retina and optic nerve) after topical administration of the commercial product as well as after administration of the cyclodextrin formulations are well above the IC_{50} values of human CA isoenzyme II. The results indicate that dorzolamide could have a direct effect on the CA isoenzyme II in retina and in optic nerve.

4. Discussion

Our results are in agreement with the results of Sugrue [5,25] who reported similar dorzolamide concentrations in the cornea, aqueous humor and iris-ciliary body after topical administration of the drug. However, Sugrue did not measure dorzolamide concentration in the vitreous gel or optic nerve. Our results show that the drug levels in the vitreous gel were always lower than those in retina and optic nerve. Possible explanation could be the binding of the drug to carbonic anhydrase and pigment in some tissues. Vitreous humor has neither pigment nor carbonic anhydrase and therefore the concentration of dorzolamide in vitreous gel could reflect the concentration of free dorzolamide. The dorzolamide levels in solid tissues may reflect the combination of free and bound dorzolamide. A more likely explanation for lower drug levels in vitreous than in retina and optic nerve is, however, that the drug reaches the optic nerve and retina through the blood stream circulation rather than by diffusing through the vitreous gel to the retina. The measured concentrations of dorzolamide are nearly always greater in optic nerve than in retina. Local diffusion through the sclera and the highly vascularized choroid is unlikely due to the high rate of blood flow and clearance in the choroid. Other studies on glaucoma drugs, such as pilocarpine, beta blockers, alpha agonists and prostaglandin analogs, have also shown lower vitreal than retinal drug levels suggesting that the drug diffusion is most likely from retina to vitreous and not the other way around [25–32].

5. Conclusion

Aqueous dorzolamide/cyclodextrin eye drop solutions, with pH 7.4 and viscosity of 3 to 5 cps, were successfully formulated and compared to Trusopt®, with pH 5.65 and viscosity of 100 cps. No irritation or other side effects could be observed after topical administration of the cyclodextrin eye drop solutions to rabbits. The topical availability of dorzolamide from the cyclodextrin-containing eye drops appeared to be good and the drug reached retina and optic nerve to give measurable concentration for at least 8 h after administration of the eye drops.

Acknowledgments

This study was supported in part by a research grant from the Icelandic Research Council.

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