REVIEW

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Topical drug delivery to the posterior segment of the eye: anatomical and physiological considerations

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Drug delivery to the posterior segment of the eye is important for potentially treating various disorders in retina, choroid, vitreous humor and optic nerve. Due to anatomic membrane barriers and the lacrimal drainage it can be quite challenging to obtain therapeutic drug concentrations in the posterior parts of the eye after topical drug administration. Since the membrane barriers cannot be altered with non-invasive methods invasive methods such as direct drug injection into the vitreous humor and subconjunctival, subtenons capsule or suprascleral injections are gaining popularity. However, invasive methods can cause discomfort for the patient and can also lead to complications that are even more serious than the disease being treated. Alternatively, novel ophthalmic formulations can be developed that specifically target topical drug delivery to the posterior segment of the eye. Anatomical and physiological barriers in the eye are reviewed as well as the theoretical model of passive drug diffusion from the eye surface into the eye. It is shown that enhanced drug delivery through conjunctiva/sclera to retina can be obtained by formulating lipophilic drugs as hydrophilic drug/cyclodextrin complex solutions. Optimization of the delivery system by formulating the drug as a low-viscosity aqueous drug/cyclodextrin complex suspension results in sustained high concentrations of dissolved drug in the tear fluid which further increases the targeted drug delivery to the posterior segment.

1. Introduction

Drug delivery to the posterior part of the eye (e.g. to retina, choroid, vitreous humour and optic nerve) is important for treating several disorders such as age-related macular degeneration, diabetic retinopathy, glaucoma, retinal venous occlusions, retinal arterial occlusion, macular edema, postoperative inflammation, uveitis, retinitis and proliferative vitreoretinopathy. Due to anatomic membrane barriers (i.e. cornea, conjunctiva and sclera) and the lacrimal drainage, as well as the distance from the front to the back of the eye, it can be quite challenging to obtain therapeutic drug concentrations in the posterior parts of the eye after topical drug administration. Since those barriers cannot be altered with non-invasive methods, the ophthalmic formulations have to be improved in some way to increase the ocular drug bioavailability (Davis et al. 2004). To date, there is no noninvasive, safe and patient-friendly drug delivery system that is both specific and effective for the posterior part of the eye. In general, drugs can enter the posterior segment of the eye via three distinctive routes, i.e. a) through conjunctiva/sclera after topical application, b) from the anterior part after topical application, and c) from the systemic circulation after topical, parenteral, oral, or intranasal applications or after other administration routes that deliver drug to the blood circulation (Hughes et al. 2005). Then drugs can be delivered to the eye via invasive methods such as direct drug injection into the vitreous humor, subconjunctival, subtenons capsule or suprascleral injections (Kojima et al. 2006; Sivaprasad et al. 2006). However, invasive methods can cause discomfort for the patient and can also lead to complications that are even more serious than the disease being treated (Delyfer et al. 2007; Fasolino et al. 2007; Kusaka et al. 2007).
Recently cyclodextrin-based eye drop formulations have been developed for topical drug delivery to the posterior segment of the eye (Sigurdsson et al. 2005; Loftsson et al. 2007a, 2007b; Sigurdsson et al. 2007). Following is a short review of the anatomical and physiological barriers towards topical drug delivery to the posterior segment of the eye and how cyclodextrin-based delivery system can be applied to overcome these barriers.

2. Structures of the eye

The eye is a relatively exposed and isolated organ and its surface is easily accessible for topical application of drugs. The eye can be divided into an anterior segment that includes cornea, aqueous humor, iris, lens and ciliary body, and a posterior segment that comprises vitreous humor, re-

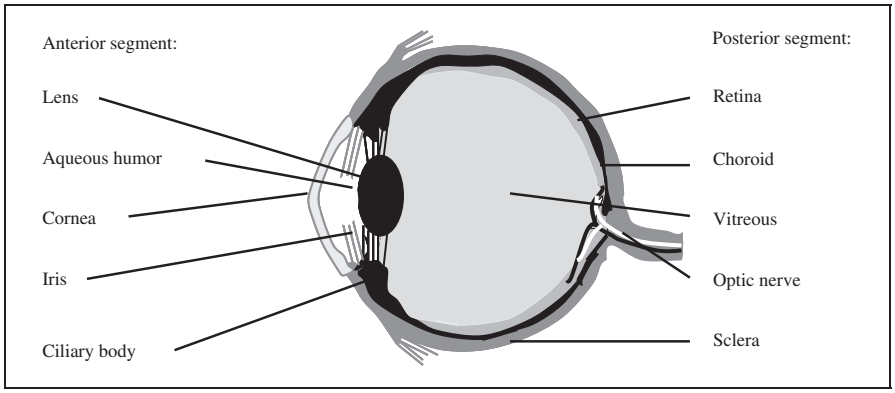


Fig. 1:
Schematic drawing of the eye

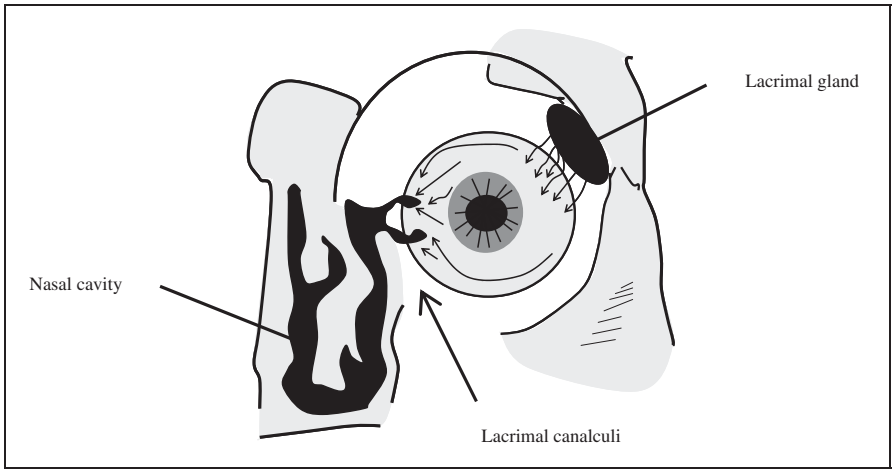


Fig. 2:
Schematic drawing of the lacrimal drainage system

tina, choroid, sclera and optic nerve (Fig. 1). The eye surface is continuously washed by the tear fluid secreted from the lacrimal glands, the main gland located at the outer portion of the eye orbit (Fig. 2). The eye is protected by the eyelids and the surrounding bone structures and adipose tissues (Sasaki et al. 1996; Moroi and Lichter 2001; Washington et al. 2001).

The cornea (Fig. 3) is a transparent five layer biomembrane. The outermost layer is the epithelium, then the Bowman's membrane, stroma (which represents about 90% of the membrane thickness), Descement's membrane and finally the endothelium. The main barrier layer towards drug penetration through the cornea is the lipophilic epithelium, which contributes about 90% of the barrier towards hydrophilic drugs and about 10% of the barrier towards lipophilic drugs. The epithelium consists of three to six layers of tightly adherent epithelial cells. The epithelial surface is covered with microvilli. Drugs penetrate the

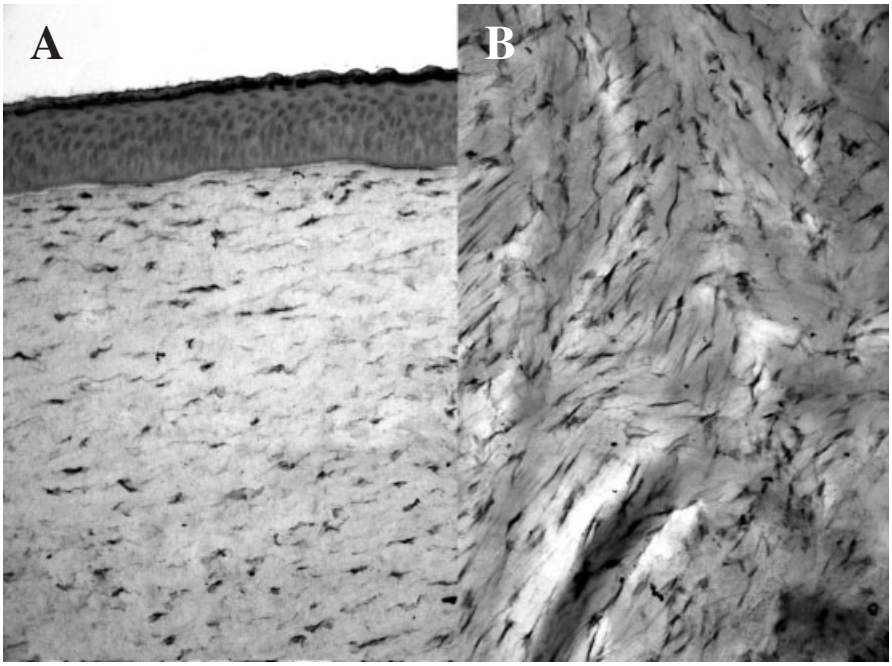


Fig. 3:
Light microscopic histology of the porcine eye
A: cornea with the outermost mucus layer (with microvilli), corneal epithelium, Bowman's membrane and stroma (with keratocytes)
B: cross section of sclera

epithelium either transcellular (i.e. through the cells) or paracellular (i.e. through pores between the cells). The transcellular route predominates for lipophilic drug molecules whereas the paracellular route predominates for hydrophilic molecules and small ions. The pore size has been estimated to be about 1 nm (permeable for drugs with molecular weight (MW) less than about 700 Da) although studies have indicated that some pores could be up to 5 nm in diameter (Hämäläinen et al. 1997b; Prausnitz and Noonan 1998). However, the porosity (i.e. the pore density on the surface) of the epithelium is rater low indicating that paracellular permeation is limited by the frequency of a drug molecule hitting a pore. It is believed that most drugs permeate the epithelium via passive diffusion and although drug transporters have been located in the epithelium their significance is still unclear (Mannermaa et al. 2006). The stroma can, from a drug delivery point of view, be considered to be an aqueous gel where collagen fibrils and glycosaminoglycans form the net structure. It offers little permeation resistance towards hydrophilic drugs but can be the rate limiting barrier for lipophilic drugs that permeate the epithelium relatively rapidly. The endothelium is a one cell layer thick membrane with large intracellular junctions. It can be regarded as a leaky lipophilic barrier that offers no permeation resistance towards hydrophilic drugs but may offer some resistance towards lipophilic drugs (Ghate and Edelhauser 2006). The Bowman's and Descement's membranes do not offer any significant resistance towards drug permeation.

The bulbar conjunctiva is a transparent mucous membrane that covers the outer surface of the sclera. It contains numerous microvilli on its surface. Within the bulbar conjunctiva are the goblet cells which secrete mucin, an important component of the tear layer that protects and nourishes the eye surface. The sclera is composed primarily of collagen fibers embedded in mucopolysaccharides matrix, an aqueous structure that resembles the structure of the corneal stroma (Fig. 3). The mean thickness of human sclera is 0.53 mm and the mean total area is 16.3 cm^2 (Ambati and Adamis 2002). The primary route for drug permeation through the sclera is by passive diffusion through an aqueous pathway. The permeability of sclera is similar to that of stroma with no apparent dependence on the drug lipophilicity, i.e. the octanol/water partition coefficient, but a strong dependence on the drug MW, i.e. the hydrodynamic radius of the permeating drug molecule, the permeability coefficient decreasing with increasing MW (Prausnitz and Noonan 1998; Raghava et al. 2004). Conjunctiva is approximately 15 to 25 times more permeable and the sclera is approximately 10 times more permeable than the cornea (Hämäläinen et al. 1997a). Both intercellular pore size and pore density in the cornea are much smaller than in the conjunctiva.

The choroid is a vascular layer that lies between the retina and the sclera. It is composed of layers of blood vessels that nourish the back of the eye. The choroidal vasculature can contribute to drug clearance from the eye and, thus, constitute a permeation barrier during drug permeation from the eye surface to the retina and vitreous. The vitreous humor is a clear aqueous gel where the matrix-forming polymer system consists mainly of collagen and hyaluronan. The collagen fibrils occur predominantly in parallel bundles of two or more fibrils with fibrils or bundle of fibrils frequently braking off to join other bundles and, thus, creating a self-sustained collagen fibrillar network (Bishop 2000; Bos et al. 2001; Gelse et al. 2003). Hyaluronan is an unbranched polymer of repeating disaccharide units composed of D-glucuronic acid and *N*-acetyl-D-glucosamine. The hyaluronan molecules are unable to form stable intermolecular associations between themselves but it is believed that they participate in the stabilization of the collagen fibrillar network (Bos et al. 2001; Ihanamäki et al. 2004). The water content of vitreous is about 99%.

Aqueous humor is the fluid that fills the anterior chamber, the space between the iris and the cornea. The aqueous humor has relatively low viscosity and its chemical composition resembles that of blood plasma. Its total volume in human and rabbit eye is between 200 and 300 µl. It is continuously secreted by the ciliary body, flows as gentle stream (2 to 5 µl/min) through the pupil and drained by the canal of Schlemm (Sasaki et al. 1996).

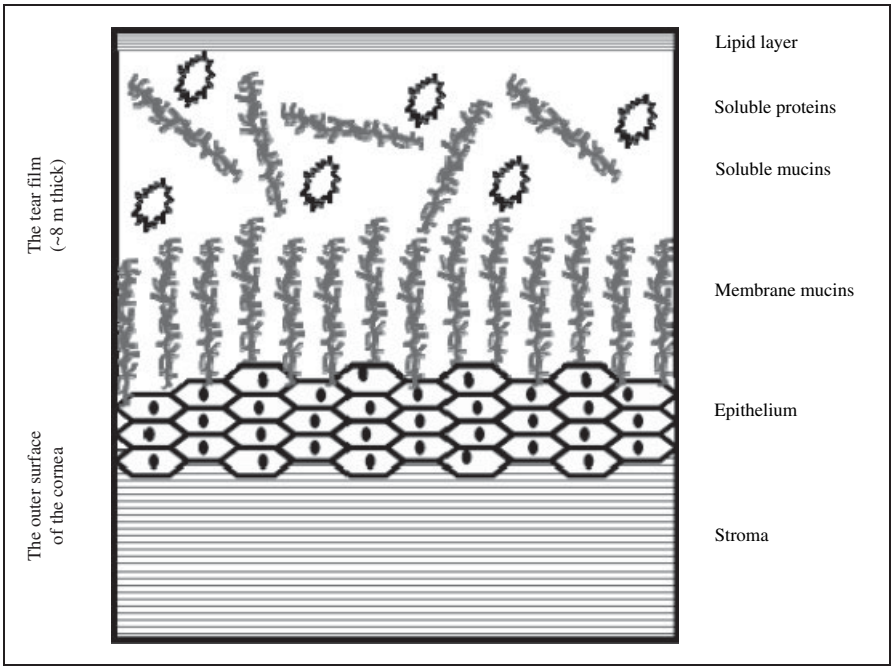


Fig. 4:
Schematic drawing of the tear film on the corneal surface of the eye

3. The tear fluid

After ocular instillation, aqueous eye drops will mix with the tear fluid and be dispersed over the eye surface. However, various pre-corneal factors will limit the ocular absorption by shortening corneal contact time of applied drugs. The most important factors are the drainage of installed solution, non-corneal absorption and induced lacrimation. The lacrimal fluid (tear fluid) cleans and lubricates the eye surface. It is produced by the lacrimal gland located in the outer portion of the orbit (Fig. 2) and accessory glands located in the conjunctiva. It consists of three layers (Fig. 4). The outermost layer is the lipid layer that retards water evaporation from the surface. Then the central aqueous layer, that contains mainly water and small amounts of other substances such as proteins. The innermost layer is the mucous a gel-like fluid containing mainly water (~95%) and mucin (Bansil and Turner 2006). Mucins are large glycoproteins with MW ranging from 0.5 to 20 MDa. Some are membrane-bound but others are not. Mucin forms hydrogen bonds with surrounding water molecules enhancing water-cluster formation and, consequently, decreased water mobility (Loftsson et al. 2007c). Following instillation of an eye drop (~35 µl) onto the pre-corneal area of the eye, the greater part of the drug solution is rapidly drained from the eye surface and the solution volume returns to the normal resident tear volume of about 7 µl. Thereafter, the pre-ocular solution volume remains constant, but drug concentration decreases due to dilution by tear turnover and corneal and noncorneal absorption. The value of the first-order rate constant for the drainage of eye drops from pre-corneal area is typically about 1.5 min^{-1} in humans. Normal tear turnover is about 1.2 µl/min in humans (Sugrue 1989) and, thus, after the initial eye drop draiage the precorneal half-life of topically applied drugs is between 1 and 3 min. As a result, only few percentages of the applied dose are delivered into the intraocular tissues. The major part (50–100%) of the administered dose will be absorbed into the systemic drug circulation which can cause various side effects. Consequently it is generally assumed that eye drops are ineffective and of little benefit in delivering drugs in therapeutic concentrations to the posterior segment of the eye (Raghava et al. 2004; Jonas 2005; Myles et al. 2005; Yasukawa et al. 2005; Sivaprasad et al. 2006).

4. Passive transport

Although drug transporters have been located in the eye their significance is still unclear and it is believed that most drugs permeate from the surface into the eye via passive diffusion. Passive drug permeation through multilayer barriers, such as through the tear fluid and through sclera or cornea, can be described as series of additive resistances analogous to electric circuits (Higuchi 1960; Flynn et al. 1972; Flynn and Yalkowsky 1972). Assuming independent and additive resistances of the individual layers, the total resistance (R_T) of a simple membrane can be defined as:

$$J = P_T \cdot C_V = R_T^{-1} \cdot C_V = (R_D + R_M + R_R)^{-1} \cdot C_V = \left(\frac{1}{P_D} + \frac{1}{P_M} + \frac{1}{P_R}\right)^{-1} \cdot C_V \quad (1)$$

where J is the flux of the drug through the membrane, P_T is the overall permeability coefficient, C_V is the drug concentration in the vehicle (i.e. donor phase), R_D, R_M and R_R, and P_D, P_M and P_R are the resistances and permeability coefficients in the unstirred water layer (UWL) at the donor side (the tear fluid), within the membrane (cornea, conjunctiva and/or sclera) and in the UWL at the receptor side, respectively (Loftsson et al. 2007c). If R_R is assumed to be negligible due to relatively rapid removal of drug molecules from the receptor side of the membrane, Eq. (2) is obtained:

$$J = \left(\frac{P_D \cdot P_M}{P_D + P_M}\right) \cdot C_V \quad (2)$$

The relationship between the permeation coefficient (P) and the diffusion coefficient (D) is given by Eq. 3:

$$P = \frac{D \cdot K}{h} \quad (3)$$

where h is the thickness (h_D, h_M or h_R) and K is the partition coefficient between the aqueous phase and the membrane. For P_D and P_R the value of K is unity. Finally D can be estimated from the Stokes-Einstein equation:

$$D \approx \frac{R \cdot T}{6\pi \cdot \eta \cdot r \cdot N} \quad (4)$$

where R is the molar gas constant, T is the absolute temperature, η is the apparent viscosity within the UWL or the lipophilic membrane, r is the radius of the permeating drug molecule and N is Avogadro's number. Thus, the diffusion constant within the UWL (D_D) will decrease with increasing viscosity of the layer as well as with increasing molecular weight of the drug. For example, small lipophilic drug molecules frequently possess a large permeability coefficient through a lipophilic membrane (i.e. large P_M value) and, thus, may be able to permeate lipophilic membrane (e.g. cornea) much faster that they can be transported through the UWL (e.g. the tear film). Under such conditions, diffusion through the UWL becomes the rate-limiting step in the absorption process. Presence of mucin in the mucus layer not only increases the thickness (h) of this UWL but also its viscosity (η) both of which will increase its resistance (R_D) and consequent decrease in permeability (P_D) (Eqs. (3) and (4)). Other surface structures, such as microvilli on the eye surface, can also increase h and η of the UWL. Studies have shown that drug diffusion through mucus is up to 100-times slower than through pure water (Khanvilkar et al. 2001).

5. The three barriers to topical drug delivery to the eye

According to Eq. (1) there are three major barriers to drug delivery into the eye. First, the rapid decrease of drug concentration (C_V) in the tear fluid due to the lacrimal drainage system (Fig. 2). The precorneal half-life of topically applied drugs in simple aqueous eye drop solutions is only between 1 and 3 min. Since passive diffusion is driven by the concentration gradient, i.e. the difference in drug concentration at the outer (C) and inner (C_{Aq}) tear layer, this decrease results in rapid decrease in drug permeability into the eye (Fig. 5). To overcome this we need to increase the precorneal half-life of topically applied drugs. Second, slow permeation of drug molecules through the UWL, the mucus layer, to the membrane surface due to low concentration gradient ($C - C_{Aq}$). To overcome this we need to increase the amount of dissolved drug in the low-viscosity external layer of the tear film (i.e. increase C in Fig. 5). Third, slow drug permeation through the membrane barrier, i.e. cornea or sclera. The only way to increase drug

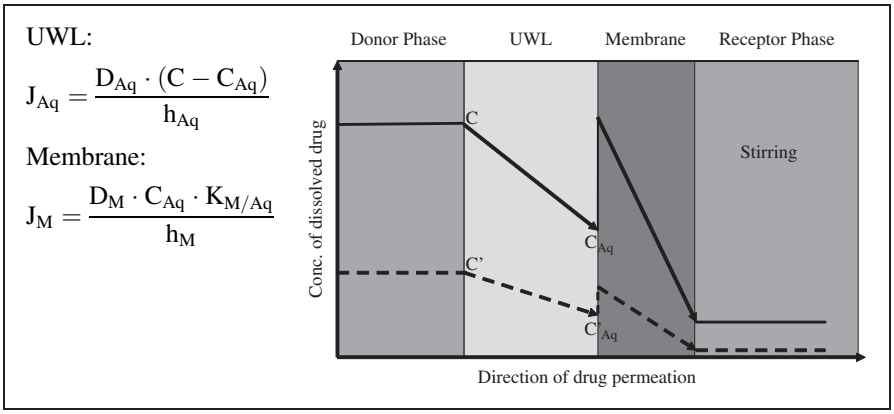


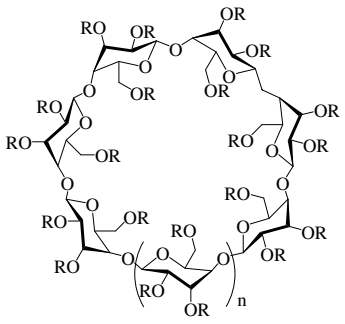
Fig. 5:
Drug permeation from the eye surface into the eye. The donor phase represents the outer low-viscosity layer of the tear film. UWL represents the inner layer of the tear film which is much more viscous due to mucus and microvilli. Membrane represents cornea or the conjunctiva/sclera membrane. The receptor phase represents the inner, more permeable, eye tissues. Drug flux through the UWL: J_{Aq}; drug diffusion coefficient within the UWL: D_{Aq}; thickness of the UWL: h_{Aq}; the drug flux through the membrane: J_M; drug diffusion coefficient within the membrane: D_M; Drug concentration immediate to the membrane surface: C_{Aq}; partition coefficient of the drug from the surface into the membrane: $K_{M/Aq}$; and thickness of the membrane barrier: h_M

permeation through the membrane, except by increasing C_{Aq}, is to increase $K_{M/Aq}$ through chemical modifications of the permeating drug molecule (e.g. through formation of prodrugs) or by increasing the diffusion coefficient (D_M) by adding permeation enhancers to the aqueous eye drop solution that temporary decrease the permeation resistance (i.e. decrease R_M in Eq. (1)). We have selected not to change the barrier properties of the membranes (i.e. cornea, conjunctiva and sclera) or the chemical structures of the topically applied drugs, e.g. through formation of hydrophilic prodrugs, but emphasized the usage cyclodextrin oligosaccharides as carriers to both increase the total amount of dissolved drug in the aqueous tear fluid (i.e. C) and to increase the concentration gradient (i.e. $C-C_{Aq}$) over the thickness (i.e. h_{AQ}) of the UWL (Fig. 5).

6. Cyclodextrins

Cyclodextrins are oligosaccharides formed by (α-1,4)-linked α-D-glucopyranose units, with a hydrophilic outer surface and a lipophilic central cavity (Brewster and Loftsson 2007; Loftsson and Duchêne 2007). The natural α-, β- and γ-cyclodextrins consist of six, seven and eight glucopyranose units, respectively (Table 1). 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 (Loftsson and Stefánsson 2007). 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 and Brewster 1996). Since the cyclodextrin molecule is hydrophilic on the outer surface the complex formation usually increases the water-solubility of lipophilic water-insoluble drugs. Thus, through cyclodextrin com-

Table 1: Structure and properties of some cyclodextrins



Cyclodextrin	n	R = H or	Subst.[a]	MW [b] (Da)	Solubility in water [c] (mg/ml)
α-Cyclodextrin (αCD)	0	−H	0	972	145
β-Cyclodextrin (βCD)	1	−H	0	1135	18.5
2-Hydroxypropyl-β-cyclodextrin (HPβCD; Kleptose® HPB)	1	$-CH_2CHOHCH_3$	0.65	1400	>600
Sulfobutylether β-cyclodextrin sodium salt (SBEβCD; Captisol®)	1	$-(CH_2)_4SO_3^- Na^+$	0.9	2163	>500
Randomly methylated β-cyclodextrin (RMβCD)	1	$-CH_3$	1.8	1312	>500
γ-Cyclodextrin (γCD)	2	−H	0	1297	232
2-Hydroxypropyl-γ-cyclodextrin (HPγCD)	2	$-CH_2CHOHCH_3$	0.6	1576	>500

[a] Average number of substituents per glucose repeat unit.
[b] MW: Molecular weight.
[c] Solubility in pure water at approx. 25 °C

plexation it has been possible to formulate lipophilic water-insoluble steroids as aqueous eye drop solutions (Usayapant et al. 1991; Kristinsson et al. 1996; Davies et al. 1997; Gavrilin et al. 1999; Loftsson and Järvinen 1999; Kearse et al. 2001; Loftsson and Stefánsson 2007). Once included in the cyclodextrin cavity, the drug molecules may be dissociated from the cyclodextrin molecules through complex dilution in the aqueous tear fluid. 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.

Cyclodextrins are able to enhance drug delivery through biological membranes. However, *in vitro* studies have shown that hydrophilic cyclodextrins can only enhance drug delivery through membranes when the permeation resistance of the UWL on the donor side is about equal or greater than the resistance of membrane barrier, i.e. $R_D \geq R_M$ in Eq. 1 (Loftsson et al. 2007c). This is frequently the case when drugs readily permeate cornea or sclera and the mucinous tear film forms the rate-determining UWL. Conventional penetration enhancers, such as benzalkonium chloride, disrupt the ophthalmic barrier (i.e. reduce R_M), whereas hydrophilic cyclodextrins enhance drug penetration into the eye by carrying the lipophilic water-insoluble drug molecules through the aqueous tear film and thereby increasing drug availability at the membrane surface (i.e. by increasing C and $C-C_{Aq}$, see Fig. 5). In addition cyclodextrins can form both nano- and microparticles and in some cases large nanoparticles can penetrate rapidly human mucus (Lai et al. 2007; Loftsson et al. 2007c).

7. Examples

Following are two examples of cyclodextrin-based drug delivery to the posterior segment of the eye.

7.1. Dorzolamide

Dorzolamide (Trusopt®) 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) (Maren 1984; Maren 1987). Furthermore, carbonic anhydrase inhibitors could be useful for medical treatment of optic nerve and retinal ischemia, potentially in diseases such as glaucoma and diabetic retinopathy (Stefánsson et al. 2005). 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 appetite, fatigue, weight loss, gastrointestinal disturbances, paresthesias and renal calculi (Pfeiffer 1997). Studies in the 1960's showed that acetazolamide did not have any IOP lowering effect when applied topically and therefore topical administration CAIs was considered impossible (Kaur et al. 2002). The concentration of dorzolamide HCl in Trusopt® eye drops 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 that results in increased corneal contact time and consequently to increased bioavailability. However, the relatively low pH and high viscosity have been shown to generate local irritation in humans after topical administration of Trusopt® eye drops (Silver 2000).

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. It is mainly in its hydrophilic cationic form at pH below 6.4 and in its hydrophilic anionic form at pH values above 8.5. Largest fraction of the lipophilic unionized form exists at pH right between the two pKa values or at pH 7.45. However, it is not possible to administer dorzolamide as free base in eye drops at pH 7.45 since the aqueous solubility of the base is very limited. Thus, RMβCD was used as a solubilizer (Sigurdsson et al. 2005). A phase solubility study was performed to determine the optimum amount of RMβCD needed to prepare eye drop solutions containing 2% and 4% (w/v) of the free base. The aqueous eye drop formulation contained dorzolamide (2.0 or 4.0% w/v), benzalkonium chloride (0.02% w/v), sodium edetate (0.1% w/v), hydroxypropyl methylcellulose 1000 (0.1% w/v) and RMβCD (7.70 or 18.7% w/v) in 0.05M phosphate buffer solution (pH 7.4). The viscosity of 2% and 4% eye drops is very low or about 3 cps and 5 cps, respectively. For comparison the viscosity of Trusopt® eye drops is around 100 cps. All three eye drop formulations were tested in rabbits. They were well tolerated by the rabbits and no macroscopic signs of irritation, redness or other toxic effects were observed (Sigurdsson et al. 2005). Dorzolamide was absorbed from all the formulations into the anterior part of the eye. The results (Table 2) 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 humour, iris and corpus ciliare). The results also show that the drug levels in the

Table 2: Concentration of dorzolamide (dorz.), in µg/g, in various parts of rabbit eye (mean ± standard deviation; n = 6) after administration of 2 and 4% (w/v) dorzolamide/RMβCD eye drop solutions and after administration of Trusopt®

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

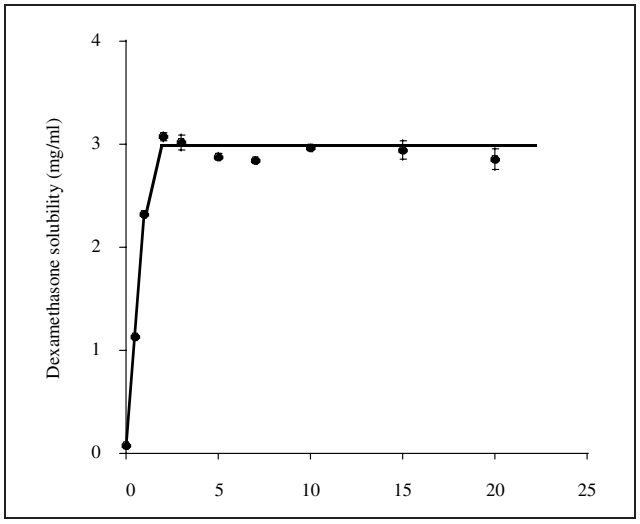


Fig. 6: Phase-solubility of dexamethasone in the aqueous eye drop formulation at 22–23 °C. The aqueous eye drop formulation contained benzalkonium chloride (0.02% w/v), sodium edetate (0.1% w/v), sodium chloride (0.45%) and various amounts of γ-cyclodextrin in pure water

vitreous humor are always lower than in those in the retina. Moreover, the measured concentration of dorzolamide is nearly always greater in optic nerve than in the retina. The results indicate that a significant part of the drug reaches the posterior part of the eye via the systemic circulation to retina and then to vitreous humor. This is supported by other studies of glaucoma drugs, such as pilocarpine, beta blockers, alpha agonists and prostaglandin analogs (Araie et al. 1982; Urtti et al. 1984a, 1988; Acheampong et al. 1995; Sugrue 1996; Sjoquist et al. 1998). This is not unexpected since the low viscosity cyclodextrin-containing eye drops are relatively rapidly drained from the eye surface and then absorbed from the nasal cavity into the systemic blood circulation (Sigurdsson et al. 2005; Loftsson et al. 2007b; Sigurdsson et al. 2007). However, although Trusopt® (cont. 2% dorzolamide) gives 40 to 200% higher drug concentrations in cornea and aqueous humor the first two hours after topical administration than the 2% dorzolamide eye drop solution there is no statistical difference between the concentrations in the retina (Table 2).

7.2. Dexamethasone

Dexamethasone is a glucocorticoid that is commonly used as an anti-inflammatory drug in ophthalmology. It is somewhat lipophilic ($LogP_{(octanol/water)}$ 1.8) and low-molecular weight drug (MW 392.5 Da) that permeates lipophilic biological membranes relatively easy (Moffat et al. 2004). However, its low aqueous solubility (0.16 mg/ml) hampers its clinical usefulness. Formation of water-soluble prodrugs, i.e. dexamethasone sodium phosphate, result is significant solubility increase but due to its hydrophilicity the prodrug does not readily permeate biological membranes, and consequently formation of a water-soluble prodrug can reduce drug penetration into the eye. Natural cyclodextrins, such as γ-cyclodextrin, are very hydrophilic ($LogP_{(octanol/water)} < -3$) and form water-soluble complexes with dexamethasone. Due to their molecular size and hydrophilicity cyclodextrins do not readily permeate biological membranes and, since the dexamethasone/cyclodextrin complex adopts many of the physicochemical properties of the cyclodextrin molecule, the intact complex does not readily permeate through biological membranes. However, cyclodextrin solubilization of dexamethasone will increase drug availability immediate to the epithelial surface (Fig. 5).

Table 3: Dexamethasone concentration ng/g (mean ± standard deviation; N = 6 (RMβCD), N = 8 (γCD)) in blood and various ocular tissues 120 min after topical administration to rabbits

Tissue	Dexamethasone concentration reaching the eye tissue via topical route (mg/ml)		Increase
	γ-Cyclodextrin	RMβCD	
Cornea	1137	1624	−30%
Sclera	381	200	90%
Aqueous humor	232	567	−59%
Iris-ciliary body	263	505	−48%
Lens	6	14	−57%
Vitreous	25	16	56%
Retina	28	9	210%
Optic nerve	85	46	85%

One eye drop containing 1.5% (w/v) dexamethasone in suspension (γ-cyclodextrin, βCD) or solution (randomly methylated β-cyclodextrin, RMβCD) was given to the left eye and the right eye left untreated. The concentration difference ($C_{left\ eye} - C_{right\ eye}$) was used to estimate how much dexamethasone reached various tissues in the left eye via topical route

Figure 6 shows the phase solubility of dexamethasone in the aqueous eye drop formulation. The aqueous solubility of dexamethasone in the formulation without the polymer was 0.08 mg/ml but the phase-solubility diagrams levels off at 3.0 mg/ml indicating that the solubility of the complex is about 2.9 mg/ml. The aqueous eye drop formulation contained dexamethasone (1.5% w/v), benzalkonium chloride (0.02% w/v), sodium edetate (0.1% w/v), hydroxypropyl methylcellulose 1000 (0.1% w/v), sodium chloride (0.2% w/v) and γ-cyclodextrin (18% w/v) in pure water. The concentration of dissolved dexamethasone was determined to be 0.1% (w/v) or between 6 and 7% of the total dexamethasone concentration in the eye drops. The mean (± standard deviation) particle size was determined to be 20 ± 10 μm. The reference eye drop formulation was an aqueous 1.5% (w/v) dexamethasone eye drop solution containing randomly methylated β-cyclodextrin as solubilizer (Loftsson et al. 2007a; Sigurdsson et al. 2007). The eye drops were administered to the left eye but the dexamethasone concentration was determined in both eyes. The concentration difference ($C_{left} - C_{right}$) was used to estimate how much dexamethasone reached various tissues in the left eye via the topical route. Table 3 shows that formulating the drug as a suspension, where the solid particles consist of not the pure drug but the drug/cyclodextrin inclusion complexes, increases the drug delivery to the posterior segment of the eye resulting in over threefold increase in the amount of drug reaching the retina. The blood dexamethasone concentration, two hours after topical administration, was also much lower when the drug was administered as a cyclodextrin complex suspension, being 45 ± 24 ng/g after administration of the eye drop solution but 10 ± 7 ng/g after administration of the suspension (Loftsson et al. 2007a). Interestingly, the amount in sclera was increased by 90% when the drug was administered as suspension while reduced by 30% in the cornea (Table 3). The cornea is a lipophilic tissue and drug permeability through cornea is much lower than through sclera. In cornea the main barrier towards drug penetration through is the lipophilic epithelium (i.e. several layers of tightly adherent epithelial cells) and the permeation rate is

affected by the drug lipophilicity. The sclera is, on the other hand, composed of collagen fibers embedded in mucopolysaccharides matrix and it is about 10 times more permeable that cornea and there is no apparent relationship between the permeation rate and drug lipophilicity (Prausnitz and Noonan 1998; Raghava et al. 2004). Thus, the results indicate that the drug/cyclodextrin delivery system specifically targets sclera and delivery of drugs through sclera to the posterior segment of the eye.

The nature of the solid drug/cyclodextrin particles is also important. Due to their size, the water-soluble drug/cyclodextrin microparticles will not be washed away from the eye surface but adhere to the surface and the surrounding tissue. The particles will dissolve rapidly enough to maintain the aqueous tear fluid saturated with the drug, i.e. the C_{Aq} in Fig. 5 will not decrease during drainage of the tear fluid. Particles of lipophilic drugs possessing limited solubility in water will dissolve very slowly in the aqueous tear fluid. Conventional suspensions, even in micronized form, will not possess sufficiently rapid dissolution rates to maintain drug saturation of the aqueous tear fluid. Formulating the drug as more water-soluble drug/cyclodextrin complexes in a microparticle will ensure rapid drug dissolution. This will maintain drug saturation of the aqueous tear fluid (i.e. high C and C_{Aq} in Fig. 5). Consequently this novel formulation technology will not only enhance the flux (J) of drug into the eye but also reduce the amount of drug reaching the systemic circulation via nasal absorption.

8. Conclusion

It is well documented that cyclodextrins can enhance topical drug delivery to the eye by increasing the amount of dissolved drug in the aqueous tear fluid. However, even under such conditions the bioavailability will be limited by the rapid removal of dissolved drug from the eye surface. Formulating the drug as drug/cyclodextrin complex suspension decreases the drug drainage from the eye surface and this leads to sustained high drug concentrations in the tear fluid and, consequently, to increased ocular drug bioavailability. Since the conjunctiva/sclera membrane is much more permeable than cornea, and since drug permeation through this membrane is not effected by the lipophilicity of the permeating molecule, the cyclodextrin complexation predominantly enhances drug delivery to the posterior segment of the eye.

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