ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 50 (2000) 237–244

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Considerations in the use of hydroxypropyl-β-cyclodextrin in the formulation of aqueous ophthalmic solutions of hydrocortisone

Andrew R. Bary*, Ian G. Tucker, Nigel M. Davies

Formulation and Drug Delivery Group, School of Pharmacy, University of Otago, Dunedin, New Zealand

Received 23 February 2000; accepted in revised form 5 May 2000

Abstract

The in vivo ocular bioavailability of hydrocortisone (HC) in the NZW rabbit was determined following topical administration of solutions containing HC (1%) with hydroxypropyl-β-cyclodextrin (HP-β-CD) alone, or containing the mucoadhesive, viscosity enhancing polymers sodium hyaluronate (0.2 and 0.5% w/v) or Carbopol 934P (0.1% w/v). A 1% HC suspension was used as control. Formulation of HC as a solution with HP-β-CD in the absence of polymer increased the bioavailability of HC in the aqueous humour by approximately 55% and cornea by 75% when compared to suspension. Inclusion of either polymer did not result in any further increase in ocular bioavailability over that noted for the polymer-free solution. The in vitro corneal permeability of HC was also evaluated. A linear relationship ($r^2 = 0.999$) was noted between corneal permeability and the concentration of free (uncomplexed) HC in solution. Permeability was greatest when formulated either as a suspension, or as an HP-β-CD solution in which the concentration of free (uncomplexed) HC is equivalent to that of a saturated solution. Thus, when using cyclodextrins in the reformulation of ophthalmic suspensions as solutions, consideration must be given to the concentration of cyclodextrin used and to the benefits of including viscosity enhancing polymers.

Keywords: Hydrocortisone; Hydroxypropyl-β-cyclodextrin; Complexation; Polymers; Ocular bioavailability; Corneal permeability

1. Introduction

Topically administered corticosteroids are used to treat various ocular inflammatory disorders. Owing to their poor aqueous solubility, they are formulated either as ophthalmic suspensions of the lipophilic parent compound or as aqueous solutions of a water-soluble derivative such as the sodium phosphate salt. Steroids formulated as suspensions or solutions have low corneal permeability due, in part, to either low aqueous solubility of the steroid in the case of suspension or poor partitioning of the hydrophilic steroid derivative into the lipophilic epithelium in the case of the water-soluble salt [1,2]. Consequently, the ocular bioavailability of topically administered steroids has been estimated at less than 0.5% [3–5]. Formulation of steroids as ophthalmic solutions, however, offers a number of advantages over suspensions. Solutions are physically stable, free from potentially irritating particles, can be readily sterilized and, being homogeneous molecular dispersions, do not suffer from variability in the administered dose [6].

To exploit the advantages of solution formulation and retain the partitioning characteristics of the lipophilic parent steroid, cyclodextrins (CDs) have received attention as potential solubilizing agents for topical ophthalmic delivery [7–10]. CDs are capable of forming water-soluble inclusion complexes with many poorly water-soluble compounds such as steroids by taking up the molecule in part or in its entirety into its relatively hydrophobic cavity [11]. No covalent bonds are formed between the drug guest and CD host and the complex is in rapid equilibrium with free drug in solution [12]. The size and hydrophilicity of CDs hinder their absorption across biological membranes [13,14]. Consequently, they can potentially serve as a drug reservoir, replenishing the free drug concentration at a membrane surface by rapid dissociation as equilibrium is disturbed following drug absorption. The driving concentration for drug absorption can thus be maintained, provided the formulation is retained at the membrane surface.

Literature reports concerning the effect of reformulating ophthalmic suspensions as solutions using CDs are, however, conflicting. Papers have reported both an increase in ocular bioavailability [7,15] and a decrease [10,16–18]

* Corresponding author. School of Pharmacy, University of Otago, Adam Building, Frederick Street, P.O. Box 913, Dunedin, New Zealand. Tel.: +64-3-479-7240; fax: +64-3-479-7034.

E-mail address: andrew.bary@stonebow.otago.ac.nz (A.R. Bary).

0939-6411/00/$ - see front matter
PII: S0939-6411(00)00108-9

when compared to suspension having the same total drug concentration. The reason for these conflicting reports is presently unclear. Previously we have reported that a 1% solution of hydrocortisone (HC), equivalent to that used clinically, can be prepared at 30°C by the addition of a minimum of 69.7 mM hydroxypropyl-β-CD (HP-β-CD), based on an association constant (K_a) of 0.636 mM^{-1} [10]. When only the minimum amount of CD is added, the concentration of free HC in solution ($[HC_f]$) is equivalent to that of a saturated solution ($[S_0]$), being 0.972 mM (or 0.035% w/v). The aim of the present study was to assess the ocular bioavailability of this HC/HP-β-CD solution compared to a suspension and to further understand some of the factors which influence the ocular bioavailability of steroid-CD systems including the effect of incorporating viscosity enhancing polymers in the HC/HP-β-CD solution formulations.

2. Materials and methods

2.1. Materials

Hydrocortisone was purchased from Sigma (MO) and 1,2,6,7-tritiated hydrocortisone (1 mCi/ml), tissue solubilizer (NCS-II) and aqueous and non-aqueous scintillation fluids (BCS and BCS-NA, respectively) were purchased from Amersham (Buckinghamshire, UK). HP-β-CD (average molar substitution 1 and MW 1541) was a gift from Cerestar Inc. (IN) and Carbopol 934P was from BF Goodrich (OH). Sodium hyaluronate was purchased from Acros Organics (NJ). All other materials were of at least reagent grade. Distilled, deionized water was used in the preparation of all aqueous solutions (Milli-Q® reagent water system, Millipore, MA). Corneas used in the in vitro permeability study were obtained from Large White/Duroc pigs 16–18 weeks of age and weighing approximately 50 kg. NZW rabbits of either sex, weighing between 3.5 and 5.0 kg, were used for the in vivo ocular bioavailability study.

2.2. Method

2.2.1. Effect of sodium hyaluronate and Carbopol 934P on HC/HP-β-CD complexation

The effect of sodium hyaluronate (SH) and Carbopol 934P (CP) on the complexation of HC with HP-β-CD was examined by phase-solubility analysis as described previously [10]. Briefly, excess HC (80 mg) was added to 5.0 ml of isotonic phosphate buffered saline (pH 7.4) (PBS) alone or containing 0.2 or 0.5% w/v SH, or 0.1% w/v CP and increasing quantities of HP-β-CD (0–100 mM). The resulting systems were ultrasonicated in a bath for 5 min and then mixed by rotating end-over-end (approximately 50 rev./min) at 30°C for 24 h. Following equilibration, the systems were filtered through 0.45 μm cellulose acetate filters (Millipore). The supernatants were immediately diluted with water and total HC analyzed by stability indicating HPLC assay. The HPLC system consisted of a 5 μm, 250 × 4.6 mm CN Econosphere column (Alltech Associates, IL, USA) and mobile phase of 40% v/v acetonitrile in water run at a flow rate of 1 ml min^{-1}. A sample of 50 μl was injected and the effluent was monitored at 254 nm. The retention time of HC under these conditions was 4.0 min, which was completely resolved from its degradation products. The intra-day and inter-day coefficients of variation for the assay were less than 1% in all cases and were not affected by the presence of HP-β-CD. The association constant (K_a) for the complex formed was estimated from the slope of the linear, ascending portion of the phase-solubility curve and the determined aqueous solubility of HC ($n = 6$).

2.2.2. Preparation and characterization of HC formulations for in vivo ocular bioavailability and in vitro corneal permeability studies

For the in vivo bioavailability study, 1.0% w/v HC in PBS was formulated either as a suspension (all particles <50 μm, as confirmed by light microscopy) or as a solution with HP-β-CD (69.7 mM), with or without polymer. Tritiated hydrocortisone ([^{3}H]HC) was used to estimate the concentration of HC in ocular tissues and fluids. Tritium labelling was accomplished by the addition of 0.6 ml of [^{3}H]HC (equivalent to 600 μCi) to 2 ml of 10 mg/ml non-tritiated HC in ethanol, mixed and evaporated to dryness under nitrogen. For suspension preparation, the dried residue was ground to a fine powder using a glass rod, and suspended with the aid of ultrasonication in 2.0 ml of PBS. For the HC/HP-β-CD solutions, 2.0 ml of PBS containing 69.7 mM HP-β-CD alone or with either 0.2 or 0.5% w/v SH or 0.1% w/v CP was added to the dried residue and ultrasonicated for 5 min to facilitate complete dissolution. The viscosity of each solution was determined using a Brookfield model DVIII rheometer (MA) operated at 30.0 ± 0.1°C, fitted with a small volume CP-42 cone and plate.

For the in vitro corneal permeability study, 0.2% w/v HC was formulated in PBS either as a suspension (all particles <50 μm) or its complex with HP-β-CD containing either 11.9, 21.3 or 39.4 mM CD. The concentration of free HC in solution ($[HC_f]$) was therefore 0.035% w/v for the suspension and 0.035, 0.018, and 0.009% w/v, respectively, for the three solutions based on the estimated K_a. Tritium labelling and preparation were performed as described above, by adding 0.2 ml of [^{3}H]HC (equivalent to 200 μCi) to 5 ml of 2 mg/ml HC in ethanol.

The concentration, chemical stability, specific activity and radiochemical purity of each formulation for both in vivo and in vitro studies was confirmed by HPLC and liquid scintillation counting (Beckman LS3801, CA). In all cases, the radiochemical purity of [^{3}H]HC was greater than 97% and the chemical purity was greater than 99%. The specific activities of HC used in the in vivo and in vitro studies were 30 and 20 μCi mg^{-1}, respectively.

2.2.3. In vivo ocular bioavailability study

The study was approved by the University of Otago Animal Ethics Committee in compliance with the Institutional Code of Ethical Conduct, as required by The Animals Protection Regulations 1987 which regulate the use of animals in research, testing and teaching in New Zealand.

Twenty-five microlitres of one formulation was instilled into the lower conjunctival cul-de-sac of both eyes of each rabbit after gently pulling the lower eyelid away from the globe to form a pocket. The eyelid was then gently returned and held against the upper lid for 30 s following instillation. Both eyes were dosed within 1 min. At 20, 40, 60, 90, 120 or 180 min post-instillation, an anaesthetic dose of sodium pentobarbitone was administered via the marginal ear vein. Tears were sampled by soaking a paper strip (Periopaper®, IDE Interstate, NY) in the lower conjunctival sac of each eye. A sample of blood was collected from the heart. Rabbits were then sacrificed by a lethal dose of sodium pentobarbitone and samples of various ocular tissues and fluids were collected. The [^{3}H]HC level in the various fluids and digested tissues was estimated by liquid scintillation counting and converted to total HC per gram of wet tissue or fluid.

The $AUC_{0-3\ h}$ ($\mu g\ min\ g^{-1}$) of the mean HC concentration ($n = 6$) versus time curve for each wet tissue or fluid was calculated according to the method of Yuan [19] and compared by multiple comparisons *t*-test with a Bonferroni adjusted level of significance of 0.017. The mean residence time for HC in each tissue or fluid was estimated by dividing the area of the first moment profile ($AUMC_{0-3\ h}$) by the $AUC_{0-3\ h}$ for each formulation. The ocular bioavailability of the HC/HP-β-CD solution containing CP was determined on a separate occasion to those containing SH. A 1% w/v suspension was included in both studies. No significant difference in bioavailability was observed between studies for the two suspension formulations and therefore the data was pooled and used for statistical comparison with the HC/HP-β-CD formulations.

2.2.4. In vitro corneal permeability studies

The diffusion of HC through porcine cornea in vitro was monitored using acrylic side-by-side diffusion cells based on those described by Camber [20]. The apparatus consisted of a donor compartment (epithelial side, 1.0 ml formulation) and receptor compartment (endothelial side, 3.0 ml glutathione bicarbonate Ringer (GBR)) maintained at 30°C. Corneas were clamped between each compartment exposing 0.785 cm^2 of the corneal surface to formulation and receptor medium. A gas mixture of 95% O_2 and 5% CO_2 was passed through both donor and receptor compartments to facilitate stirring and oxygenation. On the day of study, corneas were excised, rinsed with GBR and mounted into the cells within half an hour of sacrifice of the animal, taking care to avoid damage to the corneal surface. Test formulation and GBR were placed in the donor and receptor compartments, respectively, and at various time intervals up to 6 h, 400 μl of receptor fluid was removed with replacement and analyzed for [^{3}H]HC by scintillation counting. Corneas showed no visible signs of change over this period, which is in agreement with Camber et al. [21] who demonstrated that under the conditions described, corneas did not change and remained viable for at least 4 h. The apparent permeability coefficient of HC was estimated by non-linear regression of the cumulative mass of HC in the receptor fluid versus time profiles for each formulation during non-steady and steady state conditions using Eq. (1) modified from Crank [22]

$$Q_t = k_1 A[HC_f]\left(k_2 t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp^{-k_2 n^2 \pi^2 t}\right) \quad (1)$$

where Q_t is the cumulative mass of HC diffusing across the cornea into the receptor compartment under sink conditions at time *t*, $[HC_f]$ is the concentration of free HC, *A* is the area of cornea exposed to the donor compartment and k_1 and k_2 are given by Eq. (2) enabling the calculation of P_{app}

$$P_{app} = k_1 k_2 = \frac{DK_p}{L} \quad (2)$$

This avoids the necessity for the independent determination of the partition coefficient (K_p), diffusion coefficient (D) and the diffusion path-length (L) [23]. Implicit in this model is the assumptions that the cornea and aqueous diffusion layers are a homogeneous plane sheet, $[HC_f]$ in the donor compartment is not depleted and diffusion occurs under sink conditions.

Upon completion of the permeability studies, the cornea was removed from the cell, rinsed with GBR and blotted dry with lint-free tissue. Samples of intact cornea, cornea minus epithelium and epithelial cells (collected by gentle scraping of the corneal surface with a scalpel) were collected. The [^{3}H]HC level in the digested tissue was estimated by liquid scintillation counting and converted to total HC per gram of wet tissue. The radiochemical purity and chemical stability of the HC in the receptor phase was again confirmed as described (see Section 2.2.2).

3. Results and discussion

3.1. Effect of SH and CP on HC/HP-β-CD complexation

HC has an aqueous solubility ($[S_0]$) of 0.972 mM in PBS at 30°C and forms a 1:1 inclusion complex with HP-β-CD having a K_a of 0.636 mM^{-1} [10]. These values were confirmed in the present investigation. The slope of the phase-solubility profile, representing complexation of HC with HP-β-CD, was not affected by addition of SH at concentrations of 0.2 or 0.5% w/v, or CP at a concentration of 0.1% w/v ($P > 0.05$) although slight increases in $[S_0]$ were observed (Table 1). The observed lack of effect of these two polymers on complexation of HC with HP-β-CD at the concentrations investigated is in contrast to that

Table 1
Physicochemical properties of HC/HP-β-CD/polymer systems determined at 30°C

Polymer	Concentration (% w/v)	$[S_0]$ (mM)[a]	K_a (mM^{-1})[a]	η (mPa s)
–	–	0.972 ± 0.108	0.636 ± 0.011	1.13
CP	0.1	0.978 ± 0.056	0.619 ± 0.023	1.54
SH	0.2	0.983 ± 0.117	0.644 ± 0.074	3.63
SH	0.5	0.992 ± 0.109	0.635 ± 0.055	11.17

[a] Mean ± SD, $n = 6$.

noted for a number of CDs, guest compounds and polymers [8,24–26]. However, an optimum polymer concentration seems to be necessary to achieve such effects, with little effect being observed above or below this concentration [24]. Thus, the lack of effect noted in the present study may be a result of the polymer or concentration used.

At the polymer concentrations used, both SH and CP appeared to exhibit Newtonian flow behaviour over the shear rates studied. The viscosities of the resulting solutions, which were used in the in vivo bioavailability study, are listed in Table 1.

3.2. In vivo ocular bioavailability study

Fig. 1 illustrates the relative bioavailability ($AUC_{0-3\ h}$) of the formulations investigated in the various ocular tissues, fluids and blood when compared to a 1% w/v suspension formulation. The relative bioavailabilities and the mean residence times in the cornea and aqueous humour are documented in Table 2.

Formulation of HC as a solution with HP-β-CD in the absence of polymer significantly increases the bioavailability of HC in aqueous humour, cornea, iris and sclera compared to suspension (Fig. 2). An increase of approximately 75% is noted in the HC levels of the cornea, often the target tissue for corticosteroids, and 55% in the aqueous humour, which is traditionally used as a measure of the ocular bioavailability of ophthalmic formulations.

The increase in ocular bioavailability resulting from reformulation of HC as a solution with HP-β-CD is in contrast to that previously reported for an HC/HP-β-CD solution formulation [10]. In the present study, only 69.7 mM HP-β-CD was used to solubilize the HC as compared to 90 mM used by Davies et al. [10]. The addition of 69.7 mM HP-β-CD to a system containing 1% w/v HC is sufficient to complex only the amount of HC which is in excess of $[S_0]$. Thus, when HC is formulated with 69.7 mM HP-β-CD, $[HC_f]$ is equal to $[S_0]$, being 0.972 mM. Under these conditions, $[HC_f]$ is equivalent for both the solution and suspension formulations. The addition of more CD results in a reduction of $[HC_f]$ as the equilibrium between free and complexed guest depends on the concentration of both CD and the guest. Using a $[S_0]$ of 0.972 mM and a K_a of 0.636 mM^{-1}, it can be calculated that $[HC_f]$ following addition of 90 mM HP-β-CD is 0.668 mM, which represents a 31% reduction in the free concentration of HC in the system.

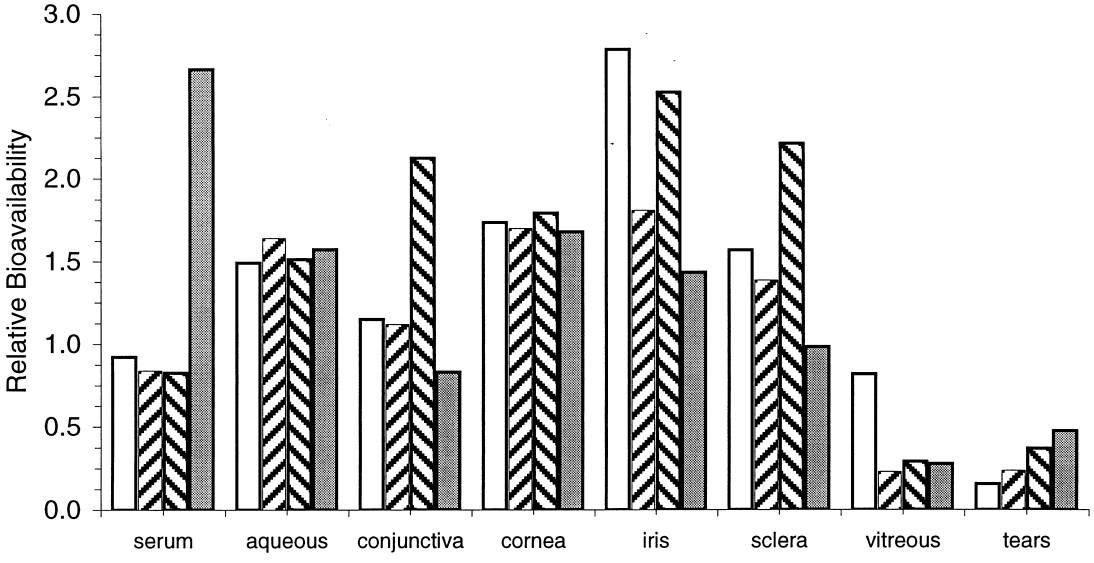

Fig. 1. Relative bioavailability of HC in various ocular tissues and fluids and serum following instillation of a 25 μl dose of HC/HP-β-CD solution containing no polymer (□), 0.2% w/v SH (▨), 0.5% w/v SH (▧) and 0.1% CP (▩) compared to suspension. Values represent mean, $n \geq 6$.

Table 2
Effect of SH and CP on the $AUC_{0-3\ h}$ (μg min g^{-1}) and MRT (min) of HC in aqueous humour and cornea when formulated as a solution with HP-β-CD relative to suspension

Formulation	$AUC_{0-3\ h}$ and (MRT) relative to suspension	
	Aqueous humour	Cornea
No polymer	1.49 (0.89)	1.74 (0.81)
SH 0.2% w/v	1.64 (0.88)	1.70 (0.95)
SH 0.5% w/v	1.51 (0.97)	1.79 (0.89)
CP 0.1% w/v	1.57 (0.99)	1.68 (1.03)

Thus, to exploit the beneficial effect of CDs in ocular drug delivery, it would appear that the amount of CD used must be optimized so that only the drug that is in excess of its solubility is complexed. Over-complexation by addition of excess CD appears to reduce ocular bioavailability.

Both SH and CP have received attention in ophthalmic drug delivery as a means of increasing the pre-corneal retention of solution formulations. Both are anionic polymers capable of interacting with the mucous overlying an absorbing membrane, which can lead to an increase in the retention of the formulation at that site. Consequently, the pre-ocular retention of solution formulations containing either polymer is prolonged to a greater extent than would be expected from viscosity effects alone [27,28]. The mucoadhesive property of these two polymers has been used with some success to increase the ocular bioavailability of a number of compounds formulated as solutions [28–35]. In the present study, no significant increase in relative bioavailability of HC was noted following inclusion of either CP or SH when compared to the polymer-free solution formulations. Further, no significant increase was noted in the MRT values in either the cornea or aqueous humour for the polymer formulations, despite obvious visual signs of the presence of a polymer film in the pre-corneal area for a prolonged period commensurate with the slight increase in the $AUC_{0-3\ h}$ of HC in the tears.

A possible explanation for this lack of effect may reside in the physicochemical properties of HC, which has an octanol/water partition coefficient of 35.7 [36]. Grass and Robinson [37] proposed that increasing the pre-corneal retention of lipophilic compounds having log partition coefficients ranging from 1 to 4 did not increase their ocular bioavailability as these compounds quickly partitioned into the corneal epithelium. Therefore, incorporation of viscosity enhancing polymers in solutions of poorly water-soluble drugs formulated with cyclodextrins may not yield the benefits reported for hydrophilic compounds. The results of Jarho et al. [17] for the limited effect of poly(vinyl alcohol) on the ocular bioavailability of the lipophilic prodrug *O′*,*O*-dipropionyl-(1,4-xylylene) bispilocarpate formulated with a modified β-cyclodextrin are in agreement with that observed in the present investigation.

Of note is that the $AUC_{0-3\ h}$ of HC in the tears was significantly higher ($P < 0.05$) following administration of the suspension compared to solution (8173 and 1279 μg min g^{-1}, respectively) suggesting that HC particles may be retained in the pre-corneal area for an extended period following instillation. This is supported by the findings of Sieg and Robinson [3] who demonstrated that significant levels of fluorometholone exist in the conjunctival sac for an extended period following ocular administration of a suspension when compared to a saturated solution. Increased pre-corneal retention of HC suspension in the pre-corneal area, however, is not translated to an enhanced bioavailability in the aqueous humour or cornea when

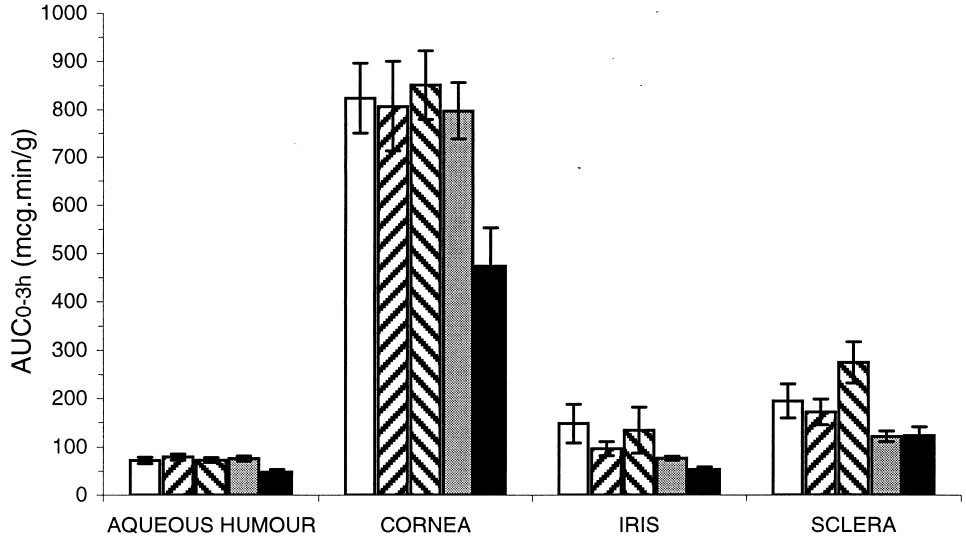

Fig. 2. $AUC_{0-3\ h}$ (μg min g^{-1}) of HC in ocular tissues following instillation of a 25 μl dose of HC/HP-β-CD solution containing no polymer (□), 0.2% w/v SH (▨), 0.5% w/v SH (▧) and 0.1% CP (▩) or suspension (■). Values represent mean ± SD, $n \geq 6$.

Table 3
In vitro corneal permeability of HC at 30°C formulated as suspension or HC/HP-β-CD solution containing different concentrations of HP-β-CD

Formulation (HC 0.2% w/v) (mM)	$[HC_f]$ (% w/v)[a]	Apparent permeability coefficient ($P_{app} \times 10^{-6}$ cm s^{-1}) (n)
Suspension	0.035	2.97 ± 0.6 (10)
HP-β-CD (11.90)	0.035	3.12 ± 0.8 (10)
HP-β-CD (21.30)	0.018	1.57 ± 0.2 (4)
HP-β-CD (39.37)	0.009	0.72 ± 0.4 (4)

[a] Calculated based on $K_a = 0.636$ mM^{-1} and $[S_0] = 0.972$ mM.

compared to an HC/HP-β-CD solution of equal concentration.

3.3. In vitro corneal permeability studies

The permeability of HC through porcine cornea formulated as a 0.2% w/v suspension and solution formulation containing various concentrations of HP-β-CD is reported in Table 3. The addition of 11.90 mM HP-β-CD to a 0.2% w/v suspension is sufficient to complex only the fraction of HC which is in excess of $[S_0]$. Thus, $[HC_f]$ in both solution and suspension formulations is equal, being 0.972 mM. Under these conditions, the corneal permeability of HC from both formulations is equivalent. The addition of greater than 11.90 mM HP-β-CD to a system containing 0.2% w/v HC results in a reduction of $[HC_f]$. The addition of 21.30 and 39.37 mM HP-β-CD results in a 50 and 75% reduction of $[HC_f]$, respectively (Table 3). Thus, a linear relationship exists between corneal permeability and $[HC_f]$ ($r^2 = 0.999$). The y-intercept of the plot of corneal permeability versus $[HC_f]$ is not significantly different from zero ($P > 0.05$), suggesting that complexed HC does not permeate the cornea and that CD does not perturb the membrane resulting in enhanced absorption. The ocular biocompatibility and apparent inability of the CD/drug complex to permeate the cornea is in agreement with previous reports [38–40]. The observed reduction of the in vitro corneal permeability of HC by decreasing $[HC_f]$ may explain the observation of Davies et al. [10] where a decrease in the ocular bioavailability of a 1% HC formulation solubilized with 90 mM HP-β-CD was observed. The addition of excess HP-β-CD in the study resulted in a reduction of $[HC_f]$ by 31%.

Table 4 summarizes the concentration of HC in the intact cornea, epithelium and combined stroma and endothelium following completion of the in vitro permeability study (6 h). The distribution of HC in the cornea is the same for both suspension and HP-β-CD solution containing 11.90 mM CD with the lipophilic epithelium having an approximately six times higher concentration of HC than the hydrophilic stroma and endothelium. Thus, addition of HP-β-CD does not alter the distribution of HC in the cornea. In all cases the total amount of HC in the cornea and receptor compartment after 6 h was less than 1.8% of $[HC_f]$, which is equivalent to 0.3% of the total HC in the donor compartment at $t = 0$.

4. Conclusion

Formulation of 1% w/v HC as an HC/HP-β-CD solution containing 69.7 mM CD results in an approximately 60% increase in ocular bioavailability compared to suspension in the NZW rabbit. In vitro, the corneal permeability of HC is directly proportional to $[HC_f]$ and is the same whether it is formulated as a suspension or HC/HP-β-CD solution, provided that $[HC_f] = [S_0]$. This suggests that the increase in ocular bioavailability in vivo is not a result of enhancement of corneal permeability. The linear relationship between permeability and $[HC_f]$ may explain the reduced in vivo ocular bioavailability of HC when formulated with 90 mM HP-β-CD compared to suspension noted by Davies et al. [10]. Thus, it is critical when formulating ophthalmic solutions of poorly water-soluble compounds using CDs that the amount of CD used in the formulation is limited to that required to complex only the fraction of drug which is in excess of $[S_0]$. As such, the free concentration, and hence driving concentration, of the drug is maintained at its maximum. The amount of CD required can be calculated knowing both the aqueous solubility of the drug and K_a, but the values should be accurately determined otherwise errors may result which can translate to reduced ocular bioavailabilities.

In the present study, the mucoadhesive, viscosity enhancing polymers SH and CP were used to increase the pre-corneal residence of HC/HP-β-CD solution formulations. Despite visual evidence of a prolonged pre-corneal residence of the polymer solutions compared to a polymer-free solution, supported by slight increases in the $AUC_{0-3\ h}$ of HC in the tear film, the inclusion of polymer did not result in any further increase in ocular bioavailability over that observed for the polymer-free solution formulation. The pre-corneal retention of drug is also enhanced by formulation as a suspension compared to a solution. However, prolonged retention of particulates in the cul-de-sac and inner canthal region does not translate to an enhanced ocular

Table 4
Concentration of HC (μg/g ± SD, $n = 8$) in the cornea following completion of permeability studies

Formulation (HC 0.2% w/v)	Intact cornea	Cornea minus epithelium	Epithelium
Suspension	97.8 ± 11.0	65.9 ± 9.5	398.4 ± 48.7
HC/HP-β-CD solution (11.90 mM CD)	106.9 ± 9.80	66.8 ± 10.1	388.6 ± 35.2

bioavailability when compared to an HC/HP-β-CD solution where $[HC_f] = [S_0]$. In conclusion, HP-β-CD can be useful in the reformulation of suspensions of poorly soluble compounds as solutions for ocular drug delivery with associated enhancement of ocular bioavailability. The mechanism for this increase in bioavailability remains to be fully elucidated.

Acknowledgements

We would like to express our gratitude to Cerestar Inc. (IL) and BF Goodrich (OH) for supply of HP-β-CD and CP, respectively. Financial support from the New Zealand Pharmacy Education and Research Foundation is gratefully acknowledged.

References

[1] H.M. Leibowitz, A. Kupferman, Bioavailability and therapeutic effectiveness of topically administered corticosteroids, Trans. Am. Acad. Ophthalmol. Otol. 79 (1975) 78–88.
[2] D.G. Musson, A.M. Bidgood, O. Olejnik, An in vitro comparison of the permeability of prednisolone, prednisolone sodium phosphate, and prednisolone acetate across the NZW rabbit cornea, J. Ocul. Pharmacol. 8 (2) (1992) 139–150.
[3] J.W. Sieg, J.R. Robinson, Vehicle effects on ocular drug bioavailability I: evaluation of fluorometholone, J. Pharm. Sci. 64 (1975) 931–936.
[4] R.D. Schoenwald, P. Stewart, Effect of particle size on ophthalmic bioavailability of dexamethasone suspensions in rabbits, J. Pharm. Sci. 69 (4) (1980) 391–394.
[5] C.N. McGhee, D.G. Watson, J.M. Midgley, M.J. Noble, G.N. Dutton, A.I. Fern, Penetration of synthetic corticosteroids into human aqueous humour, Eye 4 (3) (1990) 526–530.
[6] M. Diestelhorst, K.A. Kwon, R. Süverkrüp, Dose uniformity of ophthalmic suspensions, J. Cat. Refr. Surg. 28 (5) (1998) 672–677.
[7] A. Usayapant, A.H. Karara, M.M. Narurkar, Effect of 2-hydroxypropyl-β-cyclodextrin on the ocular absorption of dexamethasone and dexamethasone acetate, Pharm. Res. 8 (1991) 1495–1499.
[8] T. Loftsson, H. Friðriksdóttir, S. Thórisdóttir, E. Stefánsson, Effect of hydroxypropyl methylcellulose on the release of dexamethasone from aqueous 2-hydroxypropyl-β-cyclodextrin formulations, Int. J. Pharm. 104 (1994) 181–184.
[9] J.K. Kristinsson, H. Friðriksdóttir, S. Thórisdóttir, A.M. Sigurðardóttir, E. Stefánsson, T. Loftsson, Dexamethasone-cyclodextrin-polymer co-complexes in aqueous eye drops. Aqueous humor pharmacokinetics in humans, Invest. Ophthalmol. Vis. Sci. 37 (6) (1996) 1199–1203.
[10] N.M. Davies, G. Wang, I.G. Tucker, Evaluation of a hydrocortisone/hydroxypropyl-β-cyclodextrin solution for ocular drug delivery, Int. J. Pharm. 156 (1997) 201–209.
[11] J. Szejtli, Cyclodextrin Technology, 1st Edition, Kluwer, Dordrecht, 1988.
[12] A. Hersey, B. Robinson, H. Kelly, Mechanism of inclusion-compound formation for binding of organic dyes, ions and surfactants to α-cyclodextrin studied by kinetic methods based on competition experiments, J. Chem. Soc. Faraday Trans. 82 (1986) 1271–1287.
[13] K.M. Uekama, Y. Otagiri, Cyclodextrins in drug carrier systems, CRC Crit. Rev. Ther. Drug Carrier Sys. 3 (1) (1987) 1–40.
[14] M. Tanaka, Y. Iwata, Y. Kouzuki, K. Taniguchi, H. Matsuda, H. Arima, S. Tsuchiya, Effect of 2-hydroxypropyl-β-cyclodextrin on percutaneous absorption of methylparaben, J. Pharm. Pharmacol. 47 (1995) 897–900.
[15] B. Siefert, U. Pleyer, M. Muller, C. Hartmann, S. Keipert, Influence of cyclodextrins on the in vitro corneal permeability and in vivo ocular distribution of thalidomide, J. Ocul. Pharmacol. 15 (5) (1999) 429–438.
[16] P. Suhonen, T. Jarvinen, K. Lehmussaari, T. Reunamaki, A. Urtti, Ocular absorption and irritation of pilocarpine prodrug is modified with buffer, polymer, and cyclodextrin in the eyedrop, Pharm. Res. 12 (1995) 529–533.
[17] P. Jarho, K. Jarvinen, A. Urtti, V.J. Stella, T. Jarvinen, Modified β-cyclodextrin (SBE7-β-CyD) with viscous vehicle improves the ocular delivery and tolerability of pilocarpine prodrug in rabbits, J. Pharm. Pharmacol. 48 (1996) 263–269.
[18] K. Masuda, M. Tohno, Y. Konishi, H. Kojima, M. Matsumura, Ocular penetration of flurbiprofen, an anti-inflammatory drug, in rabbits after topical instillation, Atarashi Ganka 2 (3) (1985) 441–443.
[19] J. Yuan, Estimation of variance for AUC in animal studies, J. Pharm. Sci. 82 (7) (1993) 761–763.
[20] O. Camber, An in vitro model for determination of drug permeability through the cornea, Acta Pharm. Suec. 22 (1985) 335–342.
[21] O. Camber, C. Rehbinder, T. Nikkila, P. Edman, Morphology of the pig cornea in normal conditions and after incubation in a perfusion apparatus, Acta Vet. Scand. 28 (2) (1987) 127–134.
[22] J. Crank, The Mathematics of Diffusion, Oxford University Press, Oxford, 1956.
[23] H. Okamoto, H. Komatsu, M. Hashida, H. Sezaki, Effects of β-cyclodextrin and di-O-methyl-β-cyclodextrin on the percutaneous absorption of butylparaben, indomethacin and sulfanilic acid, Int. J. Pharm. 30 (1986) 35–45.
[24] T. Loftsson, H. Friðriksdóttir, A.M. Sigurðardóttir, H. Ueda, Effect of water-soluble polymers on drug-cyclodextrin complexation, Int. J. Pharm. 110 (1994) 169–177.
[25] T. Loftsson, T.K. Guðmundsdóttir, H. Friðriksdóttir, The influence of water-soluble polymers and pH on hydroxypropyl-β-cyclodextrin complexation of drugs, Drug Dev. Ind. Pharm. 22 (1996) 401–405.
[26] A.M. Sigurðardóttir, T. Loftsson, The effect of polyvinylpyrolidone on cyclodextrin complexation of hydrocortisone and its diffusion through hairless mouse skin, Int. J. Pharm. 126 (1995) 73–78.
[27] R. Gurny, H. Ibrahim, A. Aebi, P. Buri, C.G. Wilson, N. Washington, P. Edman, O. Camber, Design and evaluation of controlled release systems for the eye, J. Control. Release 6 (1987) 367.
[28] N.M. Davies, S.J. Farr, J. Hadgraft, I.W. Kellaway, Evaluation of mucoadhesive polymers in ocular drug delivery, Part 1. Viscous solutions, Pharm. Res. 8 (1991) 1039–1043.
[29] O. Camber, P. Edman, R. Gurny, Influence of sodium hyaluronate on the miotic effect of pilocarpine in rabbits, Curr. Eye Res. 6 (1987) 779–784.
[30] O. Camber, P. Edman, Sodium hyaluronate as an ophthalmic vehicle: some factors governing its effect on the ocular absorption of pilocarpine, Curr. Eye Res. 8 (1989) 563–567.
[31] M.F. Saettone, B. Giannaccini, B. Guiducci, P. Savigni, Semisolid ophthalmic vehicles, III. An evaluation of four organic hydrogels containing pilocarpine, Int. J. Pharm. 31 (1986) 261–270.
[32] M.F. Saettone, P. Chetoni, M. Torracca, S. Burgalassi, B. Giannaccini, Evaluation of muco-adhesive properties and in vivo activity of ophthalmic vehicles based on hyaluronic acid, Int. J. Pharm. 51 (1989) 203–212.
[33] M.F. Saettone, B. Giannaccini, P. Chetoni, M.T. Torracca, D. Monti, Evaluation of high- and low-molecular-weight fractions of sodium hyaluronate and an ionic complex as adjuvants for topical ophthalmic vehicles containing pilocarpine, Int. J. Pharm. 72 (1991) 131–139.
[34] S.F. Bernatchez, C. Tabatabay, R. Gurny, Sodium hyaluronate 0.25% used as a vehicle increases the bioavailability of topically administered gentamicin, Graef. Arch. Clin. Exp. Ophthalmol. 231 (1993) 157–161.
[35] M. Nomura, M.A. Kubota, R. Kawamori, Y. Yamasaki, T. Kamada, H. Abe, Effect of addition of hyaluronic acid to highly concentrated

insulin on absorption from the conjunctiva in conscious diabetic dogs, J. Pharm. Pharmacol. 46 (1994) 768–770.

[36] D.B. Jack, Handbook of Clinical Pharmacokinetic Data, Macmillan, London, UK, 1992.

[37] G.M. Grass, J.R. Robinson, Relationship of chemical structure to corneal penetration and influence of low-viscosity solution on ocular bioavailability, J. Pharm. Sci. 73 (1984) 1021–1027.

[38] B. Siefert, S. Keipert, Influence of α-cyclodextrin and hydroxyalkylated β-cyclodextrin derivatives on the in vitro corneal uptake and permeation of aqueous pilocarpine-HCl solutions, J. Pharm. Sci. 86 (1997) 716–720.

[39] T. Jansen, B. Xhonneux, J. Mesens, M. Borgers, β-cyclodextrins as vehicles in eye-drop formulations: an evaluation of their effects on rabbit corneal epithelium, Lens Eye Tox. Res. 7 (1990) 459–468.

[40] K.A. Freedman, J.W. Klein, C.E. Crosson, β-cyclodextrins enhance bioavailability of pilocarpine, Curr. Eye Res. 12 (1993) 641–647.