ELSEVIER

International Journal of Pharmaceutics 156 (1997) 201–209

international journal of pharmaceutics

Evaluation of a hydrocortisone/hydroxypropyl-β-cyclodextrin solution for ocular drug delivery

Nigel M. Davies *, Guangji Wang [1], Ian G. Tucker

School of Pharmacy, University of Otago, Dunedin, New Zealand

Received 2 April 1997; received in revised form 23 June 1997; accepted 24 June 1997

Abstract

The effect of hydroxypropyl-β-cyclodextrin (HP-β-CD) on the aqueous solubility and chemical stability of hydrocortisone (HC) was investigated with an ultimate aim of formulating a stable topical ophthalmic solution of HC. The ocular bioavailability following topical administration to rabbits of the aqueous formulation of HC was then compared to that of a suspension formulation having an equivalent HC concentration. The aqueous solubility of HC was markedly increased upon addition of HP-β-CD due the formation of a soluble 1:1 inclusion complex. The apparent association constant of the HC/HP-β-CD complex determined by phase-solubility analysis was estimated to be 0.636 mM^{-1}. The decomposition of HC in pH 7.4 phosphate buffer followed pseudo first-order kinetics having rate constants of 13.6×10^{-3} and 1.70×10^{-3} h^{-1}, respectively, in the presence and absence of disodium edetate. Complexation with HP-β-CD increased the chemical stability of HC with the respective pseudo first-order rate constants of decomposition being reduced to 6.73×10^{-3} and 0.90×10^{-3} h^{-1}. The ocular bioavailability following topical administration to rabbits of a tritium labelled 1% HC solution formulation of the HC/HP-β-CD complex was lower than that of a 1% suspension formulation. A significant reduction ($p < 0.05$) of between 25 and 40% was apparent in the cornea, aqueous humour, iris and sclera. © 1997 Elsevier Science B.V.

Keywords: Hydrocortisone; Hydroxypropyl-β-cyclodextrin; Complexation; Solubility; Stability; Ocular bioavailability

1. Introduction

Corticosteroids are widely used in ophthalmology to treat various inflammatory conditions of the eye. Owing to the poor aqueous solubility of the parent steroid, they are usually formulated as

* Corresponding author. Tel.: +64 3 4797324; fax: +64 3 4797034; e-mail: nigel.davies@stonebow.otago.ac.nz

[1] Present address: Pharmacokinetic Research Centre, College of Pharmacy, China Pharmaceutical University, Nanjing, People's Republic of China.

PII S0378-5173(97)00199-3

topical ophthalmic suspensions. Topical ophthalmic suspensions, however, have a number of limitations compared to solutions. In their formulation, suspensions present problems with regards to their physical stability and method for sterilisation, having to be prepared aseptically due to the unsuitability of other techniques. Suspensions should be adequately shaken before use to avoid incorrect dosing (Kwon et al., 1996) and the necessity for shaking can result in poor patient compliance (Apt et al., 1979). Further, it has been claimed that for suspension formulations to show any advantage over solutions in terms of ocular bioavailability, the dissolution rate of the particles must be equal to or greater than their rate of clearance from the precorneal area (Sieg and Robinson, 1975). Hence, ophthalmic suspensions are very costly in terms of the total amount of drug required to achieve the moderate increase in bioavailability.

To overcome these problems, water-soluble derivatives of the parent steroids (eg sodium phosphate salts) have been used and formulated as solutions. Due to the lipophilic nature of the corneal epithelium, however, the corneal permeability of the water-soluble derivative is reduced compared to the parent and other poorly water-soluble derivatives such as the acetates (Leibowitz and Kupferman, 1975; Musson et al., 1992). A decrease in intraocular absorption would therefore be expected for the water-soluble derivatives but the reduced permeability is compensated for by an increased driving concentration resulting from the enhanced aqueous solubility (Olejnik and Weisbecker, 1990; Musson et al., 1991).

The aim of this study was to formulate and evaluate a solution formulation of a parent corticosteroid, hydrocortisone (HC), using a derivatised cyclodextrin having good aqueous solubility. This approach could overcome many of the problems associated with suspension formulation while maintaining the lipophilicity and hence permeability characteristics of the parent corticosteroid. Cyclodextrins (CDs) are cyclic oligosaccharides which owing to their configuration have a hydrophilic outer surface and a central lipophilic cavity. The lipophilic cavity enables CDs to form inclusion complexes with a wide variety of poorly water-soluble compounds in aqueous solution while the hydrophilic outer renders these inclusion complexes water soluble (Szejtli, 1988). In addition, inclusion of molecules within the cavity of cyclodextrins may protect the guest from the external environment and hence CDs may be used to increase the chemical stability of molecules susceptible to degradation.

2. Materials and methods

2.1. Materials

HC was purchased from Sigma (St. Louis, MO) and 1,2,6,7-^{3}H-HC (37 MBq/ml), tissue solubiliser and scintillation fluids (ACS and OCS) from Amersham (UK). Hydroxypropyl-β-cyclodextrin (molar substitution 1 and average MW 1541, HP-β-CD) was gifted by American Maize Products Company. Methanol (HPLC grade) and distilled deionised water were used for the preparation of HPLC mobile phase. All other materials were of at least reagent grade. Distilled water was used in the preparation of all aqueous solutions. Albino (NZW) rabbits of either sex, weighing 1.5–2.5 kg were used for the in vivo ocular bioavailability study. The study was approved by the Otago University committee on ethics in the care and use of laboratory animals operating under NZ legislation equivalent to the NIH guide.

2.2. Methods

2.2.1. Phase-solubility studies

An excess amount of HC (80 mg) was added to 5 ml 0.1 M phosphate buffered saline pH 7.4 (PBS) to which was added increasing quantities (0–100 mM) of HP-β-CD. The suspensions were sonicated for 3 h and then rotated end-over-end (approx 50 rpm) at 30°C for a further 24 h to achieve equilibration. Following equilibration, the suspensions were filtered through a 0.45-μm cellulose acetate filter (Millipore), diluted with distilled water and analysed for total HC by a stability indicating high performance liquid chromatographic (HPLC) assay. The HPLC system consisted of a model 6000A solvent delivery unit

operated at 1.3 ml/min, and a model 441 fixed wavelength (254 nm) UV detector (Waters Associates, USA). A 5-μm, 250 × 4.6 mm CN econosphere column (Alltech Associates, USA) was used with 45% v/v aqueous methanol as mobile phase. Then, 50-μl volumes were injected and peak heights determined. The retention time of HC under these conditions was 4.45 min which was completely resolved from its decomposition products. The intra-day and inter-day coefficients of variations for the assay were less than 2% in all cases and were not affected by the presence of HP-β-CD. Phase-solubility studies were carried out in duplicate. The association constant (K_c) for the complex formed was calculated from the slope of the phase-solubility profiles and the aqueous solubility of HC (S_0) according to Eq. (1) (Grant and Higuchi, 1990):

$$K_c = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad (1)$$

2.2.2. Chemical stability studies

Stability studies were conducted at a temperature of 60°C (± 0.5). The reactions were initiated by adding an equal volume of a freshly prepared HC stock solution in water (0.55 mM) to 0.2 M PBS containing various concentrations of HP-β-CD (0.165–8.8 mM). Disodium edetate was added to one group of solutions (final concentration 0.15% w/v) to investigate the effect of trace metal ions on the chemical stability of HC. Samples were withdrawn immediately and after 48, 96, 144 and 192 h and diluted (1 in 100) with iced water and frozen until analysis by the stability indicating HPLC assay.

Pseudo first-order rate constants for the decomposition of HC were calculated by linear regression of the natural logarithm of percentage HC remaining versus time plots. The experiments were carried out in triplicate and the mean value of the rate constant determined.

2.2.3. Preparation of formulations for ocular bioavailability assessment

Tritium labelling was accomplished by adding the appropriate amount of tritiated HC to a solution (1 mg/ml) of nontritiated HC in toluene. The solvent was evaporated to dryness under nitrogen flow. The resulting tritium-labelled HC was found to have a specific activity of approximately 0.8 MBq/mg.

A 1% w/v HC suspension was prepared by adding 0.1 M PBS to the ^{3}H-HC precipitate which had been ground with a glass rod and the suspension was then probe sonicated for 25 min to achieve saturation solubility and a particle size which complied with the specifications of the British Pharmacopoeia (1993) as determined by optical microscopy. The suspension was stored at room temperature for the duration of the in vivo bioavailability study (3 weeks).

A 1% w/v solution of complex was prepared by adding 0.1 M PBS containing 90 mM HP-β-CD to the ^{3}H-HC precipitate and then vortexing to facilitate complete dissolution. The solution formulation was stored at 4°C until required for use. Then, 30 min prior to use, the solution was placed in a 30°C water bath to re-establish equilibrium at this temperature.

The radiochemical purity of ^{3}H-HC in both formulations was determined at the beginning and upon completion of the in vivo study by thin-layer chromatography (Kiesel gel 60, F254) using two solvent systems consisting of dichloromethane and acetone having a ratio of either 2.5:1.5 or 4:1. In all cases, the radiochemical purity of ^{3}H-HC was greater than 96%. The two formulations were also tested for decomposition products upon completion of the in vivo study by the stability indicating HPLC assay. No decomposition products were detected in either formulation and in both cases the HC concentration was greater than 95% of that at the beginning of the study.

2.2.4. Ocular bioavailabilty study

First, 25 μl of either formulation was topically instilled into the lower cul-de-sac of each eye of a rabbit after gently pulling the lower lid away from the globe to form a pocket. The lower lid was then slowly returned and then held against the upper lid for 20 s following instillation. Both eyes were dosed within an interval of 1 min. It had previously been shown in a preliminary study ($n = 2$) that no radioactivity could be detected in

any tissues of the untreated contralateral eye following dosing with either suspension or solution to one eye. At predetermined time intervals post-instillation (10, 20, 30, 45, 60, 90, 120 and 180 min), serum and various ocular tissues were sampled following the procedure described by Chiang and Schoenwald (1986). Three rabbits were used for each formulation at each time point. The rabbits were not restrained for the time interval between dosing and sampling.

^{3}H-HC levels in the various tissues (digested) and fluids were estimated by scintillation counting and converted to total HC concentration present per gram of wet tissue or fluid. The mean HC concentration ($n = 6$) in each tissue or fluid was plotted as a function of time. The area under the concentration–time profile up to 3 h ($AUC_{0-3\,h}$) and the associated variance for each formulation was determined according to the method proposed by Yuan (1993). The $AUC_{0-3\,h}$ for suspension and solution for each tissue were then compared via a t-test with $p \leq 0.05$ taken as the level of significance. The mean residence time for drug disposition in the various tissues was calculated by dividing the area under the first moment profile ($AUMC_{0-3\,h}$) by the $AUC_{0-3\,h}$. The maximum concentration reached (C_{max}) and time to reach maximum concentration (t_{max}) were also estimated for each profile.

3. Results and discussion

3.1. Phase-solubility studies

The phase-solubility profile of HC in aqueous HP-β-CD solutions at 30°C is shown in Fig. 1. The solubility of HC in water was 0.972 mM. The apparent solubility of HC increased linearly ($r^2 = 0.99$) upon addition of HP-β-CD up to a concentration of 80 mM giving an A_L-type phase-solubility profile having an average slope of 0.382. Some curvature was noted at HP-β-CD concentrations greater than 80 mM possibly indicating an approach to the solubility limit of the HC/HP-β-CD complex. Similar phase-solubility profiles were obtained whether water or PBS was used as vehicle indicating that the buffer salts did not affect the complexation. Assuming the formation of only a 1:1 complex, the apparent association constant of the complex was estimated to be 0.636 mM^{-1}. The estimated association constant is slightly lower than that reported by Loftsson et al. (1994a) of 1.01 mM^{-1} for the complexation of HC with HP-β-CD having a molar substitution of 0.6 at an equilibration temperature of 23°C. However, this is in agreement with their proposal that the complexing ability of HP-β-CD decreases upon increasing the degree of substitution. Of note, is that a 1% w/v solution of HC (27.6 mM), equivalent to that used in clinical practice, can be prepared by the addition of a minimum of 69.7 mM of HP-β-CD.

3.2. Chemical stability studies

The decomposition of HC in 0.1 M PBS both in the absence and presence of various concentrations of HP-β-CD followed pseudo first-order kinetics as determined by the linearity of the natural logarithm of percentage HC remaining versus time plots. Using the association constant of 0.636 mM^{-1} determined in the phase-solubility study, the percentage of HC complexed at the various concentrations of HP-β-CD used can be calculated. Fig. 2. shows the estimated pseudo first-order rate constant for the hydrolysis plotted

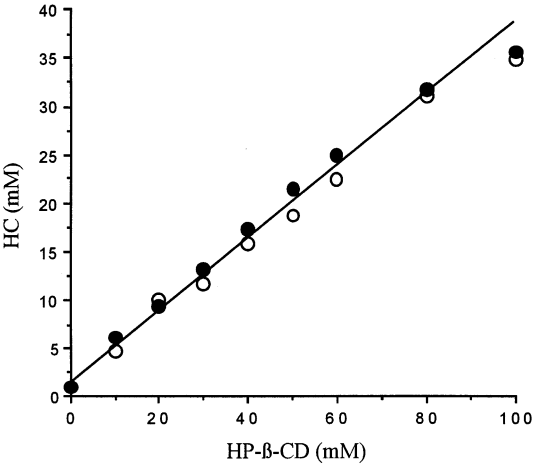

Fig. 1. Phase solubility profile of HC with HP-β-CD in PBS at 30°C ($n = 2$).

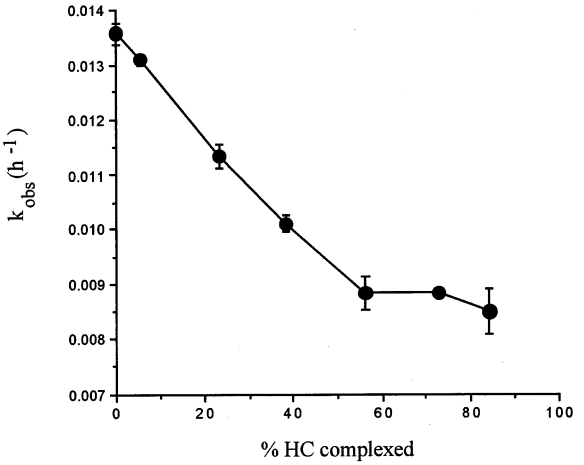

Fig. 2. Effect of complexation by HP-β-CD on the chemical stability of HC in aqueous solution at 60°C (data represent means $\pm$ S.E., $n = 3$).

against the percentage HC complexed. It can be noted that the rate constant for hydrolysis decreases with increasing percentage of HC complexed and asymptotically reaches a minimum value. Thus, the inclusion of the HC molecule within the hydrophobic cavity of the cyclodextrin affords some degree of protection against hydrolysis. The pseudo first-order rate constant of complexed HC can be estimated, assuming formation of 1:1 complex, from the expression (Loftsson and Ólafsdóttir, 1991):

$$\frac{k_0}{k_0 - k_{obs}} = \frac{k_0}{K_c(k_0 - k_c)[HP - \beta - CD]} + \frac{k_0}{k_0 - k_c} \quad (2)$$

where k_{obs} is the observed pseudo first-order rate constant of hydrolysis, k_0 is that for uncomplexed HC and k_c for complexed HC. K_c is the association constant for the HC/HP-β-CD complex. A plot of $k_0/(k_0 - k_{obs})$ versus 1/[HP-β-CD] yields a linear plot from which k_c can be estimated (Fig. 3). The pseudo first-order rate constant for hydrolysis of complexed HC was estimated to be 6.24×10^{-3} h^{-1} which compares to 13.60×10^{-3} h^{-1} for uncomplexed HC. The expression also enables prediction of the association constant which was estimated to be 0.676 mM^{-1} which compares favourably to 0.636 mM^{-1} determined by phase-solubility analysis.

The inclusion of disodium edetate further enhanced the chemical stability of HC both in the presence and absence of HP-β-CD, a result of the chelation of trace metal ions (Hansen and Bundgaard, 1980). The pseudo first-order rate constant for uncomplexed HC (k_0) was estimated to be 1.70×10^{-3} h^{-1} compared to 0.90×10^{-3} h^{-1} for complexed HC (k_c).

The stabilising effect of HP-β-CD on the hydrolysis of HC, both in the presence and absence of disodium edetate, is in contrast to that reported by Andersen and Bundgaard (1983) for the parent β-CD. They reported that inclusion complexation with the parent β-CD (in the presence of disodium edetate only) did not exhibit any significant stabilising effect on the decomposition of HC at 60°C in neutral or acidic aqueous solutions and the presence of β-CD accelerated the decomposition under alkaline conditions.

The mechanism for the complexation of HC with the parent β-CD has also been studied, however, with conflicting results (Uekama et al., 1982; Liu et al., 1990). Uekama et al. (1982) proposed that the inclusion of the HC A-ring within the β-CD was favoured in the complexation while Liu et al. (1990) proposed the inclusion of the five-member cyclopentane ring of the steroid molecule. If the major pathway for the

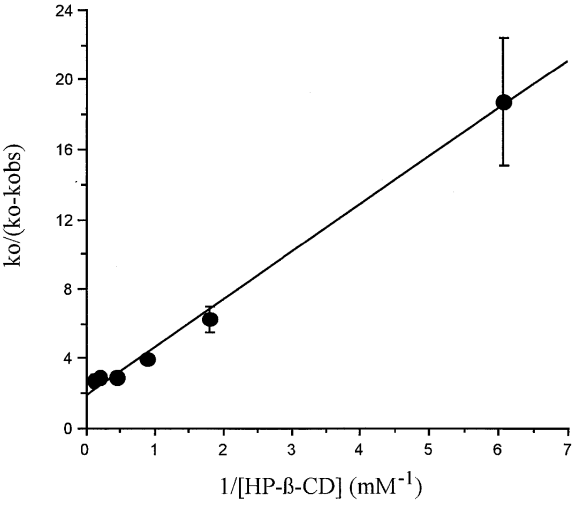

Fig. 3. Plot of decomposition rate data according to Eq. (2) (data represent means $\pm$ S.E., $n = 3$).

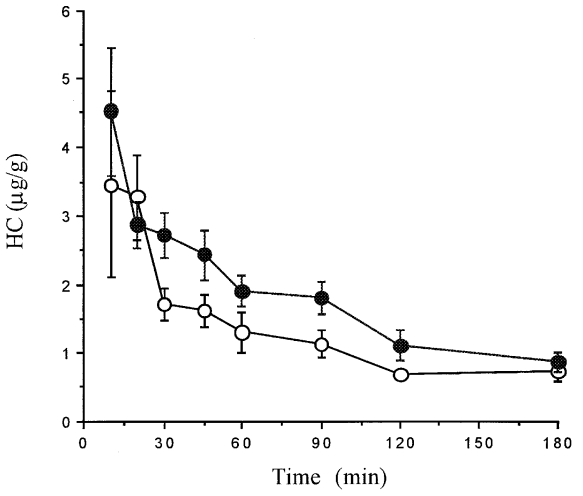

Fig. 4. Concentration of HC (μg/g) in the cornea following instillation of a 25-μl dose of HC suspension (●) or solution with HP-β-CD (○) (data represent means ± S.E., $n = 6$).

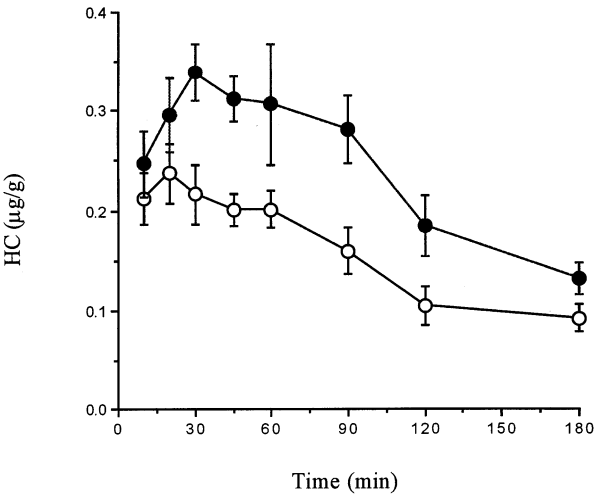

Fig. 5. Concentration of HC (μg/g) in the aqueous humour following instillation of a 25-μl dose of HC suspension (●) or solution with HP-β-CD (○) (data represent means ± S.E., $n = 6$).

decomposition of HC in neutral aqueous solutions is via the C_{17} side-chain (Hansen and Bundgaard, 1980) then the observations of Andersen and Bundgaard (1983) would also suggest the inclusion of the A-ring, as this would not be expected to influence reactions at the C_{17} side-chain. If the mechanism for the complexation of HC with the derivatised HP-β-CD is similar, then the stabilising effect observed in the present study may be a consequence of the extension of the hydrophobic cavity by the hydroxyalkylation of the primary and secondary hydroxyl groups of the cyclodextrin, thereby facilitating inclusion and consequently protection of the C_{17} side-chain. On the other hand, Das Gupta (1978) proposed that the decomposition of HC in aqueous solutions at elevated temperatures occurred via two mechanisms: attack on the C_{17} side-chain and also attack on the A-ring. This would imply that complexation would influence the decomposition of HC whatever the mechanism for complexation.

3.3. Ocular bioavailability study

Figs. 4 and 5 illustrate the concentration versus time profiles for HC determined in the cornea, often the target tissue for corticosteroids, and the aqueous humour, which is traditionally used as a measure of the ocular bioavailability of an ophthalmic formulation. Fig. 6 summarises the $AUC_{0-3\,h}$ determined for the HC concentration versus time profiles of the various ocular tissues and fluids including serum. In all cases, the bioavailability from the HC/HP-β-CD solution

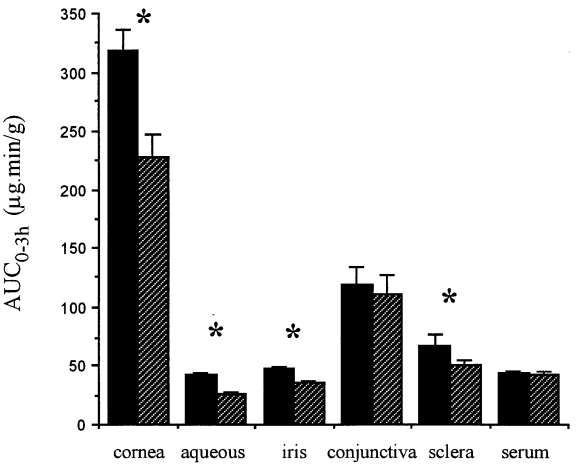

Fig. 6. Bioavailability (0–3 h) of HC (μg/min/g) in various ocular tissues following instillation of a 25-μl dose of HC suspension (filled square) or solution with HP-β-CD (hatched square). Values represent means ± S.E., $n = 6$. * Significant difference $p < 0.05$.

formulation was lower than that observed from the 1% suspension formulation with the difference being significant ($p < 0.05$) for the cornea, aqueous humour, sclera and iris ciliary body (Fig. 6).

This decrease in ocular bioavailability is in agreement with that previously reported for the ocular bioavailability of flurbiprofen when administered as an inclusion complex with β-CD (Masuda et al., 1985) and for a poorly water-soluble pilocarpine prodrug when administered as an inclusion complex with a sulphobutyl ether derivative of β-CD (Jarho et al., 1996). However, the results are in contrast to those reported for dexamethasone together with its acetate salt when complexed with HP-β-CD (Usayapant et al., 1991) and for pilocarpine when complexed with either HP-β-CD or α-CD (Freedman et al., 1993; Keipert et al., 1996). The results for pilocarpine with HP-β-CD could not, however, be confirmed by Järvinen et al. (1994).

A decrease in the relative bioavailability compared to suspension of 10% was reported for the flurbiprofen complex (Masuda et al., 1985) whereas a decrease of between 25 and 50% was reported for *O'*,*O*-dipropionyl-(1,4-xylylene)-bispilocarpate depending on the amount of cyclodextrin added to the system (Jarho et al., 1996). An increase in the relative bioavailability compared to suspension of 40 and 100% respectively for dexamethasone and dexamethasone acetate was reported by Usayapant et al. (1991). The aqueous solubility of dexamethasone and dexamethasone acetate is 63 and 10 μg/ml, respectively, with both having an association constant of 2.2 mM^{-1} with HP-β-CD, although the acetate is reported to also form a very weak secondary complex (Usayapant et al., 1991). The aqueous solubility of flurbiprofen is 66 μg/ml (Uekama et al., 1985) and has an association constant of 4.8 mM^{-1} with β-CD (Imai et al., 1984) while *O'*,*O*-dipropionyl-(1,4-xylylene)bispilocarpate, a prodrug of pilocarpine, has an aqueous solubility of 15 μg/ml and an association constant 9.8 mM^{-1} with the sulphobutyl ether derivative of β-CD (Jarho et al., 1996). These compare to 360 μg/ml and 0.636 mM^{-1}, respectively, for the aqueous solubility and the association constant of HC with HP-β-CD. Thus, there does not appear to be any relation between the effect of cyclodextrin on ocular bioavailability and the aqueous solubility of the drug or the association constant.

Since dissociation of a complex would be expected to be more rapid than the dissolution of particles (Szejtli, 1988), particularly under the rather static conditions expected in the cul-de-sac, then following drug absorption, the driving concentration of free drug for further absorption should be better maintained by the system containing the complex than by the suspension formulation. Consequently, a better ocular bioavailability would be expected from the cyclodextrin system if ocular absorption were dependent on maintenance of a driving concentration at the corneal surface and if the two formulations exhibited similar precorneal clearance profiles. However, it has been reported that suspensions have enhanced precorneal retentions compared to non-viscous solutions (Sieg and Robinson, 1975) which may have contributed to the enhanced bioavailability observed following suspension administration. Estimation of the mean residence times of each formulation in various ocular tissues in the present study did not suggest such an enhanced retention for the suspension formulation ($p > 0.05$) as did the similar times for maximum response noted for the two formulations in the various ocular tissues. However, these parameters would not be sensitive to small differences in precorneal retention times. The insignificant difference ($p > 0.05$) in $AUC_{0-3\,h}$ observed in the conjunctiva following solution and suspension administration also suggests that there is little difference in the precorneal clearance of the two formulations. Thus, it would appear that the increased ocular bioavailability following suspension administration is not a result of enhanced precorneal retention. Increased ocular bioavailability may also have occurred if the precorneal tissues were damaged by physical impact following instillation of the suspension. However, the

particle size of the suspension formulation complied to the specifications of the British Pharmacopoeia (1993) which applies to ophthalmic suspensions for use in humans and therefore damage to precorneal tissues of rabbits would not be expected. Further, no ocular discomfort was noted in rabbits receiving the suspension formulation suggesting that damage to precorneal tissues was minimal.

Encouraging results regarding the potential of cyclodextrins for increasing the ocular bioavailability of drugs have also been reported by many other investigators (Kanai et al., 1989; Loftsson et al., 1994b; Pate et al., 1995; Kistinsson et al., 1996). In these reports, however, the ability of cyclodextrins to increase aqueous solubility was exploited and the cyclodextrin formulations tested for ocular bioavailability were not compared to formulations containing an equivalent drug concentration. As such, the cyclodextrin formulations contained a higher drug concentration than the comparative formulation and therefore the effect of cyclodextrins on ocular drug absorption is difficult to ascertain from these results.

4. Conclusion

The complexation of poorly water-soluble drugs by cyclodextrins can often increase their water solubility. Consequently, cyclodextrins may be useful in the reformulation of ophthalmic suspensions as solutions thereby overcoming many of the problems associated with the formulation and use of ophthalmic suspensions. However, the potential of cyclodextrins as carriers for increasing the ocular bioavailability of poorly water-soluble compounds, at present, is unclear.

Acknowledgements

We are grateful to American Maize Products for donating the cyclodextrin and the Otago Medical and Pharmacy Schools for funding this research.

References

Andersen, F.M., Bundgaard, H., 1983. The influence of β-cyclodextrin on the stability of hydrocortisone in aqueous solutions. Arch. Pharm. Chem. Sci. Ed. 11, 61–66.

Apt, L., Henrick, A., Silverman, L.M., 1979. Patient compliance with the use of topical ophthalmic corticosteroid suspensions. Am. J. Ophthalmol. 87, 210–214.

British Pharmacopoeia, 1993. HMSO, London, pp. 736.

Chiang, C., Schoenwald, R.D., 1986. Ocular pharmacokinetic models of clonidine-^{3}H hydrochloride. J. Pharmacokinet. Biopharm. 14, 175–211.

Das Gupta, V., 1978. Effect of vehicles and other active ingredients on stability of hydrocortisone. J. Pharm. Sci. 67 (3), 299–302.

Freedman, K.A., Klein, J.W., Crosson, C.E., 1993. Beta-cyclodextrins enhance bioavailability of pilocarpine. Curr. Eye Res. 12 (7), 641–647.

Grant, D.J.W., Higuchi, T., 1990. Solubility behaviour of organic compounds. In: Weissberger, A. (Ed.), Techniques of Chemistry, vol. 21. Wiley, New York, pp. 440–473.

Hansen, J., Bundgaard, H., 1980. Studies on the stability of corticosteroids. V. The degradation pattern of hydrocortisone in aqueous solution. Int. J. Pharm. 6, 307–319.

Imai, T., Irie, T., Otagiri, M., Uekama, K., 1984. Comparitive study on inclusion complexations of antiinflammatory drug flurbiprofen with β-cyclodextrin and methylated β-cyclodextrins. J. Inc. Phenom. 2, 597–604.

Jarho, P., Järvinen, K., Urtti, A., Stella, V.J., Järvinen, T., 1996. Modified β-cyclodextrin (SBE7-β-CYD) with viscous vehicle improves the ocular delivery and toloerability of pilocarpine prodrug in rabbits. J. Pharm. Pharmacol. 48, 263–269.

Järvinen, K., Järvinen, T., Thompson, D.O., Stella, V.J., 1994. The effect of a modified β-cyclodextrin, SBE4-β-CD, on the aqueous stability and ocular absorption of pilocarpine. Curr. Eye Res. 13 (12), 897–905.

Kanai, A., Alba, R.M., Takano, T., Kobayashi, C., Nakajima, A., Kurihara, K., Yokayama, T., Fukami, M., 1989. The effect on the cornea of alpha-cyclodextrin vehicle for cyclosporin eye drops. Trans. Proc. 21 (1), 3150–3152.

Keipert, S., Fedder, J., Böhm, A., Hanke, B., 1996. Interactions between cyclodextrins and pilocarpine—as an example of a hydrophilic drug. Int. J. Pharm. 142, 153–162.

Kistinsson, J.K., Fridriksdóttir, H., Thórisdóttir, S., Sigurdardóttir, A.M., Stefánsson, E., Loftsson, T., 1996. Dexamethasone-cyclodextrin-polymer co-complexes in aqueous eye drops. Invest. Ophthal. Vis. Sci. 37 (6), 1199–1203.

Kwon, K., Diestelhorst, M., Süverkrüp, R., 1996. Dose uniformity problems of suspension eyedrops. Klinische Monatsblatter fur Augenheilkunde 209 (2-3), 144–149.

Leibowitz, H.M., Kupferman, A., 1975. Bioavailability and therapeutic effectiveness of topically administered corticosteroids. Trans. Am. Acad. Ophthalmol. Otol. 79, 78–88.

Liu, F., Kildsig, D.O., Mitra, A.K., 1990. Beta-cyclodextrin/steroid complexation: Effect of steroid structure on association equilibria. Pharm. Res. 7 (8), 869–873.

Loftsson, T., Ólafsdóttir, B.J., 1991. Cyclodextrin-accelerated degradation of β-lactam antibiotics in aqueous solutions. Int. J. Pharm. 67, R5–R7.

Loftsson, T., Fridriksdóttir, H., Sigurdardóttir, A.M., Ueda, H., 1994a. The effect of water-soluble polymers on drug-cyclodextrin complexation. Int. J. Pharm. 110, 169–177.

Loftsson, T., Fridriksdóttir, H., Thórisdóttir, S., Stefánsson, E., 1994b. The effect of hydroxypropyl methylcellulose on the release of dexamethasone from aqueous 2-hydroxypropyl-β-cyclodextrin formulations. Int. J. Pharm. 104, 181–184.

Masuda, K., Tohno, M., Konishi, Y., Kojima, H., Matsumura, M., 1985. Ocular penetration of flurbiprofen, an anti-inflammatory drug, in rabbits after topical instillation. Atarashii Ganka 2 (3), 441–443.

Musson, D.G., Bidgood, A.M., Olejnik, O., 1991. Comparative corneal penetration of prednisolone sodium phosphate and prednisolone acetate in NZW rabbits. J. Ocul. Pharmacol. 7, 175–182.

Musson, D.G., Bidgood, A.M., Olejnik, O., 1992. An in vitro comparison of the permeability of prednisolone, prednisolone sodium phosphate and prednisolone acetate across the NZW rabbit cornea. J. Ocul. Pharmacol. 8, 139–150.

Olejnik, O., Weisbecker, C.A., 1990. Ocular bioavailability of topical prednisolone preparations. Clin. Ther. 12, 2–11.

Pate, D.W., Järvinen, K., Urtti, A., Jarho, P., Järvinen, T., 1995. Ophthalmic arachidonylethanolamide decreases intraocular pressure in normotensive rabbits. Curr. Eye Res. 14 (9), 791–797.

Sieg, J.W., Robinson, J.R., 1975. Vehicle effects on ocular drug bioavailability. I. Evaluation of fluoromethalone. J. Pharm. Sci. 64 (6), 931–936.

Szejtli, J., 1988. Cyclodextrin technology. In: Davies, J.E.D. (Ed.), Topics in Inclusion Science. Kluwer, Dordrecht, pp. 336–340.

Uekama, K., Fujinaga, T., Hirayama, F., Otagiri, M., Yamasaki, M., 1982. Inclusion complexations of steroid hormones with cyclodextrins in water and in solid phase. Int. J. Pharm. 10, 1–15.

Uekama, K., Imai, T., Maeda, T., Irie, T., Hirayama, F., Otagiri, M., 1985. Improvement of dissolution and suppository release characteristics of flurbiprofen by inclusion complexation with heptakis(2,6-di-*O*-methyl)-β-cyclodextrin. J. Pharm. Sci. 74 (8), 841–845.

Usayapant, A., Karara, A.H., Narurkar, M.M., 1991. Effect of 2-hydroxypropyl-β-cyclodextrin on the ocular absorption of dexamethasone and dexamethasone acetate. Pharm. Res. 8, 1495–1499.

Yuan, J., 1993. Estimation of variance for AUC in animal studies. J. Pharm. Sci. 82 (7), 761–763.

.