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An ocular drug delivery system containing zinc diethyldithiocarbamate and HPβCD inclusion complex – corneal permeability, anti-cataract effects and mechanism studies

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Abstract

Our purpose was to study the formulation and anti-cataract effects of aqueous eye drops containing a high concentration of zinc diethyldithiocarbamate (Zn-DDC). A possible mechanism of the anti-cataract effect of Zn-DDC was also studied. Zn-DDC and hydroxypropyl-β-cyclodextrin (HPβCD) inclusion complex (Zn-DDC/HPβCD) was studied using the saturation solution method and characterized by differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (IR). Suitable formulations for Zn-DDC eye drops were established by means of in-vitro trans-corneal penetration experiments. The anti-cataract effect of the selected formulation was demonstrated by the delay in lens opacity development in hereditary shumuya cataract rats (SCRs). Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) was performed to study the effect of diethyldithiocarbamate (DDC), a metabolite of Zn-DDC, on the transcription inducible nitric oxide synthase (iNOS) mRNA in human lens epithelial cells (HLEC). In the presence of 22% (w/v) HPβCD, the solubility of Zn-DDC in water (0.2 mM) was increased almost 850 fold (to 17 mM), by the formation of Zn-DDC/HPβCD. The stoichiometry of Zn-DDC inclusion was 1:1. The Zn-DDC/HPβCD stability constant, K(s) (1:1) was estimated to be 3453 M^{-1}. The ophthalmic preparation containing 0.1% HPMC and 0.1% poloxamer 188 (P_{188}) exhibited better permeability than the others in-vitro, and significantly delayed cataract formation in SCRs compared with non-treated SCRs. DDC inhibits the transcription of iNOS mRNA in HLEC. We concluded that this drug delivery system increases both the drug solubility in aqueous eye drops and the permeability of drug through the rabbit cornea, by the formation of a drug–cyclodextrin inclusion complex and the addition of polymers and penetration enhancers. The preparation effectively prevented the development of cataracts in SCRs. DDC, the metabolite of Zn-DDC, may be one of the factors in the prevention of cataract formation because it inhibits the transcription of iNOS mRNA.

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Introduction

In the research on ophthalmic drug delivery systems, it is well recognized that the ease of drug penetration across a biological membrane generally depends on the lipophilicity of the drugs used. Usually, the drug penetration increases with an increase in drug lipophilicity until a maximum is reached. Diethyldithiocarbamate (DDC) exhibits beneficial effects in selenite-treated rat cataracts (Ito et al 1999, 2000), but is freely soluble in water and unstable in the presence of metal and oxide. Zn-DDC, a derivate of DDC, is used in this study as the prodrug of DDC (Terao & Ito 1997), because of its stability and lipophilic property (water-insoluble, soluble in benzene carbon disulfide and organic liquids). One problem with lipophilic drugs administered as aqueous eye drops is obtaining the desired drug concentration in any ophthalmic drug delivery system. HPβCD, a hydroxypropyl substituted β-cyclodextrin, is a cyclic oligosaccharide with a hydrophilic outer surface and a lipophilic cavity at its centre, which is capable of forming inclusion complexes with many lipophilic drugs by taking up the drug molecule, or part of it, into its lipophilic cavity (Muller & Brauns 1985; Kristinsson et al 1996). Some water-soluble polymers, such as hydroxypropylmethylcellulose (HPMC), are often used in aqueous eye drops to prolong the drug retention in the precorneal area by interaction with the glycoprotein at the corneal surface, thus reducing the drug loss by tear turnover and

increasing the bioavailability of the preparation. In addition, in aqueous solutions, these polymers are capable of increasing the solubilizing effect of cyclodextrins on lipophilic drugs by increasing the stability constants of the drug/cyclodextrin inclusions. In this study, we attempted to increase the concentration of a lipophilic drug in aqueous eye drops and its permeability through the cornea by the use of a cyclodextrin-based drug delivery system for eye drops, to obtain a better anti-cataract effect.

The mechanism of cataract formation has been widely studied in the past few decades, and a hypothesis accepted by most investigators is that radical oxygen species damage the lipid membrane and proteins, thereby disrupting the homeostasis of the lens, resulting in a loss in its transparency. Previous studies on selenite-induced rat cataract demonstrated that nitrogen oxide (NO) plays a role in the formation of cataract, and the development of cataracts is prevented by NO synthase (NOS) inhibitors (Ito et al 2001). This may be explained by the fact that, by inhibiting cytochrome oxidase, NO increases the production of oxygen radicals by mitochondria. These oxygen radicals may, in turn, lead to the generation of peroxynitrite (Brown 1999). These factors increase oxidative stress in the cells and, ultimately, cause lens opacity. Inducible NOS, one of the three isoforms of NOS, catalyses the oxidization of L-arginine and generates large amounts of NO (Moncada & Higgs 1993). It can be induced by agents such as cytokines, interferons and bacterial lipopolysaccharide (LPS) in a wide variety of cell types (Asano et al 1994; Griffith & Stuehr 1995; Hoffman et al 1995; Salzman et al 1998). Thus, we also carried out a study to determine whether the anti-cataract effect of DDC is related to its action on transcription inducible NOS (iNOS).

Materials and Methods

Materials

2-Hydroxypropyl-β-cyclodextrin (HPβCD) (average molar substitution, 0.6; average MW, 1380) and hydroxypropylmethylcellulose (HPMC-4000) were kindly donated by Ouchi Shinko Chemical Industries (Tokyo, Japan). Zinc diethyldithiocarbamate (Zn-DDC) and sodium N,N-diethyldithiocarbamate trihydrate (DDC) were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Benzalkonium chloride (BC) was provided by Kanto Chemical Co., Inc. (Tokyo, Japan). Brij35 was obtained from Serva Feinbiochemica Heidelberg (New York, USA). Sodium deoxycholate (DC) was obtained from Merck (Germany). Interferon-γ was purchased from PeproTech EC LTD (London, UK), and LPS purified from *Escherichia coli* was obtained from Sigma-Aldrich Co. (St Louis, MO). All other chemicals used, expect where indicated, were of the highest purity and were commercially available.

Animals

Six-week-old hereditary shumuya cataract rats (SCRs) (Tokyo Metropolitan Institute of Gerontology, Tokyo) and adult Japanese albino rabbits, 2.5–3.0 kg, were used in this study. They were housed under standard conditions (12-h fluorescent light–dark cycle, 25°C, room temperature) and allowed free access to a commercial diet (CE-2, Clea Japan Inc.) and water (rat pups were kept with their mother). All procedures were performed in accordance with the ARVO resolution on the use of animals in research.

Cell line and cell culture

The human lens epithelial cell (HLEC) line SRA 01/04 was kindly provided by Ibaraki Medical University. The cells were maintained in Dulbecco's modified Eagle medium (low glucose) (GIBCO Invitrogen Corporation, Tokyo, Japan) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, GIBCO Invitrogen Corporation, Tokyo, Japan) and 10 μg mL^{-1} gentamicin (GIBCO BRL Life Technologies, Japan) at 37°C in a humidified CO_2 incubator under 5% CO_2. The culture medium was changed every other day. Cell viability was assessed by trypan blue exclusion assay. Each treatment was carried out when the cells were 80% confluent, usually on the third day after seeding (4×10^4 cm^{-2}). The culture medium was changed just before each treatment, and any cultivation was conducted in duplicate.

HPLC assay of Zn-DDC

HPLC was used to determine the Zn-DDC concentrations. The HPLC apparatus consisted of a liquid chromatographic system (LC-10AD) (Shimadzu, Japan) equipped with a UV spectro-detector (SPD-10A) (Shimadzu, Japan). Analysis was performed on a Symmetry RP-18 column (2.0 mm × 50 mm, 3.5 μm) (Cica-Reagent, Japan) with a mobile phase composed of 0.1% trifluoroacetic acid (TFA) and 60% acetonitrile. Samples (2 μL) were eluted isocratically (flow rate, 0.35 mL min^{-1}) at room temperature. Zn-DDC was quantified at a wavelength of 215 nm and indometacin (5 μg mL^{-1}) was used as the internal standard.

Solubility studies

Solubility studies were carried out according to Higuchi et al (Higuchi & Lach 1954). Excess Zn-DDC (100 mg) was dispersed in 5 mL HPβCD solution (0–22%, w/v). The suspensions were vortexed for 10 min in 10-mL screw-capped test tubes, and then shaken in a water bath at 25°C for 72 h to establish equilibrium. Then, each sample was filtered through a cellulose nitrate membrane (0.45 μm; Sartorius Goettingen, Germany), and the filtrate subjected to HPLC assay as described above.

Preparation of the physical mixture of Zn-DDC and HPβCD

A physical mixture of Zn-DDC and HPβCD was prepared at a 1:40 weight ratio by simple dry mixing, adopting the geometric dilution method.

Preparation of the Zn-DDC/HPβCD inclusion complex

The lyophilized inclusion complex was prepared at a 1:40 weight ratio of drug to HPβCD. This involved dispersing 100 mg (0.276 mM) Zn-DDC in 40 mL 10% HPβCD solution (containing 1.104 mM HPβCD 1.523 g) at room temperature. After sonicating for 2 min in a water bath, the mixture was kept in the water bath (37 ± 1°C) under magnetic stirring until a clear solution was obtained. The solution was then filtered through a cellulose nitrate membrane (0.45 μm). The filtrate was cooled to -40°C for 30 min (PFR-1000; Eyela, Japan) and then freeze-dried at 7.6 Pa at -50°C for 8 h (FDU-1100; Eyela, Japan). This lyophilized powder was used for formation of the Zn-DDC/HPβCD inclusion complex and for preparation of eye drops.

Differential scanning calorimetry (DSC)

A Perkin-Elmer DSC-4 differential scanning calorimeter equipped with a computerized data station was used. The system was checked with indium (mp 156.6°C; ΔH_f 28.45 J g^{-1}; Perkin-Elmer, Norwalk, USA). Samples (8–10 mg; Zn-DDC, HPβCD, the physical mixture of Zn-DDC and HPβCD, or the Zn-DDC/HPβCD obtained above) were heated in sealed aluminium pans (Perkin-Elmer) at a scanning rate of 10°C min^{-1} under nitrogen flow (30 mL · min^{-1}), with a sealed empty aluminium pan as reference.

IR spectroscopy studies

The IR spectra of the freeze-dried inclusion complexes were recorded on an Infrared Fourier Spectrometer (FIS-55; Bruker, Switzerland) using the potassium bromide disk (2 mg sample in 200 mg KBr) method at a pressure of 400 kg cm^{-2} (model M; Carver, Menomonee Falls, USA). The scanning range was 450–4000 cm^{-1} and the resolution was 1 cm^{-1}.

Preparation of the Zn-DDC ophthalmic delivery system

According to the formulae shown in Table 1, 25 mg Zn-DDC, 1.0 g HPβCD, benzalkonium chloride or other components were dissolved in 10 mL 0.9% sodium chloride, followed by sonication in an ultrasonic bath for 10 min at 25 ± 1°C to produce a fine dispersion. The mixture was then stirred until a clear solution was obtained. Finally, each formulation was sterilized by passing it through a cellulose nitrate membrane (0.2 μm). The filtrate, used for Zn-DDC eye drops, was stored at room temperature.

In-vitro trans-corneal transit studies of Zn-DDC eye drops

The in-vitro trans-corneal transit studies were carried out as described by Iwata et al (1980). Male albino rabbits were killed by injection of a lethal dose of pentobarbital into the marginal ear vein. The eyes were removed and the corneas were carefully separated from other ocular tissues. The individual corneas were placed in a methacrylate cell designed for trans-corneal transit studies. The donor chamber with the exterior surface of the cornea was filled with Zn-DDC eye drop solutions. The acceptor chamber contained (in mM): 10 HEPES buffer (pH 7.4) with 136 NaCl, 5.3 KCl, 1 K_2HPO_4, 1.7 $CaCl_2$ and 5.5 glucose. The experiments were performed at 35°C and lasted for 3 h. Fifty microlitres of the sample solution was withdrawn from the acceptor chamber at indicated time intervals and replaced with the same volume of buffer. The Zn-DDC concentration of each sample was determined by HPLC as described above. The rates of hydration for each cornea were measured as described by Grass & Robinson (1988). Preliminary experiments showed that no significant changes in corneal thickness or corneal hydration were observed over the experiment time period (3 h).

Table 1 Formulations of eye drops, containing 0.25% Zn-DDC, 10% HPβCD and 0.005% benzalkonium chloride

	Polymers	Penetration enhancers
Eye Drops I	—	—
Eye Drops II	PVP (0.01%)	—
Eye Drops III	PVA (0.01%)	—
Eye Drops IV	HPMC (0.01%)	—
Eye Drops V	HPMC (0.01%)	P_{188} (0.01%)
Eye Drops VI	HPMC (0.01%)	Brij35 (0.05%)
Eye Drops VII	HPMC (0.01%)	DC (0.05%)

Administration of the Zn-DDC ophthalmic delivery system

SCR rats, 6 weeks old, were randomly divided into three groups. Two groups were treated with eye drops V with different Zn-DDC concentrations, 0.25% or 0.01%, respectively. The other group was treated with drug-free eye drops as a control. Also, another group of age-matched normal rats was treated with eye drops V. Eye drops (50 μL) for the treatment groups or drug-free eye drops for the controls were instilled into the left eye of each rat, and saline into the right eye, 4 times daily. The eyes were kept open for about 1 min to prevent the eye drops from overflowing. The treatments were continued until the rats were of 13 weeks of age.

Image analysis of cataract development

This was performed as described by Ito et al (1999). The pupils were dilated with 0.1% pivalephrine without anaesthesia 5 min before taking slit images with a photo slit lamp microscope and an anterior eye segment analysis system (EAS-1000; Nidek, Japan) equipped with a CCD camera. The area of opacity, in pixels, was analysed by a computer using image analysis software connected to EAS-1000 (Adamsons et al 1991; Kojima & Sasaki 1992). The total area of opacity of the lenses, expressed as pixels, was calculated by the following equation:

$$\text{Pixels within opacity} = \text{pixels within outline} - \text{pixels within transparent area} \quad (1)$$

The decrease of opacity was identified as follows:

$$\text{Decrease of opacity} = (\text{Area}_{\text{opacity in the right eye}} - \text{Area}_{\text{opacity in the left eye}})/\text{Area}_{\text{opacity in the right eye}} \quad (2)$$

Semiquantitative RT-PCR

Total cellular RNA was extracted and purified from HLEC with a RNeasy Mini Kit and RNase-Free DNase Set (QIAGEN K.K., Tokyo, Japan) according to the manufacturer's instructions. The RNA level was quantified by measuring the absorbance of a sample at 260 nm. The samples with a 260/280 nm ratio greater than 1.8 were subjected to RT-PCR using an RNA PCR Kit (TAKARA BIO. INC., Tokyo, Japan). To calibrate the amount of cDNA input, the mRNA level of constitutively expressed G3PDH was determined in parallel. The primers used in the study were: iNOS, sense: 5′-CCAGT GACAC AGGAT GACCT TCAG-3′, antisense: 5′-TGCCA TTGTT GGTGG AGTAA CG-3′; human G3PDH, sense: 5′-CATCA CCATC TTCCA GGAGC GAGA-3′, antisense: 5′-CCACC ACCCT GTTGC TGTAG CCA-3′). Cycling conditions were: 35 cycles for iNOS and 20 cycles for GAPDH at 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min for amplifying 603-bp and 752-bp products, respectively. Then, the product extension was performed at 72°C for 10 min. PCR-amplified samples were run on 1.5% agarose gels and visualized using ethidium bromide, and then photographed under UV light.

Statistical analysis

All values were presented as the means ± s.e. in a total of 3–5 experiments. In the in-vitro transit studies, Student's *t*-test was carried out to determine the significance of Zn-DDC concentrations in acceptor chamber containing different formulations versus eye drops I. One-way analysis of variance was performed to determine the statistical significance of the decrease of opacity in SCRs treated with eye drops V in two different doses versus drug-free eye drops.

Results and Discussion

Characterization of Zn-DDC/HPβCD inclusion complex

Solubility studies

The phase solubility diagrams of Zn-DDC in HPβCD solutions (0–160 mM) at 25°C were plotted according to Higuchi & Kristiansen (1970) (Figure 1). The stoichiometry and solution stability of the inclusion complex can be determined from the slope and intercept of the phase solubility plot of Zn-DDC solubility as a function of HPβCD concentration. The phase solubility diagram obtained under our experimental conditions is type A_L,

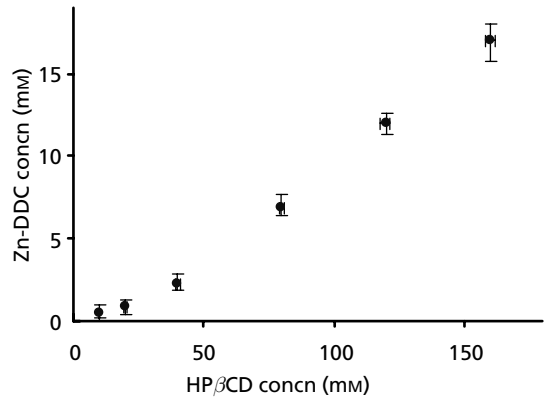

Figure 1 Phase-solubility diagram of Zn-DDC with HPβCD in de-ionized water at 25 ± 1°C. Each point represents the mean ± s.e. of 3 experiments.

implying a linear increase in solubility with unchanged stoichiometry. This complex is highly water-soluble at room temperature, since no precipitation was observed even at HPβCD concentrations as high as 0.35 M. The solubility of Zn-DDC increased 850 fold at the highest HPβCD concentration used. The stability constant K' for the Zn-DDC/HPβCD complex was calculated to be 3453 M^{-1}, using equation 3:

$$K' = \text{slope}/S_0(1 - \text{slope}) \quad (3)$$

where S_0 is the solubility of Zn-DDC in the absence of HPβCD. Experimentally, K' values between 200 and 5000 $L \cdot mol^{-1}$ are considered as the most suitable for improving the bioavailability of poorly water-soluble drugs (Castillo et al 1999).

DSC analysis

The DSC isotherms of free Zn-DDC, HPβCD, their physical mixture (1:40 w/w) as well as the Zn-DDC/HPβCD inclusion complex (1:40 w/w) are shown in Figure 2A. A sharp endothermic peak at 178°C, corresponding to drug melting, characterizes the DSC isotherm of free Zn-DDC, while the isotherm of HPβCD shows no endothermic peak from 60 to 300°C. Also, the physical mixture of Zn-DDC and HPβCD exhibits an endothermic peak at 178°C suggesting the presence of free Zn-DDC although the Zn-DDC/HPβCD complex showed no endothermic peak for free Zn-DDC. The disappearance of the endothermic peak for Zn-DDC was taken as an indication of the formation of an inclusion complex.

IR study

Although the DSC test was positive for Zn-DDC complexation with HPβCD, IR was used to further confirm the inclusion of Zn-DDC within the HPβCD cavity. As shown in Figure 2B, in the spectrum of the inclusion complex (d), the clear methyl peak at 2966 cm^{-1} in the spectrum of Zn-DDC (a) and physical mixture (c) was not

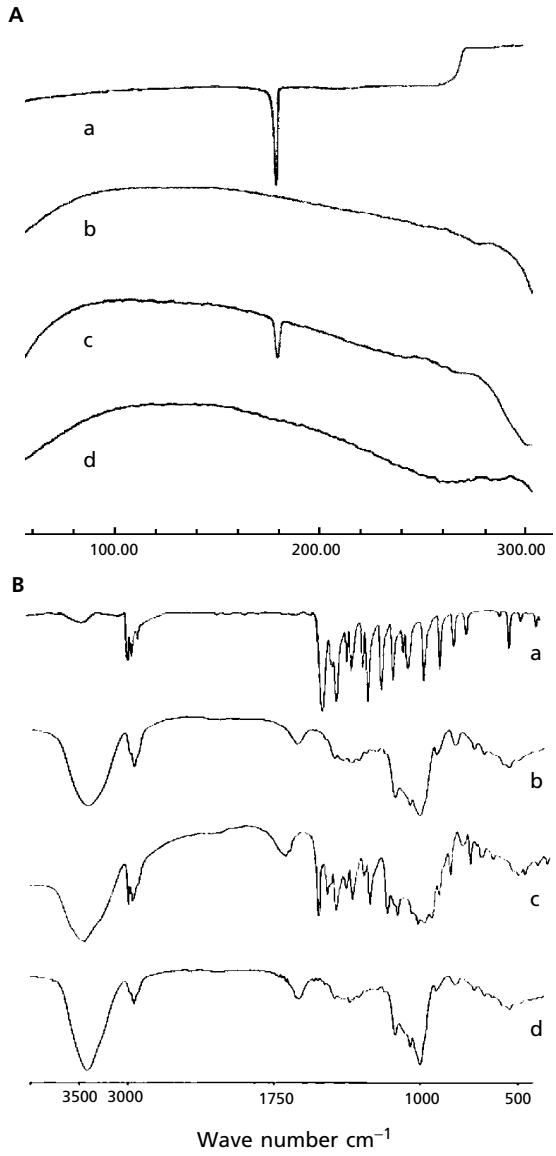

Figure 2 DSC profiles (A) and FT-IR spectra (B) for Zn-DDC crystalline powder (a), HPβCD powder (b), physical mixture prepared at a 1:40 weight ratio by simple dry mixing of Zn-DDC and HPβCD (c), and inclusion complex powder obtained by freeze-drying the inclusion solution at 7.6 Pa and -50°C for 8 h (d).

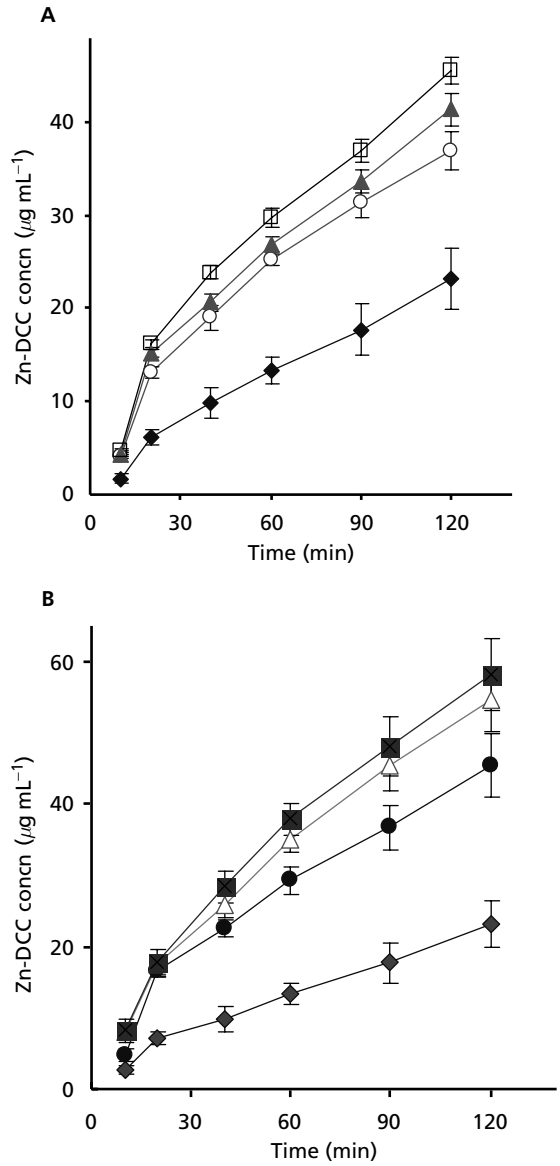

Figure 3 Zn-DDC concentration in the acceptor chamber at indicated times in the in-vitro trans-corneal studies. The donor chamber was filled with: eye drops I (♦); eye drops II (○); eye drops III (▲); eye drops IV (□); eye drops V (■); eye drops VI (△); eye drops VII (●). Each point represents the mean ± s.e. of 3 experiments. *$P < 0.05$ vs eye drops I by Student's t-test.

present in the spectrum of the inclusion complex (d), which demonstrates the entrapment of the methyl group in Zn-DDC in the lipophilic cavity of the cyclodextrin.

Effect of formulations on in-vitro transcorneal permeability

The Zn-DDC concentrations in the receptor were plotted as a function of time. The slopes of the linear part of each curve were determined by linear regression. Then, the slopes of one formulation were averaged and used to calculate the permeability coefficient as described by Grass & Robinson (1988). The permeability coefficients for eye drops I to VII were 4.5 ± 1.9, 6.7 ± 2.7, 8.6 ± 4.1, 11.2 ± 3.9, 15.4 ± 4.4, 14.6 ± 4.1, $10.9 \pm 3.9 \times 10^{-6}$ cm · s^{-1}, respectively.

The penetration of Zn-DDC through the cornea is increased by the addition of macromolecular polymers, especially HPMC, as shown in Figure 3A. It is believed that the water-soluble polymers interact with drug/CD complexes in the same way as polymers interact with micelles forming drug–CD–polymer aggregates (i.e. complex

formation between several drug/CD complexes and a polymer chain) (Loukas et al 1997). Such macromolecular clusters would be more readily adsorbed to biological membranes than the individual drug/CD complexes, and the polymers adhering to the outer surface of the cornea may promote the release of drug molecules from the cyclodextrin inclusion complex into the solution leading to a high concentration of drug molecules at the corneal surface, resulting in enhanced permeability (Loftsson et al 1994; Castillo et al 1999).

At the same concentration, P_{188} is better at enhancing the corneal permeability in-vitro than Brij 35 and DC, as shown in Figure 3B. These penetration enhancers used in these studies are also commonly used in transdermal drug delivery systems. The reason for the enhanced permeability is related to their molecular structure. P_{188} and DC, following insertion into the lipid bilayer, change the fluidity of the cell membrane, which facilitates drug transport through the cornea barrier. In the case of Brij, it mainly increases protein and lipid production in the corium and removes these newly synthesized compounds, thus producing an incompact structure on the membrane, and enhancing drug transport.

In-vivo, viscosity is another important factor that contributes to the resistance of eye drops on the corneal surface. However, in the in-vitro transcorneal studies, because of the sink conditions, the effect of viscosity cannot be detected as it is in-vivo that the retention time at the cornea surface is prolonged, thus increasing the amount of drug in the receptor with time. So, formulation V, with the highest permeability coefficient, may not be the best formulation when applied in-vivo. More research into this needs to be performed in the near future.

Effect of Zn-DDC delivery systems on cataract development

The Shumiya cataract rat (SCR) is a hereditary cataract model in which lens opacity appears spontaneously in the nuclear and perinuclear portions of the lens at 11–12 weeks of age (Okano et al 1993). In this study, SCRs were treated with eye drop V (containing 0.25% or 0.10% Zn-DDC) or drug-free eye drops from the age of 6 weeks onwards. In non-treated eyes (right), lens opacity appeared first in the perinuclear portions of the lens at 10 weeks of age, while in treated eyes (left), lens opacity was delayed for 8 to 15 days. In age-matched normal rats, no lens opacity was observed (both right and left) from the beginning to the end of the study.

As shown in Figure 4, the reduction in lens opacity in SCR rats treated with eye drops V was observed from 49 to 80 days. The biggest difference in opacity between drug-treated eyes (left) and saline-treated counterparts was observed at nine weeks of age. After 13 weeks, almost all the lenses had undergone opacification, including those treated with Zn-DDC eye drops. Hence, these Zn-DDC-containing eye drops can not completely stop the development of cataracts, but prolong the onset and development of cataracts. Because of the ageing of the world's population, the pressure on cataract surgery is larger and larger, so the identification and trial of preventive drugs such as Zn-DDC are a real challenge and have real meaning.

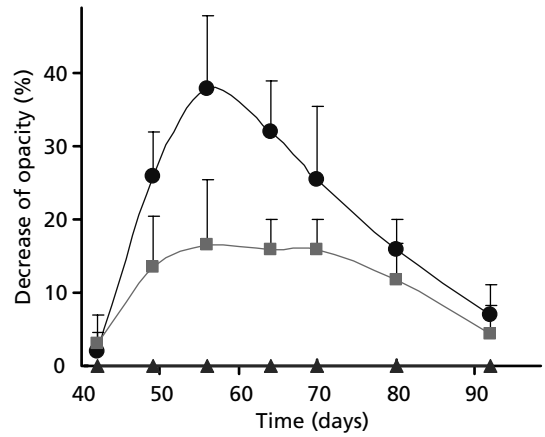

Figure 4 Decrease of lenses' opacity in SCRs treated with: eye drops V (containing 0.25% Zn-DDC (●); 0.10% Zn-DDC (■) or drug-free eye drops (eye drops V without Zn-DDC (▲)). Lens opacity was examined using software supplied with the EAS-1000. Each value represents the mean ± s.d. of 4∼6 rats. $P < 0.01$.

Inhibitory effect of DDC on iNOS expression

As shown in Figure 5, incubation with LPS or IFN-γ or DDC alone failed to induce iNOS mRNA, while the combination of LPS + IFN-γ resulted in the expression of iNOS. However, the induction of iNOS by LPS and IFN-γ was attenuated in HLEC that had been loaded with DDC. The synthesis of iNOS is mainly regulated at the transcriptional level, and the promoter region of the iNOS gene from different species has been reported to contain binding sites for several transcription factors, including nuclear factor-κB (NF-κB) sites located both in the enhancer and basal promoter (Xie 1994). Dithiocarbamates are well-known NF-κB inhibitors, and DDC, a dithiocarbamate analogue, had a concentration-dependent biphasic effect in inhibiting NF-κB activation in cerebral endothelial cells (Kim et al 2000). So, it appears that the inhibitory effect on NO production may be due to the inhibition of NF-κB. Further studies are needed to clarify the anti-cataract mechanism.

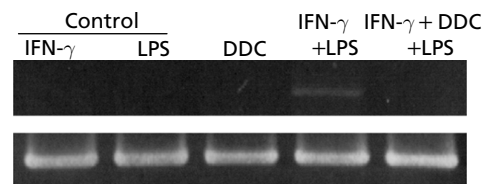

Figure 5 Inhibitory effect of DDC on iNOS mRNA transcription stimulated with IFN-γ and LPS. Semiquantitative RT-PCR analysis of the iNOS mRNA level in HLEC preincubated with or without IFN-γ (1000 U) in the presence or absence of DDC (1 mM) for 3 h and followed by a 24-h co-incubation with LPS (100 ng mL^{-1}). Representative results of one of the three independent experiments are shown.

Conclusions

This cyclodextrin-based drug delivery system increases both the drug solubility in aqueous eye drops and the drug permeability through the rabbit cornea, following the formation of a drug–cyclodextrin inclusion complex and the addition of polymers and penetration enhancers. The preparation effectively prevented the development of cataracts in SCRs. The metabolite of Zn-DDC, DDC, inhibits the transcription of iNOS gene, resulting in reduced NO production and prevention of the formation of cataracts. This is the basis of the anti-cataract mechanism.

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