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Hydrocortisone delivery to healthy and inflamed eyes using a micellar polysorbate 80 solution or albumin nanoparticles

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Abstract

Hydrocortisone was loaded onto albumin nanoparticles by sorption. Two systems were tested, one comprised of a 0.03% saturated drug solution, the other of a 0.2% micellised drug solution. In both cases 45–70% of the originally available drug was bound to the carrier surface. The systems were further tested by measuring the in vitro transport of radiolabelled hydrocortisone through porcine cornea. Nanoparticles led to sustained drug transport through the cornea. The distribution of both 0.2% hydrocortisone preparations (nanoparticles and solution) was then evaluated under in vivo conditions in healthy and inflamed eyes of rabbits. In all tissues the level of drug was higher in the inflamed than in the healthy eye due to increased cell permeability as a result of inflammatory processes. The application of nanoparticles led to lower hydrocortisone tissue concentrations than the reference solution due to the strong binding of hydrocortisone onto the particle system and the resulting slow release. An exception occurred with the reference solution in the conjunctiva, as less drug was found in the inflamed than in the normal tissue, since enhanced lacrimation led to increased drug drainage. In contrast, the corresponding nanoparticle preparation was more efficiently retained at the inflamed than at the normal conjunctiva. Consequently, in the inflamed eye, hydrocortisone-loaded nanoparticles enabled targeting to the precorneal area away from the inner segments of the eye.

Key words: Hydrocortisone; Albumin nanoparticles; Drug delivery system; Ophthalmology; In vitro diffusion; Porcine cornea; Distribution study; Normal eye; Inflamed eye

1. Introduction

Hydrocortisone represents a frequently used steroid in ophthalmology. It is employed for the treatment of allergic ocular diseases, most non-pyogenic inflammations in the anterior (e.g., disorders of lids, conjunctivitis, keratitis, uveitis, anterior scleritis) or posterior (e.g., retinitis, optic neuritis posterior scleritis, etc.) segments of the eye, and for the reduction of scarring from certain types of severe injury.

Two prominent ocular side effects of steroids

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in the eye are the development of elevated intraocular pressure, eventually leading to glaucoma, and the irreversible formation of cataracts which restrict their medical use to severe disorders in the eye. Therefore, for some precorneal diseases such as inflammation or allergic processes, drug targeting away from the inner segments of the eye to external tissues might be advantageous.

Therapeutically used steroids in ophthalmology can be administered systemically or topically. Topically, they have been applied in the form of solutions (use of soluble salt modifications), suspensions (Leibowitz and Kupferman, 1974; Sieg and Robinson, 1975; Schoenwald and Stewart, 1980) or ointments, e.g., petrolatum-based preparations (Sieg and Robinson, 1975). In order to increase the therapeutic effect, periocular administration, such as subconjunctival (McCarthy, 1965; Hyndiuk, 1969; Wine et al., 1969; Jan and Srivastava, 1978), anterior sub-tenon, posterior sub-tenon, or intravitreal (Graham and Peyman, 1974) injections have also been suggested.

The use of steroid-containing gels, such as carbopol, polyethylene glycol, and methylcellulose (Schoenwald and Boltralik, 1979; Kupferman et al., 1981; Kassem et al., 1986), bioadhesive polymers, e.g., cross-linked acrylic acid (Hui and Robinson, 1985), pre-soaked contact lenses (Hull et al., 1974), or soluble and insoluble inserts releasing the steroids over a controlled period of time (Dohlman et al., 1972; Heller and Baker, 1973; Lerman et al., 1973; Allansmith et al., 1975; Keller et al., 1976) has been described with the therapeutic goal of minimising adverse reactions and of obtaining sustained constant levels of medication.

Owing to the unsatisfactory bioavailabilities achieved with other ophthalmic drug delivery systems, the potential of biocompatible particulate carrier systems for the eye has been evaluated during recent years (Gurny et al., 1987; Kreuter, 1987). The active principle (drug) either may be added during particle formation, resulting in the incorporation or adsorption of the drug, or the drug might be adsorbed onto the surface of preformed particle systems.

Drug encapsulation efficiency, drug release properties, interaction and affinity of the carrier system with the corneal and conjunctival tissues, precorneal residence time, stability of the particulate system in the conjunctival sack, and tear fluid and distribution of drug within the carrier all have an influence on the properties and the efficacy of the developed particulate systems. A high incorporation ratio, together with a considerable amount of free drug in order to attain an adequate initial drug level, as well as sustained drug release during the residence time of the carrier systems are required for ophthalmic use (Li et al., 1986).

To date, some studies with steroid-containing liposomes have been carried out (Singh and Mezei, 1983; Tanigushi et al., 1987). The aforementioned investigations suggested the possible superiority of liposomes in ocular drug delivery. Studies with progesterone-loaded polyhexylcyanoacrylate nanoparticles, however, led to lower corticoid concentrations in the tissues of the eye. These relatively low concentrations were due to the high percentage (99%) of binding of this drug and to the resulting very slow release (Li et al., 1986).

The goal of the present work was to create a nanoparticle-drug system that allows enough drug to be adsorbed at its surface, while at the same time ensuring that sufficient drug is free in solution. Additionally, differences in the distribution behaviour of hydrocortisone in the eye under normal and pathological conditions, as well as differences in drug distribution within the various tissue compartments between nanoparticle-bound hydrocortisone and reference were evaluated. Since Diepold et al. (1989) showed that radiolabelled nanoparticles were retained for more than 6 h on inflamed conjunctival tissue, nanoparticulate dosage forms may hold promise as inflammation-targeted drug delivery systems for the eye.

2. Materials and methods

2.1. Nanoparticle preparation

500 mg albumin (bovine serum albumin, fraction V, Sigma Chemicals, St. Louis, U.S.A.) was

dissolved in 40 ml of distilled water (DAB 10). About 60 ml of absolute ethanol (Merck, Darmstadt, Germany) was added, until the onset of protein desolvation was visually observed as a rise in turbidity. The system was then cross-linked by the addition of 0.1 ml of 25% glutaraldehyde (Fluka Chemicals, Buchs, Switzerland) and agitated for 1 h with a magnetic stirrer (IKA, Heidelberg, Germany). Unreacted glutaraldehyde was detroyed by adding 0.5 ml of an aqueous 12% sodium metabisulfite solution (Merck, Darmstadt, Germany).

After 3–4 h, excess ethanol was evaporated under vacuum (Rotavapor, Büchi, Switzerland). The preparation obtained was then further purified by column gel filtration (Sephacryl G 1000, Pharmacia, Sweden).

After the addition of 100 mg glucose (Merck, Darmstadt, Germany), the resulting particle suspension was lyophilised for about 16 h (Lyovac, Heraeus, Hanau, Germany).

2.2. Size determination

Photon correlation spectroscopy was carried out at a 90° angle (BI-90, Brookhaven Instruments Corp., Holtsville, U.S.A.). Nanoparticle suspensions were diluted with demineralised water (HPLC grade, filtered through 0.2 μm cellulose nitrate filters, Sartorius, Karlsruhe, Germany), at a ratio of 1:100. In order to ensure homogeneity, samples were subjected to ultrasonication prior to use.

2.3. Hydrocortisone loading

Hydrocortisone (Synopharm GmbH, Barsbüttel, Germany) (0.0298 g or 0.2 g dissolved in 5.0 ml polysorbate 80, Tween® 80, Atlas Chemie, Essen, Germany) was dissolved in phosphate buffer pH 7.0 to a volume of 100.0 ml. Blank samples without drug were also prepared.

Dissolution in the surfactant was carried out under moderate heating followed by dilution with buffer solution (phosphate buffer pH 7.0, 80 mOsm: sodium dihydrogen phosphate dihydrate, 2.07 g/l, disodium hydrogen phosphate, 2.83 g/l; Merck, Darmstadt, Germany).

After adjusting the buffer volume to a final concentration of 0.2% hydrocortisone, the loading capacity was determined by increasing the nanoparticle concentrations from 5 to 60 mg/ml. In order to avoid drug adsorption, the membrane filters were saturated with drug prior to use. Furthermore, the reference solution was also filtered to ensure that concentration differences were not due to hydrocortisone-containing polysorbate 80 micelles entrapped in the filter membrane.

The solutions obtained were divided into two parts. One part served as the reference value, while to the other half various amounts of nanoparticles (5, 10, 20, 40 and 60 mg/ml) were added. The system then was allowed to equilibrate for 24 h, while being stirred continuously with a magnetic stirrer (IKA, Heidelberg, Germany).

After drug saturation of the filter membranes (cellulose nitrate 0.01 μm, Sartorius GmbH, Göttingen, Germany), the samples were subjected to ultrafiltration (Ultrasart cell 10 SM166 66, Sartorius GmbH, Göttingen, Germany). The filtrates were assayed spectrophotometrically against the corresponding empty nanoparticle control at 241 nm to determine the hydrocortisone content. The abovementioned reference solutions and the corresponding blanks were treated in the same way. The amount of drug in the filtrate then was calculated.

2.4. Preparation of the radioactive hydrocortisone samples

Radioactive hydrocortisone preparations were produced by dissolving 500 mg of non-radioactive material in absolute ethanol. 250 μCi [1,2,6,7-^{3}H]hydrocortisone, radiochemical purity > 98.55% (Amersham Buchler GmbH, Braunschweig, Germany) dissolved in 250 μl toluene/ethanol (9:1) was added.

The solvent was then evaporated under a gentle nitrogen stream. The hydrocortisone preparation finally obtained had an activity of about 0.5 μCi/mg and was stored at −20°C until use.

2.5. Hydrocortisone diffusion study through isolated porcine cornea

Corneas were obtained from pigs (Deutsches Hausschwein, Hybrid) of age between 6 and 8 months. Within 1 h after death of the animal, the eyeballs were excised at the slaughterhouse and transported to the laboratory in ice-cooled GBR buffer pH 7.65 (O'Brien and Edelhauser, 1977: sodium chloride, 12.400 g/l; potassium chloride, 0.716 g/l; monobasic sodium phosphate monohydrate, 0.206 g/l; sodium bicarbonate, 4.908 g/l; calcium chloride dihydrate, 0.230 g/l; magnesium chloride hexahydrate, 0.318 g/l; glucose, 1.800 g/l (all Merck, Darmstadt, Germany); oxidised glutathione, 0.180 g/l (Fluka Chemicals, Germany).

Six corneas with a 3 mm ring of sclera left were dissected, while the tissue was kept moistened with GBR buffer. The corneal tissue was then mounted between two half-cells of a side-by-side perfusion apparatus (Fig. 1).

GBR buffer of 32–34°C was added into the donor (4.0 ml) and receiver (7.0 ml) compartments and the system was kept at 32–34°C, corresponding to the corneal temperature. The system was gassed with carbogen (95% O_2, 5% CO_2) to maintain a constant pH of 7.65 throughout the experiment. After 15 min of equilibration, the GBR buffer in the donor compartment was withdrawn and substituted with GBR buffer containing the radiolabelled hydrocortisone preparation.

Samples of 1.0 ml were taken from the receiver side at 20 min intervals over a period of 280 min. The sample volume was immediately replaced with an equal amount of GBR buffer at 32–34°C. The samples were transferred into 6 ml polyethylene vials, mixed with 5 ml of Ready Solv® (Beckman Instruments, München, Germany) and counted against a known standard concentration of labelled hydrocortisone (Beckman Scintillation Counter LS, Beckman Instruments, München, Germany).

After the experiment was terminated, the corneas were removed from the chambers and then rinsed with GBR buffer in order to wash off adhering radioactivity. Subsequently, the sclera was carefully trimmed. The corneas were put into 20 ml glass vials and were digested at 60°C by adding 2 ml of BTS 450® tissue solubiliser (Beckman Instruments, München, Germany). Three drops of H_2O_2 were added to decolorise the dissolved tissue samples, two to three drops of acetic acid being used for acidification. Then the samples were mixed with 10 ml of Ready Organic® (Beckman Instruments, München, Germany).

Prior to counting, the samples were allowed to stand for at least 24 h in the dark in order to minimise chemiluminescence.

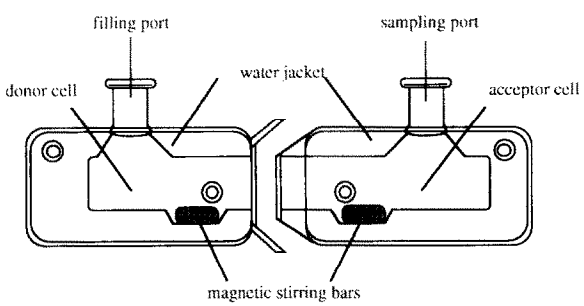

Fig. 1. Design of the perfusion cell.

2.6. Hydrocortisone ocular distribution study

Male New Zealand albino rabbits (2.5–3.0 kg, Elevage Scientifique des Dombes, Romans, France) were chosen. For each time point 7–10 animals were used. 24 h before administering the radioactive test sample, an inflammation was artificially induced in one eye of the rabbit, while the other eye served as a control. For this purpose, 25 μl of proxymetacain HCl 0.55% (Ophthetic® Allergan, Karlsruhe, Germany) were applied to anaesthetise the eye, and then 50 μl of clove oil (Caelo, Hilden, Germany) were instilled. During the following 24 h, a severe inflammation was induced in the precorneal area. On the following day, 25 μl of the respective radioactive hydrocortisone preparation (40000 dpm) was instilled into the lower cul-de-sac of both eyes of the rabbit.

After predetermined time intervals (5, 15, 30, 60, 90, 120, 180 and 240 min), the animals were killed by rapidly injecting 1.0 ml T 61® (Hoechst AG, Hoechst, Germany) into the marginal ear

vein. The eyes were rinsed with normal saline, blotted dry in order to remove adhering drug, and then were quickly dissected into the following components: cornea, conjunctiva, iris-ciliary body, vitreous humour, and aqueous humour.

The samples were then placed into preweighed scintillation vials and weighed. After the addition of 1–2 ml of tissue solubilizer they were digested at 60°C until completely dissolved. Three drops of H_2O_2 30%, three to four drops of acetic acid, and 10 ml Ready Organic® scintillation cocktail (Beckman Instruments, München, Germany) were added.

Before counting, the samples were stored for at least 24 h in the dark. The results were expressed as μg hydrocortisone/g tissue (wet weight).

3. Results and discussion

3.1. Albumin nanoparticles

Albumin nanoparticles were produced by employing a slightly modified desolvation process (Knop et al., 1974; Marty and Oppenheim 1977). Nanoparticles were yielded as a white fluffy powder which was readily resuspended in buffer solution. The resulting particle sizes were between 100 and 300 nm.

3.2. Hydrocortisone loading

A 0.03% hydrocortisone solution was used for determination of the loading parameters onto nanoparticles. Since the lowest concentration in commercially available solutions (Optef®, Upjohn, U.S.A.) contains 0.2% hydrocortisone solubilised with tyloxapol, a micellar solution containing 0.2% hydrocortisone and 5% polysorbate 80 was also tested in addition. Polysorbate 80 was chosen as surfactant as it has been reported to be relatively non-toxic and non-irritating to the rabbit eye up to a concentation of 10% (Hazleton, 1952).

Various concentrations of particles ranging from 5 to 60 mg/ml were examined to optimize loading conditions. Table 1 depicts the results obtained by the loading experiments. With the 0.03% hydrocortisone solution, a maximal amount of about 42–52% of the initial drug concentration of the lipophilic drug (hydrocortisone solubility in aqueous media 0.00298 mg/ml, corresponding to approx. 0.03%) was adsorbed onto the particles. An adsorption plateau resulted above a particle concentratin of 20 mg/ml. In order to increase the amount of soluble hydrocortisone to a therapeutically effective concentration, the drug was solubilised by the addition of polysorbate 80, forming a 0.2% micellar solution. Absolute loading ranged between 2 and 18 mg/100 mg carrier and had its optimum at a particle concentration of 5 mg/ml. Again an adsorption plateau was attained at particle concentrations above 20 mg/ml, with a maximal percentual hydrocortisone adsorption of about 70% of the initial cocentration. Consequently, solubilisation of hydrocortisone with polysorbate 80 substantially enhanced drug loading onto the albumin binding sites compared to the saturated solution.

Table 1
Relative and absolute hydrocortisone loading onto nanoparticles

	Particle concentration (mg/ml)				
	5	10	20	40	60
Relative hydrocortisone loading 0.03%	16.34 (±6.31)	26.21 (±11.31)	45.36 (±11.54)	52.12 (±7.33)	47.13 (±6.84)
Absolute hydrocortisone loading 0.03%	0.18 (±0.07)	0.29 (±0.12)	0.45 (±0.12)	0.57 (±0.08)	0.52 (±0.07)
Relative hydrocortisone loading 0.2% in micelles	46.22 (±5.90)	52.67 (±11.00)	70.55 (±4.30)	66.77 (±5.54)	66.35 (±1.81)
Absolute hydrocortisone loading 0.2% in micelles	18.48 (±2.36)	10.53 (±2.20)	7.05 (±0.43)	3.34 (±0.27)	2.21 (±0.06)

Number of experiments $n = 4$. Numbers in parentheses represent the standard deviation.

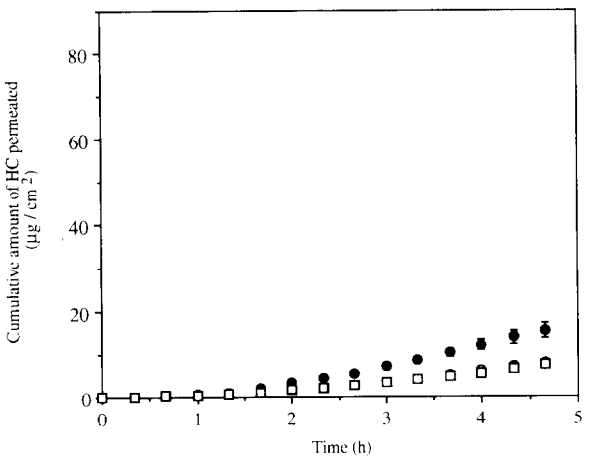

Fig. 2. Diffusion of 0.0298% hydrocortisone through isolated pig cornea. (○) 0.03% + 20 mg/ml nanoparticles; (□) 0.03% + 40 mg/ml nanoparticles; (●) 0.03% reference.

3.3. *Hydrocortisone diffusion through isolated porcine cornea*

The efficacy of drug delivery of hydrocortisone-loaded nanoparticles was assessed by measuring the transcorneal flux through isolated porcine cornea. Porcine eye was chosen since it has been reported to possess structural similarities to the human eye: except for the absence of a distinct Bowman's membrane and the proportions of polyhedral and squamous cells, porcine cornea seemed to have most characteristics in common with the human cornea (McTigue, 1967). The rabbit cornea used so far in most diffusion studies suffers from the disadvantage of being dissimilar to human cornea (Ehlers, 1970; Greiner et al., 1985). Compared to rabbit cornea the flux of a number of drugs was retarded in the porcine cornea, probably due to its increased corneal thickness. Camber et al. (1987) validated porcine cornea as a membrane for in vitro drug diffusion studies and showed that in a perfusion apparatus only small alterations in thickness, hydration, and fine structure resulted for at least 4 h.

Fig. 2 depicts the results of the present experiments obtained with a 0.03% hydrocortisone solution compared to nanoparticle-containing preparations. The flux of the hydrocortisone reference was determined to be 4.65 (μg cm^{-2} h^{-1}). The permeability of the cornea for hydrocortisone was 4.334 10^{-6} cm/s which was in good correlation with the permeability data for hydrocortisone (4.70 10^{-6} cm/s) obtained by Camber (1985). Also, the lag time of 86.73 min correlated well with their results.

Addition of nanoparticles at two different concentrations (20 and 40 mg/ml) to the system led to a significant reduction ($p = 0.01$; Student's t-test) of the flux to 2.52 μg and 2.31 μg cm^{-2} h^{-1}, respectively, as well as to a significant increase ($p = 0.01$; Student's t-test) in lag time (94.33 and 92.66 min, respectively). The hydrocortisone content of the perfused corneas was decreased to a significant extent after particle addition ($p = 0.01$; Student's t-test).

As mentioned above, polysorbate 80 seems to be relatively non-irritating to the rabbit eye (Hazleton, 1952), although non-ionic surfactants like polysorbate 80 used at concentrations above the critical micelle concentration have been shown to promote penetration (Marsh and Maurice, 1971; Tanigushi et al., 1988a,b). This was also observable with hydrocortisone in the pig cornea.

Fig. 3 shows the results of the present study. The 0.2% hydrocortisone solution containing 5%

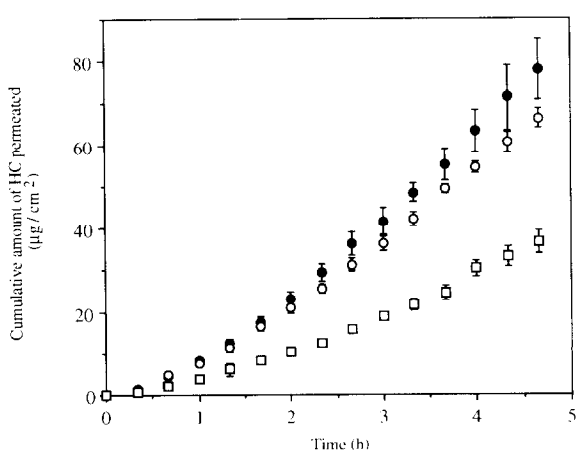

Fig. 3. Diffusion of 0.2% hydrocortisone solubilised in 5% polysorbate 80 (Tween® 80) through isolated pig cornea. (○) Tween® 80, 0.02% h.c. + 20 mg/ml nanoparticles; (□) Tween® 80, 0.02% h.c. + 40 mg/ml nanoparticles; (●) Tween® 80, 0.02% reference.

polysorbate 80 yielded a flux of 21.03 $\mu g\ cm^{-2}\ h^{-1}$ which was significantly higher than that of the 0.03% saturated hydrocortisone solution (4.65 $\mu g\ cm^{-2}\ h^{-1}$). The lag time was also significantly decreased from 86.73 min (0.03% solution) to 67.25 min (polysorbate 80-containing 0.2% solution).

The addition of nanoparticles (20 and 40 mg/ml) led to a decrease in hydrocortisone flux through the cornea to 17.69 and 10.03 $\mu g\ cm^{-2}\ h^{-1}$, respectively. The lag time with the nanoparticle-containing preparations (55.60 and 64.20 min, respectively), however, was not significantly decreased compared to the reference (67.25 min). An explanation for these effects appears to be the high percentual loading of hydrocortisone onto the particles. Only the remaining free portion of hydrocortisone was able to penetrate the tissue rapidly. Consequently, hydrocortisone-loaded particles are likely to possess sustained release properties in the precorneal area of the eye. Enhanced drug transport through the cornea by the particles was not observed.

3.4. In vivo hydrocortisone distribution in rabbit eyes

The distribution of hydrocortisone loaded onto nanoparticles in comparison to an aqueous micellar reference solution was examined under normal and inflamed conditions. A possible irritating effect of polysorbate 80 during the study was excluded by visual inspection of three rabbits which received daily a 25 μl dose of a 5% polysorbate 80-containing solution in the right cul-de-sac. No enhanced tear flow, redness, swelling or at least visible irritation were observed. These findings correlated with the results of Hazleton (1952).

The concentration-time profiles are plotted in Fig. 4–8. Each time point represents the average of 7–10 individual measurements. The pharmacokinetic parameters are summarized in Table 2.

For both preparations (NAN, REF) the concentration of hydrocortisone in the segments of the inflamed eye such as cornea, aqueous humour, vitreous humour and iris-ciliary body was found to be significantly greater than in the healthy eye.

The inflammation of the tissue after administration of clove oil significantly reduced the barrier offered to the transcorneal diffusion of drugs like hydrocortisone. The corneal epithelium which was particularly exposed to the external insult was partly destroyed which led to a considerable increase in permeability. Other studies have also demonstrated that a general inflammation of the globe, as a result of infection or irritation, caused a rise in corneal permeability, e.g., of prednisolone phosphate (Kupferman and Leibowitz, 1974) or dexamethasone phosphate (Cox et al., 1972; Hamard et al., 1975; Leibowitz et al., 1978).

However, after inflammation, drug delivery towards the eye compartments was relatively greater with the nanoparticle preparation than with the solution. This suggests that the inflammatory processes seem to lead to a greater extent of nanoparticle-mediated hydrocortisone tissue adhesion or even tissue uptake than after administration of a reference solution.

Interestingly, the measured AUC of the drug concentration vs time profile and the drug concentrastion enhancement due to inflammation in

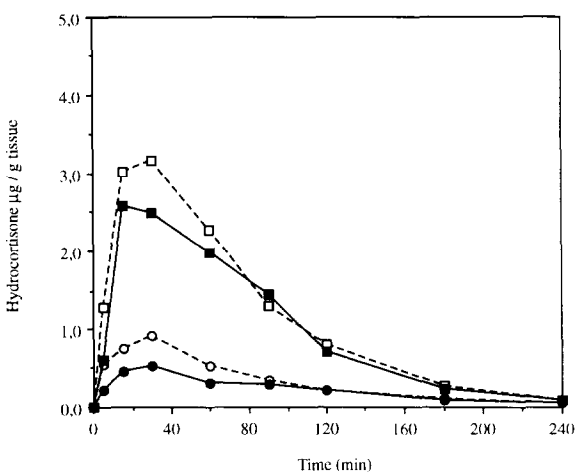

Fig. 4. Hydrocortisone distribution in aqueous humour. (●) Nanoparticle preparation, normal eye; (■) nanoparticle preparation, inflamed eye; (○) reference solution, normal eye; (□) reference solution, inflamed eye. Standard deviation error bars have been omitted for the sake of clarity.

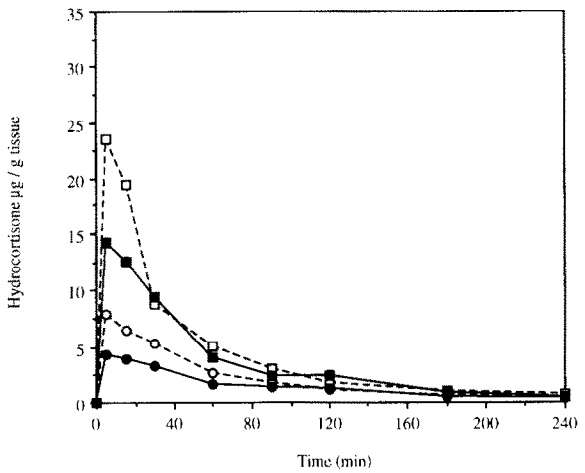

Fig. 5. Hydrocortisone distribution in cornea. (●) Nanoparticle preparation, normal eye; (■) nanoparticle preparation, inflamed eye; (○) reference solution, normal eye; (□) reference solution, inflamed eye. Standard deviation error bars have been omitted for the sake of clarity.

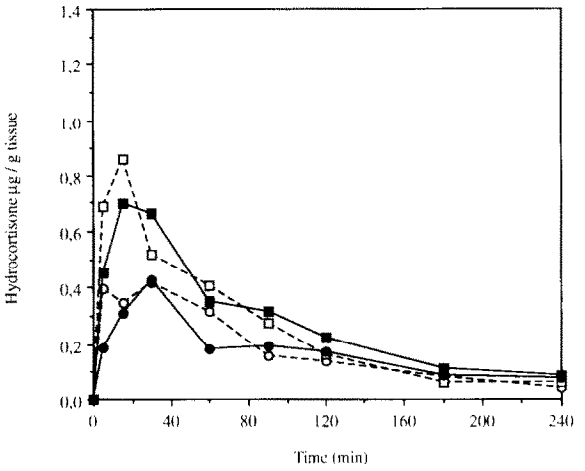

Fig. 7. Hydrocortisone distribution in iris-ciliary body. (●) Nanoparticle preparation, normal eye; (■) nanoparticle preparation, inflamed eye; (○) reference solution, normal eye; (□) reference solution, inflamed eye. Standard deviation error bars have been omitted for the sake of clarity.

the conjunctiva showed different behaviour. With the reference solution, the AUC was found to be much lower in the inflamed than in the healthy conjunctiva, which might be due to excessive tear flow and drainage occurring in the irritated eye. After administration of the nanoparticle preparation, the AUC in the inflamed tissue was marginally greater than that calculated for the healthy eye. Consequently, the particulate system adhered to a slightly larger extent than the reference solution in the inflamed eye. Thus, the drainage of drug bound to particles did not ap-

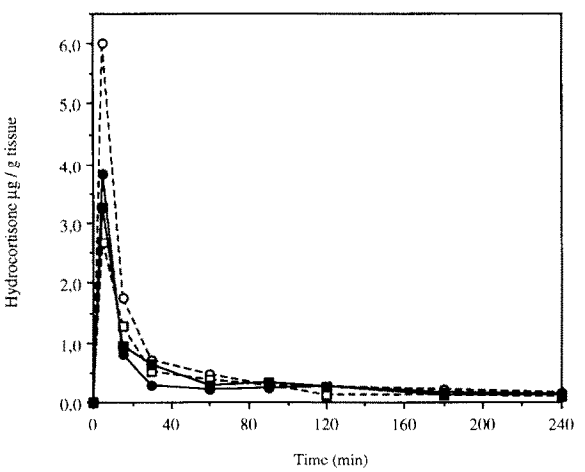

Fig. 6. Hydrocortisone distribution in conjunctiva. (●) Nanoparticle preparation, normal eye; (■) nanoparticle preparation, inflamed eye; (○) reference solution, normal eye; (□) reference solution, inflamed eye. Standard deviation error bars have been omitted for the sake of clarity.

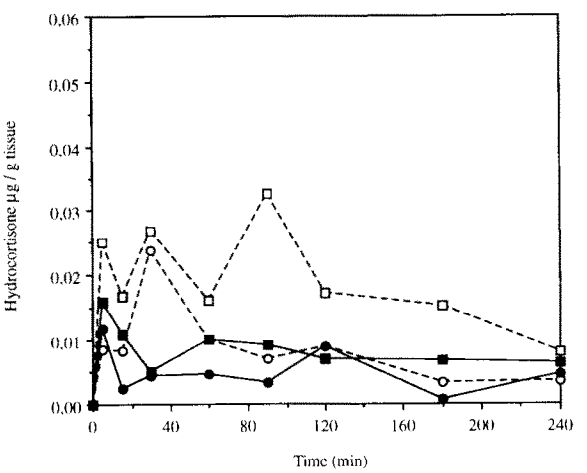

Fig. 8. Hydrocortisone distribution in vitreous humour. (●) Nanoparticle preparation, normal eye; (■) nanoparticle preparation, inflamed eye; (○) reference solution, normal eye; (□) reference solution, inflamed eye. Standard deviation error bars have been omitted for the sake of clarity.

pear to occur as quickly as observed after instillation of an aqueous reference. This might in part be due to a partial blockade of the nasolacrimal duct caused by swollen conjunctival tissue (Primbs et al., 1961). Moreover, the particles are believed to be coated with fibrous inflammatory products which might have increased adhesion to the cell surface (Diepold et al., 1989).

The amount of hydrocortisone in the healthy eyes determined as the drug concentration vs time profile (AUC) decreased in the following rank order: cornea > conjunctiva > aqueous humour > iris-ciliary body > vitreous humour. As shown in Table 3, after administration of the reference solution the major part of hydrocortisone accumulated in the cornea. The values for the conjunctiva and the aqueous humour only reached 27.25 and 15.38%, respectively, of the corneal concentration, whereas the AUC calculated for the iris-ciliary body which was in the range of 43 μg min g^{-1} (8.6%). The hydrocortisone level in the vitreous humour, however, was only marginal (0.38%).

With hydrocortisone-loaded nanoparticles, the

Table 2
Summary of the pharmacological data obtained in the hydrocortisone distribution study

Tissue/preparation	AUC (μg min g^{-1})	c_{max} (μg/g)	t_{max} (min)	k_{el} (min^{-1})	$t_{1/2}$ (min)
Aqueous humour					
Normal NAN	54.76 (22.40)	0.52 (0.28)	30	0.011	60.93
Normal REF	78.35 (32.87) [a]	0.91 (0.46)	30	0.012	56.08
Inflamed NAN	245.76 (128.71)	2.58 (1.40)	15	0.015	44.20
Inflamed REF	282.68 (128.21)	3.16 (1.58)	30	0.017	40.26
Vitreous humour					
Normal NAN	1.13 (0.65)	0.11 (0.01)	5	–	–
Normal REF	1.97 (1.17) [a]	0.02 (0.01)	30	–	–
Inflamed NAN	1.94 (0.92)	0.01 (0.01)	5	–	–
Inflamed REF	4.50 (3.22) [a]	0.03 (0.01)	90	–	–
Cornea					
Normal NAN	350.85 (154.61)	4.36 (1.84)	5	0.011	63.93
Normal REF	509.47 (220.43) [a]	7.91 (3.46)	5	0.012	56.49
Inflamed NAN	854.43 (471.11)	14.29 (5.96)	5	0.014	47.35
Inflamed REF	1036.28 (23.56)	23.56 (9.71)	5	0.014	47.31
Iris-ciliary body					
Normal NAN	42.62 (17.82)	0.42 (0.18)	30	0.007	94.31
Normal REF	43.89 (22.20)	0.41 (0.21)	30	0.010	65.23
Inflamed NAN	67.87 (30.24)	0.67 (0.24)	15	0.009	71.79
Inflamed REF	61.91 (22.29)	0.86 (0.37)	15	0.012	56.12
Conjunctiva					
Normal NAN	85.90 (44.95)	3.83 (1.39)	5	0.103 [b]	6.73
				0.005 [c]	142.29
Normal REF	138.84 (78.26) [a]	6.01 (3.04)	5	0.082 [b]	8.37
				0.003 [c]	190.88
Inflamed NAN	91.88 (48.68)	3.26 (2.36)	5	0.062 [b]	11.07
				0.006 [c]	107.38
Inflamed REF	85.17 (37.31)	2.67 (1.37)	5	0.064 [b]	10.73
				0.001 [c]	502.44

Numbers in parentheses represent the standard deviation, $n = 7–10$. – no values calculated.
[a] Significant difference ($p < 0.05$) between solution and nanoparticles.
[b] k_{el} values calculated between 5 and 30 min.
[c] k_{el} values calculated between 120 and 280 min.

Table 3
Relative distribution of hydrocortisone in the eye

	AUC REF	(%)	AUC NAN	(%)
Normal eye				
Cornea	509.47	100.00	350.85	100.00
Conjunctiva	138.84	27.25	85.90	24.48
Aqueous humour	78.35	15.38	54.76	15.60
Iris-ciliary body	43.89	8.61	42.62	12.14
Vitreous body	1.97	0.38	1.13	0.32
Inflamed eye				
Cornea	1036.28	100.00	854.43	100.00
Conjunctiva	85.17	8.21	91.88	10.75
Aqueous humour	282.68	27.27	245.76	28.75
Iris-ciliary body	61.91	5.97	67.87	7.94
Vitreous body	4.50	0.43	1.94	0.23

AUC of the corneal tissue again was highest, although the overall drug level was lower than that obtained with the hydrocortisone reference. The relative concentrations for the conjunctiva were 24.48%, respectively, and that of the aqueous humour 15.60% of the corneal concentration. The percentual AUC determined for the iris-ciliary body was 12.14%, while the value calculated for the vitreous humour again was negligible (0.32%).

In the inflamed eye (Table 3) the following rank order of AUC was determined: cornea > aqueous humour > conjunctiva > iris-ciliary body > vitreous humour. The irritation of the cornea as result of the instillation of clove oil led to AUC values that were 2–4-fold higher than those evaluated under normal conditions. The permeation rate of drug through the cornea into the aqueous humour increased drastically. The AUC of hydrocortisone calculated for the conjunctiva, however, decreased by about 50% (from 138.84 to 85.17 μg min g^{-1}, Table 3) compared to the percentual AUC value in the healthy eye. Compared to the cornea (100%), 8.21% was found in the conjunctiva, 27.27% in the aqueous humour, 5.97% in the iris-ciliary body and 0.43% in the vitreous humour. For the nanoparticle preparation, the corresponding values were cornea 100%, conjunctiva 10.75%, aqueous humour 28.75%, iris-ciliary body 7.94%, and vitreous humour 0.23%. Again the percentual distribution between the nanoparticle and the reference preparation was similar.

4. Conclusion

Hydrocortisone, a lipophilic drug, can be bound effectively onto albumin nanoparticles. In vitro drug diffusion studies through isolated porcine cornea suggested the occurrence of sustained release of the particle-bound hydrocortisone.

Under in vivo conditions, hydrocortisone-loaded nanoparticles exhibited neither higher AUC values nor prolonged release patterns with respect to an aqueous reference. It seemed that the amount of drug bound was not released sufficiently rapidly. Particle drainage occurred faster than drug desorption.

However, in contrast to the solution, in the inflamed conjunctival tissue the application of nanoparticles did not reduce the hydrocortisone concentrations. Moreover, the drug level was even slightly increased. Thus, in the inflamed eye hydrocortisone-loaded nanoparticles enabled the targeting of the hydrocortisone away from the inner compartments of the eye where it could lead to adverse reactions such as enhanced intraocular pressure and led to relatively higher concentrations in the precorneal aea, the desired site of action of this drug.

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