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A γ-scintigraphic evaluation of microparticulate ophthalmic delivery systems: liposomes and nanoparticles

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Summary

The ocular deposition of microparticulate systems in the size range 100–300 nm was investigated in rabbit using the technique of γ-scintigraphy. Small unilamellar liposomes (SUV) and reverse-phase vesicles (REV) were prepared with positively or negatively charged or neutral surfaces. They were characterised and radiolabelled prior to use. The in vivo behaviour of these colloids was compared to that of ^{111}In-labelled nanoparticles prepared from poly(butyl-2-cyanoacrylate). The present studies illustrate that a positive surface charge significantly increased the residence of liposomes in the precorneal region; retention improving with a smaller mean particle size. The nanoparticles had similar drainage rates to the positively charged REV, but had a slower clearance than negatively charged or neutral SUVs.

Introduction

Sustained therapy for the treatment of chronic eye diseases such as glaucoma and Keratitis sicca has been a therapeutic goal for many years. Ointments or inserts have been the main choices of formulators to achieve prolonged treatment, although both systems have disadvantages. Ointments cause blurring of vision and sticking of the lids which exacerbates problems of visual acuity. Inserts although an elegant formulation strategy providing controlled release may not be tolerated by some patients. The ideal formulation would be dispensed like an eye-drop, but have a prolonged residence in the eye.

The potential biocompatible, polymeric microparticulates as delivery systems have been investigated by many groups of researchers. The colloidal systems investigated have included liposomes (Schaeffer and Krohn, 1982; Fitzgerald, 1986) and nanoparticles based on polycyanoacrylates (Wood et al., 1985). Polycyanoacrylates have been used as surgical adhesives for a number of years. Nanoparticles are formed via an anionic polymerization mechanism in the presence of steric stabilizers such as dextrans and non-ionic surfactants (Douglas et al., 1985). Owing to the biocompatibility and adsorptive nature of nanoparticles

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their use in topical ocular drug delivery is creating interest. Wood et al. (1985) using nanoparticles formed from ^{14}C-labelled poly(hexyl-2-cyanoacrylate), studied their distribution in ocular tissues after topical application. The nanoparticles were rapidly removed from the precorneal region, but cleared at a slower rate than labelled solutions. It was suggested that their retention was due to binding of nanoparticles to the mucin network or the buoyancy in the precorneal fluid (density ≈ 1 g/ml; Kreuter, 1983).

In a recent study (Fitzgerald et al., 1987), the drainage of mutilamellar liposomes formed from egg lecithin or dipalmitoyl choline were investigated in the rabbit using the technique of γ-scintigraphy. The behaviour of the formulations was followed by inclusion of indium-111-labelled chelate and the charge altered by incorporation of stearylamine or dicetylphosphate. Increase in surface charge significantly affected liposomal drainage rate, whereas an increase in size restricted drainage from the inner canthal regions.

In the present study the behaviour of these particles in vivo has been compared with liposomes of a similar size range. SUVs were prepared from dipalmitoylphosphatidylcholine (DPPC), cholesterol and either stearylamine or dicetyl phosphate to confer positive and negative surface charges respectively. These liposomes ranged in size from 100 to 130 nm. Larger liposomes, REVs, were prepared with a positive surface charge and an average size of 330 nm.

Materials and Methods

(DL)-Dipalmitoylphosphatidylcholine (DL-DPPC), stearylamine, dicetyl phoshate, nitrilotriaacetic acid, 8-hydroxyquinoline, Dowex 1-X8 resin and Stannous chloride [anhydrous] were all obtained from Sigma Laboratories. Other materials were obtained from the following suppliers: cholesterol (BDH, Poole), egg lecithin (Lucas Meyer), indium-111 chloride (spec. act. < 0.1 Ci/ml), Amersham International, Amersham, U.K., technetium-99m pertechnetate–Elumatic 111, [CIS (UK)]., London, U.K., butyl-2-cyanoacrylate monomer, (Sichel-Wereke), Hanover, F.R.G., dextran 70 (mol. wt, 70,300) (Sigma).

Preparation of formulations

(i) Small unilamellar vesicles (SUV) Multilamellar vesicles were firstly prepared from synthetic phospholipid (DPPC), cholesterol and either stearylamine or dicetyl phosphate in the molar ratio 5 : 2 : 1, respectively, to produce charged vesicles, or from DPPC and cholesterol alone (molar ratio 9 : 1). The lipid films were resuspended in 3 ml of sodium chloride (0.9% w/v). The suspensions were then sonicated under nitrogen using a probe-type sonicator in a water bath at 50 °C. One-minute cycles of sonication were followed by 1 min cooling periods for a total of 5 periods of 10–15 min. Sonication was terminated when the suspension appeared to have a pale blue opalescence. The suspension was then annealed for 1 h at 50 °C. The contaminating titanium was removed by centrifuging at 2000 *g* for 10 min and removing the supernatant containing the SUVs. All liposomal formulations were stored at 4 ° C to prevent degradation and fusion occurring.

(ii) Reverse phase vesicles (REV) were prepared by the method of reverse-phase evaporation (Szoka and Papahadjopoulos, 1978). A positively charged preparation of liposomes was prepared from egg lecithin and stearylamine in the molar ratio of 4 : 1, respectively.

(iii) Nanoparticles of a mean size of approximately 250 nm were prepared using a method developed by Douglas et al. (1985). 50 mg Dextran 70 was dissolved in 9.9 ml 0.01 M HCl (pH 2.25) in a 25 ml stoppered flask and 0.1 ml butyl-2-cyanoacrylate monomer was then added dropwise. The mixture was stirred until polymerization was complete (2–3 h depending on the chain length of the polymer; Couvreur et al., 1984). The resulting opaque, white nanoparticle suspension was then neutralized with 0.1 M NaOH and filtered through a 1 μm filter (Whatman) prior to characterization.

Particle size determination

The liposomal and nanoparticle formulations were sized by photon correlation spectroscopy using a helium neon laser (wavelength = 441.6 nm) and a measuring angle of 90 °. The instrument was calibrated using latex particles in the appropriate size range to determine optimum sampling times

and corresponding experimental durations to obtain statistically accurate data. Ten determinations were made for each sample and the mean Stokes diameter and polydispersity indices calculated using the Cumulants technique.

Radiolabelling techniques

The preformed SUVs were labelled with technetium-99m pertechnetate using a stannous chloride complex (Farr et al., 1983). A dilute solution of stannous chloride (2.5 mg/ml) was prepared in de-oxygenated distilled water and the pH reduced to 1.0 by the addition of 0.1 N HCl.

1.0 ml of each liposome suspension was shaken with 0.1–0.5 ml stannous chloride solution followed by the addition of 0.5 ml technetium-99m pertechnetate (75–100 MBq/0.5 ml) in sterile saline. The mixture was vortexed briefly and allowed to incubate at room temperature for 1 h. Excess activity was removed by an ion-exchange method. Pre-washed Dowex Ag 1-X8 resin was added to each suspension and shaken. The suspension was decanted from the resin which was then washed with two further fractions of saline. The washing fractions were combined and centrifuged at 2000 g for 10 min to remove any tin colloid that may have formed. The liposomes in the supernatant were then centrifuged at 140,000 g (4°C) for 1 h to concentrate the sample. Ten μl aliquots were removed and assayed at each stage of the labelling process to determine the efficiency of labelling (> 70%). The integrity of the [^{99m}Tc]stannous chloride label was also measured by equilibrium dialysis (Richardson et al., 1978). Negligible loss of label occurred over a 24-h period. Positively charged REVs were labelled with [^{111}In]oxime (10–20 MBq/0.5 ml) using the method of Hwang (1982). A labelling efficiency of > 80% was achieved.

Nanoparticles were also labelled with [^{111}In] oxime. The complex was freshly prepared from [^{111}In]indium chloride and 8-hydroxyquinoline, and 200 μl were added dropwise to 2.0 ml of the neutralized filtered nanoparticle suspension. To determine the extent of [^{111}In]indium association with the preparation, samples of the suspension were centrifuged at 140,000 g and 10 μl samples of the supernatant counted and compared to the activity of a similar volume of original suspension. An average binding capacity of 95 ± 1.3% was achieved.

NZW rabbits were used as the test species; each colloidal preparation being tested in a group of 6 rabbits. 25 μl of the suspension was instilled directly onto the corneal surface and the animal positioned in front of the pinhole collimator of the γ-camera. Sequential images were accumulated at 15 s intervals for a period of 10 min and stored on computer for analysis. Further processing was carried out by summation of individual dynamic frames to produce a complete picture of label distribution over the 10 min imaging period. The anatomical regions of interest via the cornea and inner canthus were defined and the percentage of activity remaining in each region as a function of time determined.

Results

Particle size

The particle sizes as determined by photon correlation spectroscopy are summarized in Table 1.

Lacrimal scintigraphy

The drainage of colloids from the cornea was found to be multiphasic in nature, with an initial rapid phase (150 s) followed by a slower phase. The inner canthal drainage profile, for all the particles, were monophasic and exponential and were significantly slower ($P < 0.05$, ANOVA) than solutions of the free isotopes. Table 2 summarizes

TABLE 1

Mean hydrodynamic diameter and polydispersity indices for each colloidal preparation

Preparation	Mean size ± S.D. (nm)	Polydispersity index ± S.D.
SUV POS	102 ± 1.3	0.22 ± 0.02
SUV NEG	126 ± 5.0	0.25 ± 0.02
SUV NEU	156 ± 3.0	0.32 ± 0.02
Nanoparticles	248 ± 18.0	0.42 ± 0.04
REV POS	360 ± 7.0	0.43 ± 0.04

TABLE 2

Half-lives for corneal and inner canthus clearance in the rabbit for the preparations studied (mean ± S.D.)

Preparation	Corneal T_{50} (min)	Inner canthus T_{50} (min)
SUV POS	3.77±0.29	7.70±1.90
SUV NEG	1.06±0.46	14.04±4.40
SUV NEU	1.14±0.02	14.30±2.20
Nanoparticles	2.15±0.09	9.27±5.00
REV POS	3.30±0.42	9.25±1.60
111 In-oxime *	1.3 ±0.1	5.0 ±1.3
99m Tc-DTPA *	1.4 ±0.1	5.0 ±0.8

* Reported by Fitzgerald et al., 1987.

the clearance half-times determined constants obtained from $\log_{10}$ percentage remaining versus time profiles for each corneal and inner canthal region.

Discussion

From the precorneal clearance results it appears that size and surface charge are important factors in determining residence times. Positively charged small unilamellar vesicles were retained significantly longer ($P < 0.05$, ANOVA) than negatively-charged or neutral small unilamellar vesicles on the corneal surface. The electrostatic attraction of small positively charged particles to the negatively charged epithelium is enhanced by their large surface area thus enabling greater surface contact. However, small unilamellar vesicles have a low encapsulation volume which might negate any advantage gained due to electrostatic attraction.

As size increases the results indicate that the clearance rate decreases. Positively charged small unilamellar vesicles, positively charged reverse evaporation vesicles and nanoparticles drain significantly slower than solutions of isotopes ($P <$ 0.05, analysis of variance). Therefore, as shown previously (Fitzgerald et al., 1987), particle retention occurs mainly in the inner canthal and extra-ocular regions of the eye and not on the corneal surface where significant drug absorption takes place. This study also demonstrates that nanoparticles do not appear to have any advantages over liposomes ranging in size from 100 nm to 4.0 μm in ocular drug delivery, but they may improve the delivery of certain lipophilic compounds to extra-ocular tissues.

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