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Pilocarpine bioavailability from a mucoadhesive liposomal ophthalmic drug delivery system

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Summary

The influence of a mucoadhesive polymer (Carbopol 1342) on the in-vitro release and in-vivo ocular bioavailability of pilocarpine nitrate entrapped in liposomes has been investigated. Coating of reverse phase evaporation vesicles (REVs) by Carbopol 1342 was achieved by incubating pre-formed vesicles in a 0.05% pH 5.0 polymer solution for 5 min. The in-vitro release phase of pilocarpine was extended by the presence of the polymer coating. The adsorbed film was therefore shown to provide a substantial barrier to drug release. Bioavailability was evaluated in albino rabbits by measuring the intensity and duration of the miotic response. Carbopol 1342 coated REVs showed a larger area under the miotic intensity curve (AUC) and a longer duration of action compared to uncoated REVs. No significant difference of area under the miotic intensity curve and duration of action was found between coated REVs and phosphate-buffered saline (PBS) solution containing the same concentration (0.5% w/v) of pilocarpine nitrate.

Introduction

As a conventional ophthalmic dosage form, eye drops have two major disadvantages. Drug penetration into the ocular tissues is often poor; and if absorption does occur only a short duration of action is attained. The latter is due to a 'pulse-entry' of the drug as seen by rapid rise followed by a rapid decline in ocular drug concentration. Effective therapy with eye drops can only be achieved by providing a high level of pulse, so that the drug effect is prolonged for a longer period of time, or by more frequent administration. Alternatively, 'controlled' drug delivery systems (Shell, 1984) have been developed to minimize 'peak and valley' effects, and thus maintain an effective drug concentration for a desired period of time.

Various approaches have been used to prolong the action of ophthalmic drugs and to reduce the undesirable side effects such as myopia and miosis (Brown et al., 1976). These arise as a result of transient peak ocular drug concentrations following frequent administration of massive doses of pilocarpine. Also, since most of the applied drug is not productively absorbed and is available for

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systemic absorption from the nasolacrimal duct (Patton and Francoeur, 1978; Salminen et al., 1984), concerns about toxicity may also arise.

Many of these new systems (e.g., hydrophilic matrix, soluble polymers, Ocusert, gels) prolong ocular bioavailability, but cannot control drug penetration through the cornea. Consequently, the drug concentration at the site of action might remain inadequate. Therefore, it is necessary to develop safer, efficacious and more acceptable ocular therapeutic systems.

The administration of drugs entrapped in liposomes to the eye was first studied by Smolin et al. (1981) who showed that liposome-associated idoxuridine is advantageous over the solution form of the drug in the treatment of herpes simplex keratitis in the rabbit. The treatment consisted of eye drops administered three times a day. Megaw et al. (1981) in their in-vitro and in-vivo studies demonstrated that lectin-mediated binding of liposomes to intraocular tissues may be useful as a specific drug delivery system to ocular tissues. Schaeffer et al. (1982) also studied lectin-mediated attachment of liposomes to freshly excised cornea and its influence on transcorneal drug flux. From in-vitro experiments, they concluded that liposomes containing ganglioside increased drug binding to, and enhanced the flux across, the cornea. Their data also supported the potential usefulness of liposomes as a vehicle for topical drug flux enhancement.

Mezei and Singh (1983) and Singh and Mezei (1983, 1984) investigated the potential of liposomes in an ophthalmic drug delivery system. The studies of Singh and Mezei (1983, 1984) and Stratford et al. (1983) suggest that liposome encapsulation alters drug disposition depending on the type of liposomes and the physicochemical properties of the encapsulated drugs. The hypothesis of Singh and Mezei (1984) is that the association between the drug molecules and the lipid vesicles can be a major factor influencing drug disposition. Kafalieva et al. (1990) investigated the topological distribution of atropine in dipalmitoylphosphatidylcholine (DPPC) liposomes. They found that approx. 31% of the added atropine was entrapped in the liposomes. They concluded from their results that atropine does not interact with the hydrophobic region of the lipid bilayer but is entrapped in the aqueous compartment of DPPC liposomes.

Benita et al. (1984) encapsulated 0.2% w/v pilocarpine into small multilamellar liposomes and compared it to 1 and 2% w/v pilocarpine solutions by measuring changes in intraocular pressure and pupil diameter. They suggested that liposomal formulation was unable to enhance the corneal penetration of pilocarpine to reach satisfactory therapeutic levels when administered in lower doses than normally used. The in-vitro clearance of radiolabelled liposome formulations was investigated by Fitzgerald (1985) employing γ-scintigraphy. Multilamellar lipid vesicles (MLVs) were found to have a prolonged precorneal retention as compared to small unilamellar vesicles of the same composition with positively charged liposomes having a prolonged residence as compared to negatively charged or neutral liposomes. In 1988, Guo et al. examined the precorneal retention of lipid vesicles containing between 20 and 50 mol% of a positively charged lipid. These vesicles were shown to enhance precorneal retention with as much as 50% of the preparation remaining associated with the ocular tissues 60 min post-administration. Meisner et al. (1989) studied the potential of liposomes as an ophthalmic drug delivery system for atropine base and atropine salt. Atropine base entrapped in MLVs with a positive surface charge showed a prolonged effect up to 12 h, whereas in solution form the pupil dilatation lasted for 7 h. MLVs with neutral and negative charges maintained the effect for 9 h. These studies demonstrate the importance of liposomal charge in controlling precorneal vesicle retention.

An alternative strategy is to use mucoadhesive polymers in order to retain vesicles in the precorneal area for extended time periods. Davies et al. (1991) demonstrated that Carbopol 934P was cleared more slowly than an equiviscous solution of the non-mucoadhesive polymer poly(vinyl alcohol). γ-Scintigraphy was later used by these workers to study the precorneal clearance of uncoated, Carbopol 934P coated and Carbopol 1342 coated liposomes in rabbit eye (Davies et al., 1992). The coated liposomes at pH 5 remained

for a longer time on the corneal surface compared to those formulated at pH 7.4.

The objective of this study was to incorporate pilocarpine, which is the drug of choice in glaucoma, into liposomes and to investigate its bioavailability from aqueous solution, uncoated and Carbopol 1342 coated reverse phase evaporation vesicles (REVs).

Materials and Methods

Materials

Acetone, EPC (egg phosphatidylcholine), chloroform and diethyl ether (Analar) were obtained from BDH Ltd (Poole, U.K.). Dipalmitoylphosphatidylcholine (DPPC), cholesterol (Chol) and pilocarpine nitrate were supplied by Sigma (St. Louis, USA).

Preparation of REVs

REVs were prepared according to the method of Szoka and Papahadjopoulos (1978). Lipid components and cholesterol were weighed and dissolved in chloroform:diethyl ether (1:1) in a 50 ml round bottom flask fitted with a long neck extension. Aqueous phase (PBS, pH 5) containing pilocarpine nitrate 4% was added such that the organic-to-aqueous phase ratio was 6:1. The flask was sealed under nitrogen, and the mixture sonicated for 4–6 min at 40°C (in the case of EPC) or 50°C (DPPC) in an ultrasonic bath. A stable emulsion was produced, from which the organic solvent was slowly removed at 45°C by a rotary evaporator. At the end of the process the residue decreased in viscosity to give an REV preparation having a volume slightly less than the original volume of the aqueous phase. The traces of the organic solvent were removed by nitrogen flushing and the liposomal preparation annealed at 45°C for 1 h.

Determination of drug entrapment in liposomes (REVs)

Aliquots of 1 ml liposome suspension containing pilocarpine nitrate were centrifuged at 140000 $\times g$ (4°C) for 1 h. The supernatant containing traces of lipid vesicles was recentrifuged to remove the small lipid fragments. The clear supernatant was analysed for pilocarpine nitrate spectrophotometrically (λ_{max} 300 nm).

Assessment of efflux rate

1 ml of liposomal suspension (REVs) containing pilocarpine nitrate was centrifuged at 140000 $\times g$ (4°C) for 1 h. The supernatant was completely removed from the lipid plug which was subsequently kept inverted for 24 h. The lipid plug was then resuspended with a similar volume of fresh saline (pH 7.4). Following resuspension, 0.4 ml of the liposomal suspension was incubated for 5 min in 3.6 ml of 0.05% w/v Carbopol 1342 solution or 0.4 ml of saline, both having a pH of 5. The suspension was subsequently added to 36 ml of 0.9% w/v NaCl pH 7.4 and maintained at 33°C in a shaking water bath (Grant Instrument Ltd, U.K.). Samples were taken every 30 min and the aqueous phase separated from the liposomal vesicles by ultracentrifugation. The aqueous phase was then assayed at 300 nm.

Characterization of coated and uncoated liposomes

The mean size of REVs in the liposome suspension was determined by photon correlation spectroscopy (PCS) using a Zeta Sizer III Particle Electrophoresis and Multi-angle Particle Size Analyser (Malvern Instruments, U.K.) employing an AZ4 cell. Prior to measurement a small aliquot of the coated/uncoated liposome suspension was diluted with 0.9% w/v NaCl to give a final lipid concentration of 0.1 mg/ml. Size measurements were carried out at a 90° angle and a temperature of 25°C.

Bioavailability studies

The bioavailability studies were conducted on five unanaesthetized preconditioned, male New Zealand White rabbits (3–4 kg). Lighting and audio stimuli were maintained constant. A 20 μl dose buffered at pH 5 was administered to the eye of each rabbit which was photographed alongside a reference scale every 10 min. The pupil diameter was measured from the developed prints using a travelling microscope. The relative miotic response intensities (IR_t) at time t for the three different formulations (PBS, uncoated and

TABLE 1

Mean diameter and polydispersity indices for coated and uncoated REVs (n = 5)

Formulation	Mean size (±SE) (nm)	Polydispersity index (±SE)
REVs uncoated [a]	365(±28.8)	0.471(±0.19)
REVs coated [a]	504(±46 1)	0.195(±0.05)
REVs uncoated [b]	397(±43 8)	0.219(±0.03)
REVs coated [b]	676(±58 7)	0 480(±0 10)

[a] EPC formulation.
[b] DPPC.Chol formulation.

coated liposomes) were calculated from the equation $IR_t = (I_0 - I_t)/I_0$, where I_0 is the average baseline diameter and I_t the diameter at time t.

Results and Discussion

The manufacturing process yielded REVs, the mean particle size of which varied in the range of 314–528 nm for the uncoated REVs and 415–750 nm for Carbopol 1342 coated REVs (Table 1). This corresponds to a polymer film surrounding the vesicles equivalent to 70–140 nm in thickness assuming that the polymer induces no expansion of the outermost bilayer.

Encapsulation of pilocarpine nitrate in REVs composed of EPC was 6–7% of the original drug concentration, increasing to 20–23% for DPPC:Chol 1:1 REVs. This contrasts with work performed by Benita et al. (1984) where 3.80% of pilocarpine HCl was encapsulated in MLVs composed of, phosphatidylcholine (PC) and cholesterol (7:2) at pH 5, and 3.56% of drug in 7:0 (PC:Chol) vesicles. These workers found that increasing the cholesterol ratio to 7:7 (PC:Chol) increased the encapsulation capacity to 8.06%. They suggested that the higher cholesterol content reduced the membrane permeability of the liposomes towards active ingredients, thus ensuring that the pilocarpine was retained within the vesicles.

Fig. 1 shows the in-vitro release profile of pilocarpine nitrate from Carbopol 1342 coated and uncoated vesicles in pH 7.4 phosphate-buffered saline at 33°C from the two lipid formulations. In Fig. 1a, the pilocarpine nitrate was released within 90 min from the uncoated EPC REVs but was prolonged over a period of 160 min for the coated formulation. Drug release was similarly retarded by the presence of the polymer coat for DPPC/Chol REVs. For uncoated REVs the complete efflux took place in 120 min which extended to 280 min in the case of the coated REVs (Fig. 1b). For both vesicle formulations, the Carbopol 1342 coat provides an extra barrier to pilocarpine nitrate diffusion. This demonstrates that the rate of pilocarpine release is dependent on the fluidity of the lipids composing the vesicle membrane. EPC provides a relatively fluid membrane, ($T_c = -15$°C) whereas DPPC ($T_c = 41$°C), particularly with the inclusion of cholesterol, forms a more rigid bilayer. At a molar ratio

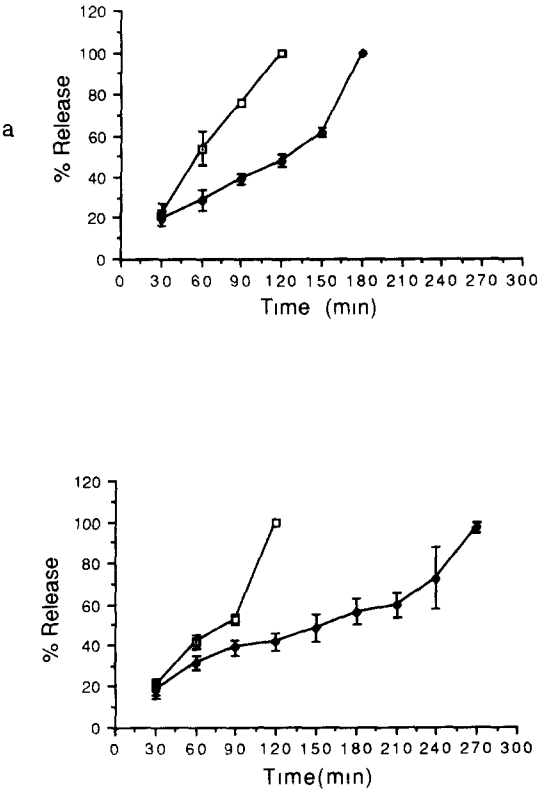

Fig 1 Efflux of pilocarpine nitrate at 33°C pH 7 4 from REVs uncoated (□) and coated (◆) composed of (a) EPC and (b) DPPC·Chol (1 1) (±SE, $n = 3$)

of 1:1 cholesterol abolishes the transition temperature of DPPC and forms a more stable vesicle. For both liposome compositions, the Carbopol 1342 film provided an extra barrier against pilocarpine nitrate diffusion from the vesicles. With the exception of EPC uncoated vesicles, an increase in the release rate was observed toward the end of the time scale. A speculative explanation for this may be that changes are occurring in the composition of the bilayer. The formulations were also tested in vivo on the basis of extent and duration of pupil miotic effect after topical instillation of the three formulations into albino rabbit eyes.

Fig. 2 compares the miotic effect of three different formulations of pilocarpine nitrate (0.5%) at pH 5. A marked reduction in pupil diameter was observed with the PBS formulation as compared to vesicular formulations. The duration of action for PBS and uncoated REVs was similar but prolonged for Carbopol 1342 coated REVs. The latter exhibited a biphasic response which was probably due to the presence of free drug (initial peak of AUC 13.02), followed by a sustained release phase (AUC 12.68) during which the pilocarpine nitrate diffused from the vesicles. The maximum effect was obtained with a PBS solution of pilocarpine nitrate (Table 2), but the duration of effect was 110 min whereas for the coated REVs it was extended to 180 min. The small AUC displayed by the uncoated REVs is believed to be the result of the rapid clearance of these vesicles from the ocular surface before efflux of pilocarpine nitrate occurs.

Duncan's new multiple range test was employed to check the significance amongst the three formulations. A statistical difference in AUCs was shown when coated and uncoated REVs were compared, but this was not significant between coated REVs and PBS solution of pilocarpine nitrate. With respect to duration of drug release, coated REVs were statistically different from both a PBS solution of the drug and uncoated REVs ($P < 0.05$). However, no difference was found to exist between the drug in PBS solution and uncoated REVs, nor in the miotic response demonstrated by the three formulations.

The vehicle pH is an important parameter for

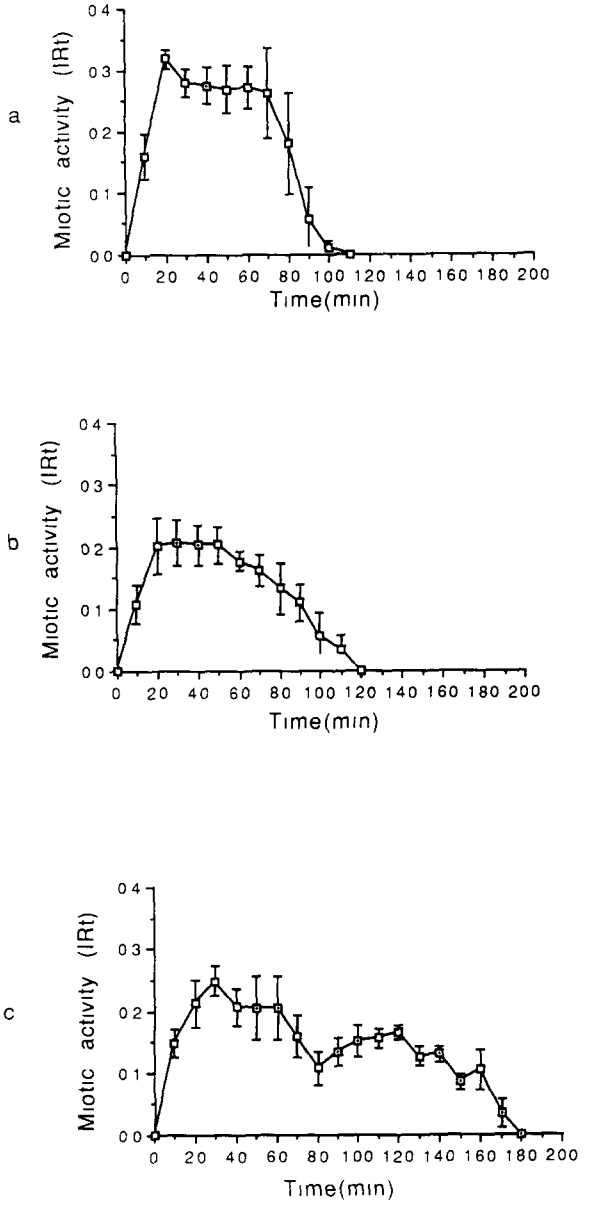

Fig 2 Effect of three different formulations of pilocarpine nitrate at pH 5.0 on the pupil diameter of rabbit (a) Phosphate buffer solution of pilocarpine nitrate, (b) REVs uncoated, (c) REVs coated with Carbopol 1342

the passive diffusion of ionisable compounds into and through the cornea (Swan and White, 1942). The pH partition hypothesis predicts that maximum corneal penetration occurs if the alkaloidal drug is administered in the free base form. This is

TABLE 2

Summary of the main bioavailability parameters (±SE, n = 5)

	IR_t (max)	Duration (min)	AUC
PBS	0.319(±0.0131) [a]	110(±4 90) [a]	20 87(±1 44)
REVs (uncoated)	0.207(±0.0271)	120(±4 90) [a]	15.97(±3.08) [a]
REVs (coated)	0.248(±0.0345)	180(±4.47)	25 70(±2 29)

[a] Significantly different from REVs coated ($P < 0.05$)

because in this form the drug is more lipid soluble than the ionised species and will then readily diffuse through the corneal epithelium. The results of Seig and Robinson (1977) show that corneal penetration of pilocarpine is increased 2–3-fold when the pH is raised from 5 to 8. Instillation of a solution in the acid pH range stimulates lacrimation which clears the solution from the preocular surface. However, the dilution of the instilled solution by the lacrimal fluid returns the pH to the physiological range. Glycerin, which is a non-ionisable compound that should not derive any direct benefit from pH manipulations for its corneal penetration, is also affected by changes in pH from 5 to 8. Such behaviour strongly suggests that some mechanism other than a direct pH effect on the drug molecule is operating to produce this increase and that this mechanism must also be responsible for the increase observed for pilocarpine. However, Riegelman and Vaughan (1958) reported no statistical difference in pilocarpine miosis in the pH range 4.2–6.6. Thus, apparently pilocarpine activity is not affected whether applied from nearly physiological pH or from more acid vehicles.

Conclusion

It has been shown that Carbopol 1342 coated liposomal formulations increase the bioavailability of pilocarpine in the rabbit eye compared with an uncoated preparation. These studies suggest that polymer coated vesicles or similar preparations may provide the basis for improved ocular drug therapy in the future.

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