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Liposomal ophthalmic drug delivery. III. Pharmacodynamic and biodisposition studies of atropine

Dale Meisner, Joan Pringle and Michael Mezei

Dalhousie University, College of Pharmacy, Halifax, N.S. (Canada)

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Summary

The potential of liposomes as an ophthalmic drug delivery system was investigated by comparing disposition and pupillary dilatory effect of atropine base and atropine sulphate in solution and various liposomal forms when topically instilled to the rabbit eye. Atropine base entrapped in multilamellar lipid vesicles (MLVs) with positive surface charge displayed the most prolonged effect, lasting up to 12 h. MLVs with neutral and negative charges maintained the effect for 9 h while atropine in solution form was effective for only 7 h. Preparations containing atropine sulphate displayed a similar pattern although were shorter-acting than corresponding base products. All preparations, whether salt or base, were equally effective in producing maximal response. In vivo drug disposition studies indicated the liposomal form produced significantly higher drug levels in the anterior tissues of the eye up to 8 h after instillation. Increased ocular bioavailability of atropine to these tissues was attributed to enhancement of pulse entry with little evidence of sustained drug release. It could be concluded that liposome encapsulation extended the duration of action and favourably altered disposition of atropine when topically instilled to the rabbit eye.

Introduction

To achieve effective ophthalmic therapy, an adequate amount of an active ingredient must be delivered and maintained at its site of action within the eye. In this regard, the ophthalmic solutions and suspensions are compromised in their effectiveness by several limitations. In solution form many drugs display poor penetration through the corneal barrier. Rapid nasolacrimal drainage of the instilled drug from tear fluid and non-productive absorption through the conjunctiva may lead to a short duration of action and unwanted entrance of the drug into the systemic circulation. Tear turnover and drug binding to tear fluid proteins are additional precorneal factors that contribute to the poor ocular bioavailability of many drugs when instilled in the eye in the solution dosage form. Ophthalmic drops also rely on the pulse entry effect (Shell, 1984). The rate of release of the drug from the tear fluid to ocular tissues is initially high but rapidly declines. This results in a transient period of overdose and the associated risk of side-effects followed by an extended period of subtherapeutic levels before the next dose is administered. The need for an ocular drug delivery system which has the con-

Correspondence: D. Meisner, Dalhousie University, College of Pharmacy, Halifax, N.S., B3H 3J5, Canada.

venience of a drop but will localize and maintain drug activity at its site of action is apparent.

A liposomal preparation may have the inherent properties to fulfill these requirements. Liposomes have the ability to entrap hydrophilic compounds in the aqueous compartments and to incorporate hydrophobic molecules in the lipid bilayers (Gregoriadis, 1976). Variation in the method of preparation and alteration in factors such as lipid composition and surface charge can produce extensive differences in physical properties (Kimelberg and Mayhew, 1979; Stamp and Juliano, 1979; Fendler and Romero, 1980; Szoka and Papahadjopoulos, 1980). The simplicity of preparation and the versatility in physical characteristics confer a unique and potentially useful property to liposomes for use as an ophthalmic drug delivery system.

Studies investigating liposomes as an ophthalmic drug delivery system has produced some promising results. Smolin et al. (1981) reported that idoxuridine entrapped in liposomes was superior to the solution form in treating herpes simplex keratitis in the rabbit eye. Corneal penetration of liposomal idoxuridine was later shown to be significantly enhanced due to liposomal encapsulation (Dharma et al., 1986). A 4-fold increase in the passage of penicillin G across rabbit corneas and a 10-fold enhancement of indoxole passage across rat corneas was observed when liposomal preparations were compared with solutions of the respective compounds (Schaeffer and Krohn, 1982). The corneal and conjunctival absorption of liposomal epinephrine was reduced by 50% after topical instillation while that of encapsulated inulin was increased 10 times when compared to the solution form (Stratford et al., 1983). Pharmacological comparison of 0.2% pilocarpine entrapped in liposomes and commercial products containing 1% and 2% of the drug as a hydrochloride salt demonstrated that the liposomal vehicle was unable to improve sufficiently the corneal penetration of the drug to supply therapeutic concentrations (Benita et al., 1986). However, liposomes containing acetylcholinesterase were found to be effective in providing significant protection against diisofluorophosphate-induced miosis (Shek and Barber, 1987).

Previous study in our laboratory suggested that the alteration of drug disposition observed with liposomal encapsulation may be dependent on the physicochemical properties of the entrapped species. Liposomal triamcinolone acetonide, a model lipophilic compound, provided higher drug concentrations in ocular tissues when compared to the suspension dosage form (Singh and Mezei, 1983); however, liposome-encapsulated dihydrostreptomycin sulphate, a hydrophilic compound, produced lower drug levels in ocular tissues compared to its solution form (Singh and Mezei, 1984). The present study was conducted in order to establish the effect liposomal encapsulation has on the biological fate and biological activity of the entrapped compound. Since the pharmacological response of atropine could be objectively measured, the base and sulphate salt were selected as model compounds for lipophilic and hydrophilic drugs. Thus atropine in solution and various liposomal forms were compared on the basis of extent and duration of pupil dilatory effect and in vivo biodisposition after topical instillation to the rabbit eye.

Materials and Methods

Formulation

DL-Dipalmitoyl phosphatidylcholine (DPPC), cholesterol (CHOL), stearylamine (SA), dicetylphosphate (DCP), atropine sulphate, and atropine base were purchased from Sigma Chemicals, St Louis, MO. Tritiated atropine base was obtained from Amersham Co., UK. Neutral MLVs were composed of 5% lipid, DPPC/CHOL (2:1 molar ratio). A net positive or negative charge was introduced by the addition of either SA or DCP to the lipid composition (here the final molar ratio was 7:4:1). All formulations were prepared by the method described by Mezei and Nugent (1984). Briefly, the lipids and atropine were weighed into a round-bottomed flask and dissolved in a minimal amount of chloroform/methanol (2:1 v/v) solvent. Glass beads were added to provide a larger surface area for film formation. The organic solvent was completely removed on a rotary evaporator under reduced pressure at 30 °C until

a thin smooth lipid film formed on the beads and walls of the flask. The film was hydrated with the required volume of 8 mM $CaCl_2$, at 52°C for 20 min while being shaken at 200 rpm in a Lab-line Orbit Environ-Shaker. The MLVs formed as a result of the procedure were filtered through a Buchner funnel (without filter paper) to remove the glass beads. Evaluation under optical microscope revealed MLVs ranging from 0.4 to 4.2 μm in diameter. Free drug was separated form encapsulated drug by repeated centrifugation at 22,000 × g for 30 min.

Pupil dilation studies

MLVs with positive, negative and neutral charges and solutions containing either 1% atropine base or sulphate based on total mass were formulated. New Zealand white rabbits (2.6–3.2 kg) were divided into 4 groups, each composed of 8 rabbits. Under standard lighting conditions the baseline pupil diameter of each rabbit was established. Each group was designated to receive one of the base preparations, i.e. base in solution, positive, negative, or neutral MLVs. Each rabbit received a single 25 μl dose of test preparation in one eye. The contralateral eye received a single 25 μl dose of either normal saline or empty liposomes. After instillation, pupil diameter in both eyes was measured at 15, 30 and 60 min then every hour until the eye returned to baseline diameter. After a week washout period the experiment was repeated using atropine sulphate preparations.

In vivo drug disposition studies

The specific activity of the [^{3}H]atropine base stock solution used for both the liposomal and solution formulation was 250 μCi/ml, i.e. 25 μCi/mg atropine. New Zealand white female rabbits weighing 2.6–3.2 kg were divided in 2 groups, each composed of 12 rabbits. Each rabbit of group A received a single 25 μl dose of atropine in solution form in both eyes. Each rabbit in group B received a single dose of atropine base encapsulated in neutral MLVs in both eyes. Immediately prior to sacrifice, blood samples were collected from the marginal ear vein. The rabbits were sacrificed by an injection of sodium pentobarbital into the marginal ear vein at 0.5, 1, 4 or 8 h after drug administration. Aqueous humour samples were removed from the anterior chamber of the eye using a 26.75-guage needle attached to 1 ml tuberculin syringe and transferred to preweighed counting vials. The conjunctiva was surgically stripped from the bulbar and lid surfaces, rinsed in normal saline, blotted dry and placed in preweighed vials. The eye globes were then enucleated and immediately frozen in liquid nitrogen to prevent post-mortem diffusion. Samples of liver and spleen were collected.

The frozen eye globes were bisected behind the lens (Abel and Boyle, 1976). Vitreous body, lens, iris/ciliary body, cornea, and sclera were separated, rinsed in normal saline (excluding vitreous body), blotted dry and placed in preweighed vials. The vials were reweighed and the weight of each sample recorded. Each internal organ was homogenized and aliquot samples were weighed. The tissues were digested overnight at 50°C by the addition of a calculated volume of NCS tissue solubilizer (Amersham Co., Canada). Dissolved tissues were decolourized (if necessary) with 0.3 ml benzoyl peroxide (20%) (BDH Chemicals, Canada) in toluene and adjusted to neutral pH with glacial acetic acid before adding Beckman-MP scintillation fluid. The sample were dark-adapted for 48 h and then counted for radioactive content in a Beckman LS 3133T counter.

Results and Discussion

Fig. 1 graphically compares the pupil dilatory effect of atropine base in solution and various liposomal forms after topical instillation to the rabbit eye. Similarly Fig. 2 depicts atropine sulphate products. Atropine base entrapped in positively-charged MLVs displayed the most prolonged effect, lasting up to 12 h. It required 9 h for eyes treated with atropine entrapped in neutral and negatively charged vesicles to return to baseline diameter. For the solution form this occurred only 7 h after instillation. Evaluation of atropine sulphate products revealed similar results although all were shorter acting than the corresponding base formulations. Fig. 2 demonstrates the most

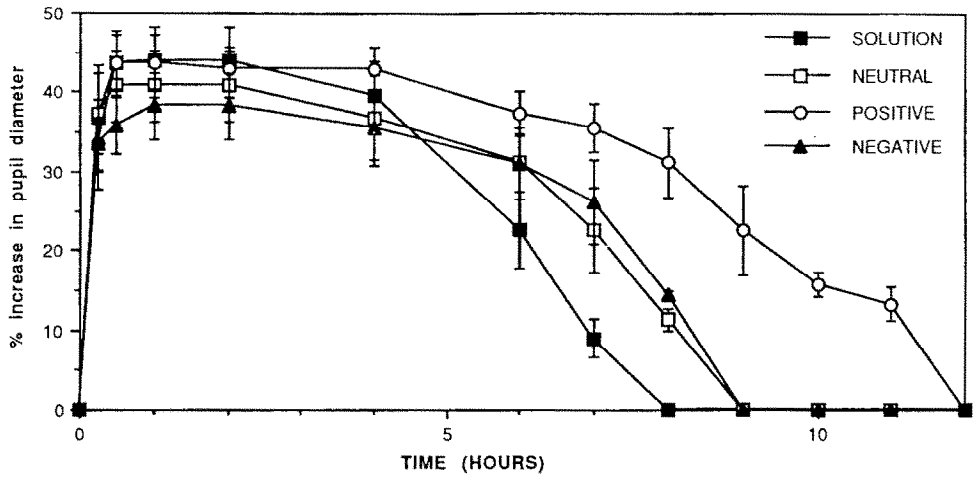


Fig. 1. Pupillary dilation effect of atropine base preparations.

effective sulphate preparation in terms of maintaining a dilated state was achieved in the order of positive, negative, neutral liposomes and solution. All preparations of both base and salt were equally effective in producing maximal response, 35–45% increase in pupil diameter. Instillation of normal saline or empty liposomes showed no significant dilatory effect.

The observation that atropine base encapsulated in positively-charged liposomes provided the longest pharmacologically effect is not surprising. Schaeffer et al. (1983) demonstrated in vitro that positively-charged liposomes have a higher binding affinity to the corneal surface than neutral or negatively-charged vesicles. Hence positively-charged vesicles can enhance and maintain cornea–liposome interaction to a greater extent and subsequently provide longer cornea–drug interaction. Furthermore, preliminary results have indicated retention of atropine in tear fluid was greatest for positively-charged MLVs (unpublished data). Fitzgerald et al. (1987) previously demonstrated that MLVs with a positive surface charge were drained more slowly than MLVs with either a negative or no surface charge. Secondly, the ability of drugs to diffuse into the corneal

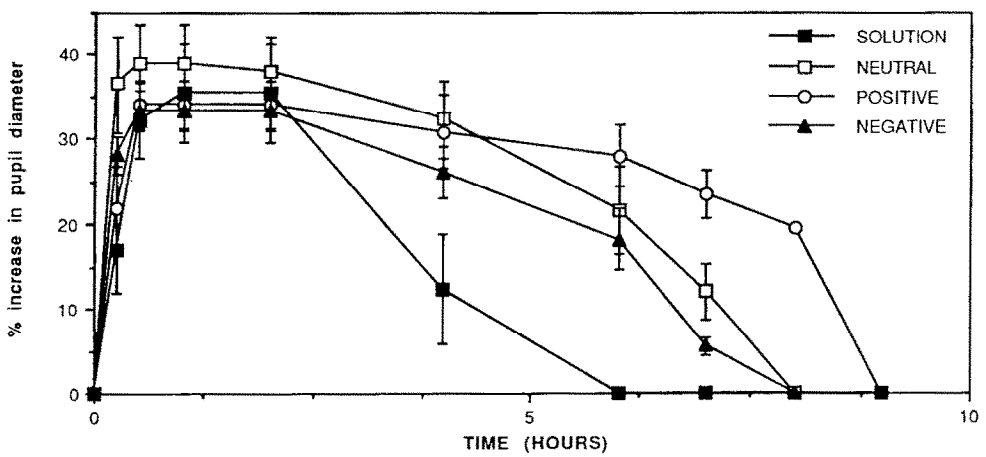


Fig. 2. Pupillary dilation effect of atropine sulphate preparations.

epithelium is influenced by its partition coefficient. The epithelium has been demonstrated to be a greater barrier to hydrophilic rather than lipophilic compounds (Huang et al., 1983); thus, atropine base is more readily absorbed than the sulphate salt. The longer duration of action observed with the base preparations was indirectly influenced by enhanced corneal absorption and augmented drug loading to intraocular tissues.

The drug concentration in ocular and internal tissues at different time intervals after a single dose of atropine entrapped in neutral liposomes or in solution form are shown in Figs. 3 and 4. The liposomal formulation provided significantly higher levels ($P < 0.05$) of atropine in the aqueous humour (Fig. 3a) and iris/ciliary body (Fig. 3b) as compared to the solution for up to 4 h. In the cornea (Fig. 3c), higher drug levels were maintained up to 8 h. The atropine concentration in the sclera (Fig. 3d) was higher at 30 min; however, this was not observed at the longer time intervals studied. This may suggest an initial attraction of the liposomes to this particular tissue of the eye. In the conjunctiva (Fig. 3e), the liposomal form maintained a higher drug concentration most prevalent at 8 h. There are two possible consequences of prolonged liposomal attachment to the conjunctiva. MLVs could slowly release drug (Mezei, 1988) into tear fluid for subsequent ocular absorption or drug could slowly diffuse through the conjunctiva and enter the systemic circulation. Obviously the first scenario is preferable in ophthalmic therapy. The posterior tissues of the eye, i.e. lens (Fig. 3f) and vitreous body (Fig. 3g),

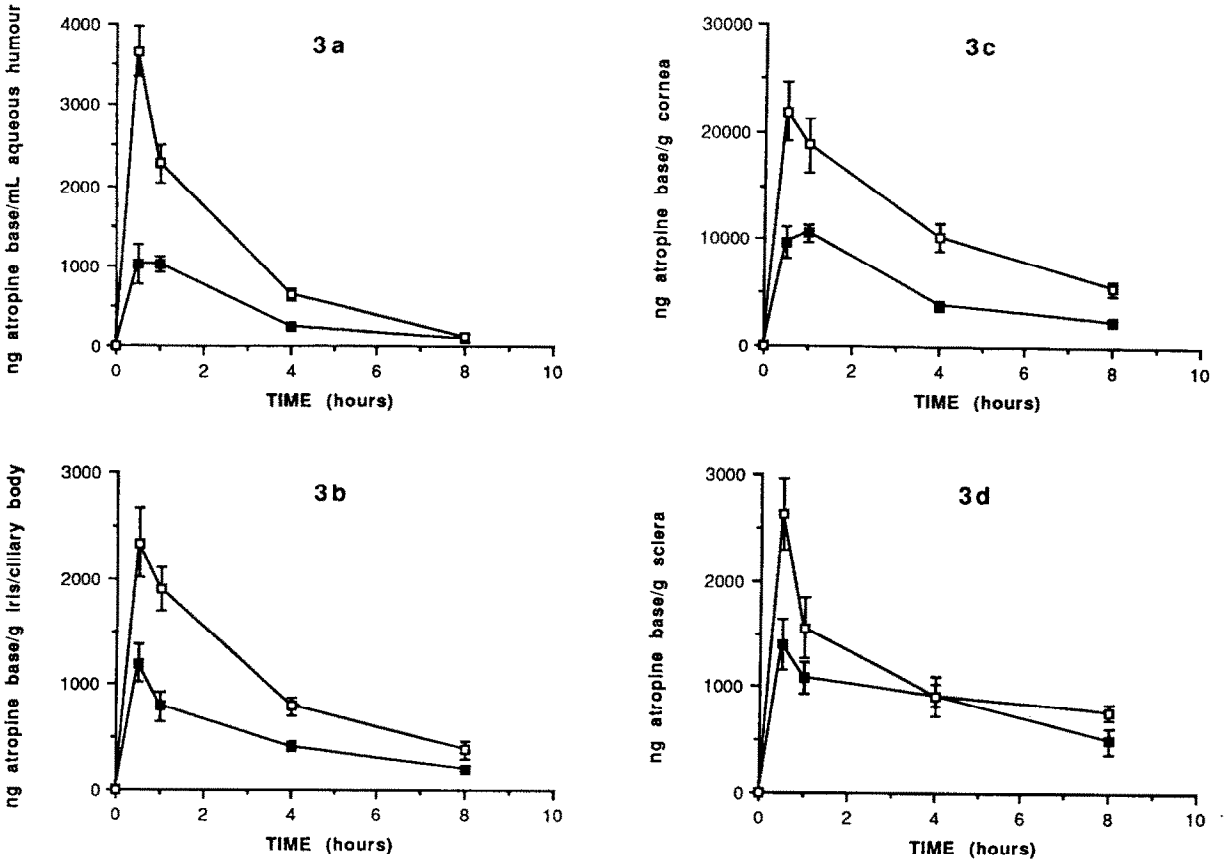
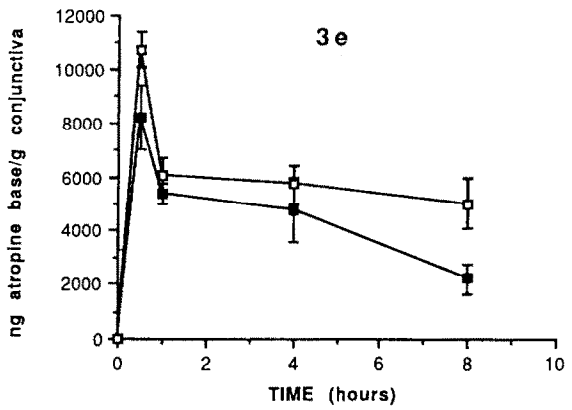
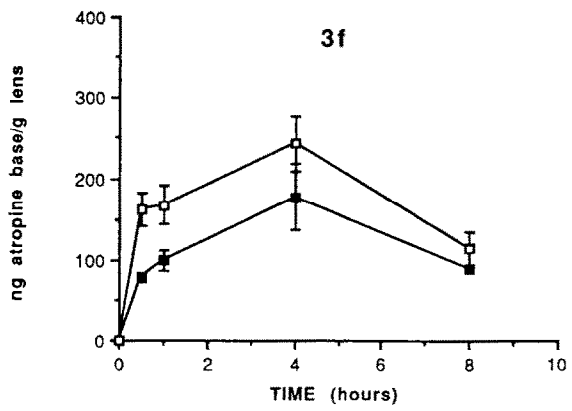


Fig. 3. Concentration of atropine base in ng/g tissue in aqueous humour (a), iris/ciliary body (b), cornea (c), sclera (d), conjunctiva (e), lens (f) and vitreous body (g) after topical instillation to the rabbit eye in solution (■) and liposomal form (□). Values represent mean ± S.E.M. ($n = 6$).





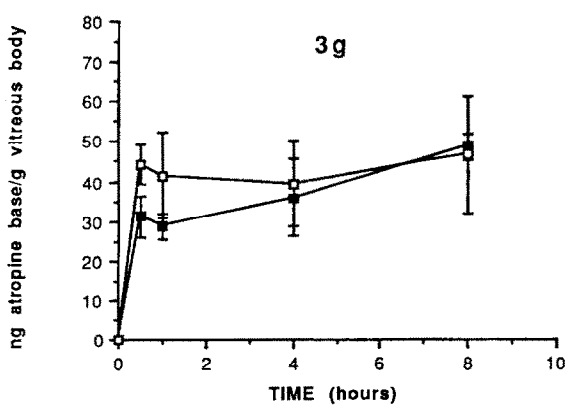
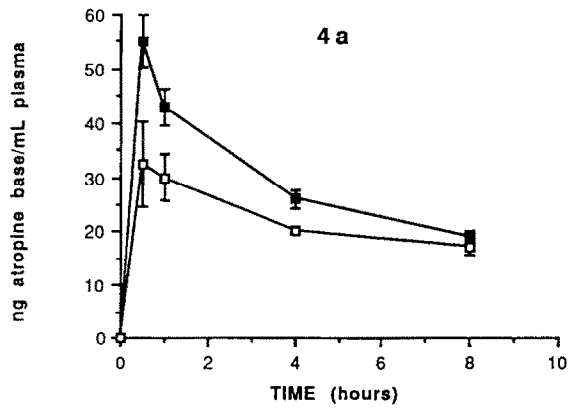
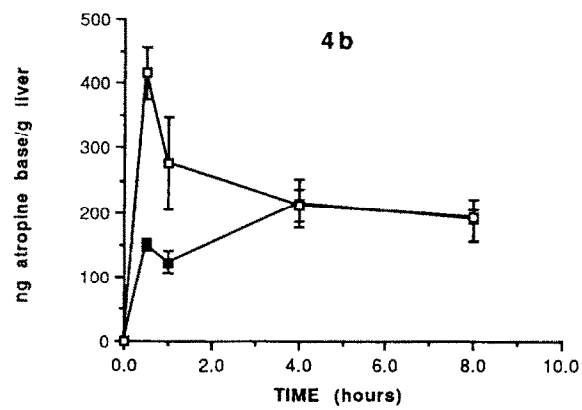


Fig. 3. (continued).

displayed low levels of drug regardless of the preparation instilled. In these tissues no significant differences were demonstrated between preparations except in the lens at 30 and 60 min at which times higher atropine concentrations ($P < 0.05$) were produced by the liposomal preparation.

Statistical tests showed a significant difference ($P < 0.05$) between drug levels produced in plasma





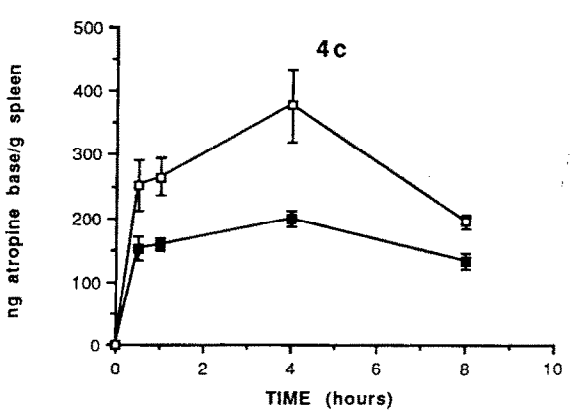


Fig. 4. Concentration of atropine base in ng/g tissue in plasma (a), liver (b) and spleen (c) after topical instillation to the rabbit eye in solution (■) and liposomal form (□). Values represent mean ± S.E.M. ($n = 6$).

(Fig. 4a) at 0.5, 1 and 4 h by liposomal and solution products equivocally establishing that less drug reaches the general circulation after topical instillation of the liposomal form. However, after 30 min rabbits treated with liposome-encapsulated drug showed significantly higher atropine levels ($P < 0.05$) in liver (Fig. 4b) and spleen (Fig. 4c). This suggests that liposome–drug fragments, or possibly intact liposomes, entered the systemic circulation and selectively accumulated in the reticuloendothelial tissues of the body. If atropine is absorbed systemically still associated with lipid, a change in the pharmacokinetic profile of the drug would be anticipated; however, this was not apparent as the plasma curves for the liposomal drug and "free" drug are practically superimposable. Drug reaching the systemic circulation after topical instillation in liposomal form could be absorbed in 2 forms, i.e. free drug released from liposomes into tear fluid and then systemically absorbed, and drug absorbed still associated with liposomes or with lipid fragments. This can explain the similarity of plasma curves and the higher drug levels concurrently observed in the liver and spleen. Endocytic uptake by the conjunctiva and drainage through the nasolacrimal system are the most probable routes by which liposomes could enter the systemic circulation. It is highly unlikely that liposomes could transflux the cornea, which displays weak phagocytic activity (Nilsson and Latkovic, 1981) intact, and reach tissues such as liver and spleen rapidly by this route.

The concentration–time profiles of cornea, aqueous humour, and iris/ciliary body (Fig. 3a, b, c) indicated liposomal encapsulation increased the pulse entry of atropine into these tissues of the eye. Area under the curve calculations indicated that only 1.9% of drug administered in solution form reached the iris/ciliary body, the site of action of atropine. Liposome encapsulation increased the bioavailability of drug in iris to 3.9%. The approximate 2-fold increase was also observed in cornea and aqueous humour. The increased pulse of atropine was probably due to enhanced association of drug and cornea provided by the inherent lipophilicity of the liposomal bilayers and the corneal epithelium. Although it has been demonstrated in vitro that positively-charged liposomes have the highest binding affinity for cornea (Schaeffer et al., 1983), Lee et al. (1984) presented evidence that neutral MLVs can loosely adsorb to the corneal surface. The ability of liposomes to accumulate the drug at the absorptive site, i.e. the epithelium of the cornea, may be the underlying mechanism by which the liposomal preparation was able to supply more atropine to the anterior compartments of the eye, i.e. cornea, aqueous humour and iris/cililary body. Positive liposomes, having a higher binding affinity, were more efficient than other products, in this regard. Since it was unlikely that liposomes penetrated the corneal barrier intact, the posterior tissues did not reflect any differences in drug concentrations. The higher concentrations in the lens at 30 and 60 min was likely due to more drug being available to diffuse from adjacent anterior tissues to the lens.

The concentration–time profiles also reveal that although the peak was augmented by liposome-encapsulation, the elimination rates of atropine for both products were similar. For example, first-order elimination constants for iris/ciliary body were 0.216 h^{-1} and 0.223 h^{-1} for atropine in solution and liposomal form, respectively. This suggests while the liposomal atropine had an increase in loading of the drug to the cornea, it did not display sustained-release of the drug into the tear fluid. Although drainage of liposomes from the precorneal area is somewhat slower than solution forms (Fitzgerald et al., 1987), residence time near the absorptive site was still inadequate to allow slow-release of drug to occur. The similar elimination profiles also indicated that the drug was no longer associated with the liposomes when it reached the interior structures of the eye. If this was the case, a difference in ocular disposition and metabolic removal of drug would be expected. Subsequently, atropine provided from the liposomal formulation would not be eliminated at the same rate as that of the solution form.

Conclusions

The mechanism by which liposomal encapsulation was able to augment the pulse of atropine to the anterior tissues of the eye remains unclear,

although, enhanced corneal attraction may play the most important role. Positively-charged liposomes previously shown to bind more effectively to corneal epithelium than neutral or negatively-charged vesicles (Schaeffer et al., 1983) is consistent with the finding that they were the most effective product in maintaining pharmacological activity. Corneal epithelium does not offer the majority of resistance to penetration of lipophilic drugs. Conversely, for hydrophilic drugs, the main barrier to penetration is the epithelium of the cornea (Huang et al., 1983). As suggested by Lee et al. (1985), this implies that liposomal encapsulation should benefit hydrophilic compounds to a greater extent than lipophilic compounds. In our experiments, this is not the case. Singh and Mezei (1983, 1984) reported that lipophilic compounds are better candidates for liposomal encapsulation. It seems apparent that release of drug from liposomes can increase its local concentration at the corneal surface; however, after release from the vesicles, molecules rely on passive diffusion to cross the corneal barrier. Subsequently the physicochemical nature of the compound still plays an important role in determining the extent and rate of ocular absorption. Furthermore physicochemical properties of the entrapped species can influence vesicle stability and drug release in tear fluid. It is imperative to understand this relationship (Barber and Shek, 1986) and to establish the mechanism of liposome–cell interaction at all possible absorptive sites, i.e. conjunctiva, cornea and sclera, in order to define the liposomal properties required to optimize ocular drug delivery. Studies related to this interaction have been designed and are presently being conducted in our laboratory.

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