International Journal of Pharmaceutics, 34 (1986) 163–167
Elsevier

IJP 01133

Short Communications

Selective intraocular delivery of liposome encapsulated inulin via the non-corneal absorption route

Imran Ahmed * and Thomas F. Patton

Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045 (U.S.A.)

(Received 29 August 1985)
(Modified version received 17 February 1986)
(Accepted 26 June 1986)

Key words: Liposome; Ocular; Topical selective drug delivery; Albino rabbit; Non-corneal absorption

It has been reported that entrapment of inulin in multilamellar liposomes has the effect of excluding inulin from the aqueous humor while increasing its concentration in the conjunctiva, cornea and iris-ciliary body (Stratford et al., 1983a and b). The increase in corneal and conjunctival concentration of inulin was attributed primarily to adsorption of liposomes onto the epithelial surfaces. It was further suggested that facile association of liposome encapsulated inulin with the adsorptive surfaces, coupled with the slow release rate of the drug from the liposomes was responsible for the absence of inulin in the aqueous humor (Lee et al., 1984). However, these studies did not address the potential reasons for the presence of inulin in the uveal tract despite the absence of appreciable levels in the aqueous humor. Since these observations appear to be in agreement with a non-corneal route of absorption of inulin as reported in a recent study (Ahmed and Patton, 1985), the present work was undertaken to investigate whether the presence of inulin in the uveal tract could be attributed to its selective intraocular delivery across the conjuctiva and sclera when entrapped in liposomes.

Multilamellar, neutral liposomes were prepared by dissolving 7.5 mg of L-α-phosphatidylcholine (Type V-E from egg yolk; purity 99%; Sigma Chemical Co., St. Louis, MO) and 1.11 mg of cholesterol (Sigma Chemical Co., St. Louis, MO) in 500 μl of chloroform in a 5 ml round-bottom flask. The solvent was evaporated, under nitrogen, to form a thin film of lipid on the walls of the flask. A 2.5% inulin solution in 0.0667 M phosphate buffer (pH 7.0) (750 μl) containing both unlabeled and tritium-labeled drug (spec. act. 199 μCi/mg; Amersham Corp., Arlington Hts., IL) was added to the flask. The film was dispersed into the aqueous solution by vortexing at low speeds for 15 min. The resulting suspension was agitated in a shaker bath for 30 min and allowed to equilibrate at room temperature for an additional 1 h. This crude liposomal preparation was centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant, containing unentrapped drug, was decanted and saved for radioactivity counting. The pellet was resuspended in 1 ml of phosphate buffer and centrifuged to remove additional, unentrapped drug. This washing procedure was repeated a total of 3 times, beyond which no further removal of unentrapped drug occurred, as judged by the constancy of the radioactivity counts in the supernatant. After the third wash, the pellet was

* *Present address*: College of Pharmacy and Health Sciences, Northeast Louisiana University, Monroe, LA 71209, U.S.A.
Correspondence: T.F. Patton, The Upjohn Co., 7000 Portage Rd., Kalamazoo, MI 49001, U.S.A.

resuspended in 500 μl of phosphate buffer. The radioactivity of this liposomal suspension was measured in a 10 μl aliquot and determined against a known standard. From counts due to tritiated drug, the percent of drug initially present in the buffer found to be associated with the phospholipid pellet was assessed to be 1.25% ± 0.29% (n = 8). In a separate set of experiments, using [^{14}C]dipalmitoyl phosphatidylcholine (spec. act. 100 mCi/mmol; Amersham Corp., Arlington Hts., IL) as a lipid-phase marker, it was found that there was no significant loss of lipid during preparation. Therefore, based on mass balance calculations, the capture efficiency was approximately 6.83 μg of inulin/μmol of lipid. The final volume of the preparation was adjusted by adding the required volume of buffer such that each ml of the final liposomal preparation which was instilled in the rabbit eye contained 15 μCi of radioactivity.

The release of inulin was studied using a method reported by Forssen and Tokes (1979). The release was studied in isotonic phosphate buffer, pH 7.0, using a multicavity dialysis cell (Bel-Art Products, Pequannock, NJ). Standard cellulose tubing (Spactrapor, Spectrum Med Ind., Los Angeles, CA) with a molecular weight cut-off between 12,000 and 14,000 was used to separate the two compartments of the cell. While about 8% of inulin escaped into the dialysate from an aqueous solution of the drug during the first 3 h, only 1% was detected in the dialysis medium for the liposomal formulation. Intact liposomes were unable to diffuse across the dialysis membrane owing to their size. No effort was made in these experiments to size the liposomes.

The intraocular penetration of inulin was investigated using an in vivo method developed previously (Ahmed and Patton, 1985). Briefly, this consisted of topically instilling 25 μl of inulin, either as an aqueous solution or as a liposomal suspension, in the rabbit eye, and allowing for selective access of drug to the cornea, conjunctiva or both, by means of a mechanical blocking technique. The total dose of inulin instilled was 8.5 μg, and in all cases the liposomal preparation contained approximately 550 nmol of lipid. Twenty minutes following drug instillation, rabbits were sacrificed with an intravenous injection of sodium pentobarbital. The eye was dissected and aqueous humor, cornea and iris-ciliary body samples were obtained. The tissues were digested (protosol, New England Nuclear, Boston, MA) and following work-up, their drug content was determined by scintillation counting (Ahmed, 1985). The values were reported as micrograms of drug normalized for the tissue weight.

The results of the study, showing inulin levels in the aqueous humor, cornea and iris-ciliary body at 20 min following instillation of an equal dose of either a liposomal or an aqueous inulin preparation are summarized in Table 1. The 20-min timepoint was selected for evaluation since the comparative concentrations at 20 min post-instillation were reflective of the results at each time point in the concentration versus time profile carried out for a period of 4 h (Ahmed and Patton, 1985). Under all three experimental conditions listed, inulin levels in the cornea were higher when the drug was encapsulated in the liposomes as compared to an aqueous solution. The enhancement in the corneal drug levels due to liposomal entrapment when the instilled formulation was in direct contact with the cornea, was similar in magnitude to that reported in an earlier study (Stratford et al., 1983a). Although conjunctival levels were not measured in all cases in the present study, liposomal encapsulation has been shown to cause up to a 15-fold increase in inulin concentration in this tissue (Stratford et al., 1983a). This increased uptake of inulin by the cornea and conjunctiva has been attributed to physical adsorption of the lipid vesicles onto the epithelial surface of the membranes (Lee et al., 1984; Stratford et al., 1983b). In both cases, when the drug was in contact with the conjuctiva, the liposomal preparation resulted in no drug in the aqueous humor, but significantly elevated drug levels (1.6–7-fold) in the iris-ciliary body. Finally, when drug contact was restricted to the cornea, appreciable aqueous humor and iris-ciliary body levels of inulin were noted with the solution, but not for the liposomal preparation, despite higher corneal levels in the latter case.

In a separate study, the entire drug dose was administered intravenously to determine if the observed results could be accounted for by drug reaching intraocular tissues via systemic return. As

TABLE 1

IN VIVO DISTRIBUTION OF 0.034% INULIN IN THE RABBIT EYE 20 min POST-INSTILLATION, ADMINISTERED EITHER AS A LIPOSOMAL SUSPENSION OR AN AQUEOUS SOLUTION, pH 7.0, WITH VERSUS WITHOUT CORNEAL ACCESS

Tissue	Concentration in $\mu g/g$ of tissue								
	With both corneal and conjunctival access			With conjunctival access only			With corneal access only		
	Aqueous	Liposomal	P [a]	Aqueous	Liposomal	P	Aqueous	Liposomal	P
Aqueous humor	0.05 (0.005; 6) [c]	N.D. [b] ($n = 16$) [d]	< 0.001	N.D. ($n = 6$)	N.D. ($n = 16$)	NS	0.14 (0.006; 6)	N.D. ($n = 16$)	< 0.001
Cornea	0.29 (0.103; 6)	1.13 (0.186; 16)	< 0.02	N.D. ($n = 6$)	N.D. ($n = 16$)	NS	0.48 (0.010; 6)	1.25 (0.176; 16)	< 0.02
Iris-ciliary body	0.16 (0.020; 16)	0.25 (0.043; 16)	< 0.02	0.03 (0.010; 6)	0.22 (0.011; 16)	< 0.001	0.05 (0.016; 6)	N.D. ($n = 16$)	< 0.001

[a] Probability based on a two-tailed t-test.

[b] N.D. = not detectable or not statistically different from zero.

[c] Standard error of the mean and the number of determinations, respectively.

[d] The sample size.

expected, this was found not to be the case because inulin, being a large molecular weight polar compound, does not readily diffuse across the blood–ocular barrier (Davson, 1969). Additionally, a formulation consisting of empty liposomes coadministered with the drug solution behaved similarly to the drug solution itself (data not presented). This suggested that firstly, drug entrapment was necessary for the effects observed with liposomal inulin, and secondly that the effects are not due to alteration in membrane properties by the lipid vesicles. These results were consistent with those reported by other investigators using similar lipid carriers (Stratford et al., 1983a and b; Schaeffer and Krohn, 1982).

Liposomes as selective drug delivery systems for the topical route of administration have been studied previously (Singh and Mezei, 1983; Mezei and Gulasekharam, 1980; Mezei and Gulasekharam, 1982; Smolin et al., 1981). A unique property of liposomes that is revealed in this study is their apparent ability to suppress transcorneal drug penetration while promoting non-corneal drug penetration. A possible explanation for this effect may be proposed by taking into account the physicochemical property of the inulin molecule, affinity of inulin for the liposomes and the differential permeabilities of the corneal and conjuctival membranes. First, when presented in liposomes, inulin is localized in the corneal epithelium, associated with liposomes that adsorb to the corneal surface (Lee et al., 1984). However, despite high corneal levels, the weak association of the liposomes with the adsorbing surface, slow release of inulin from the liposomes and the strong barrier to diffusion afforded by the corneal epithelium (Maurice and Mishima, 1984; Benson, 1974) preclude the permeation of inulin across the cornea. This results in the absence of inulin in the aqueous humor, and hence the iris-ciliary body. On the contrary, the conjunctival epithelium offers substantially less resistance (Maurice and Mishima, 1984; Thombre and Himmelstein, 1984) and a larger surface area for diffusion to inulin than does the cornea (Ehlers, 1965). Furthermore, the contact time of liposomes may be longer with the conjunctiva than it is for the cornea, as is the case for ophthalmic solutions (Wilson et al., 1983). Therefore, increased inulin levels in the iris-ciliary body upon liposomal encapsulation may be a result of a higher drug flux across the conjunctival and scleral membranes due to markedly elevated inulin concentration at the conjunctival surface. It is highly unlikely that intact liposomes can permeate across either the cornea or the conjunctiva (Stratford et al., 1983a).

In conclusion, it may be stated that although the results presented herein may be peculiar to inulin, there is a real potential to develop liposomal dosage forms which selectively promote non-corneal drug absorption. Such delivery systems may provide enhanced drug levels in certain intraocular tissues while minimizing high concentrations of drug in the anterior chamber.

References

Ahmed, I. and Patton, T.F., Effect of pH and buffer on the precorneal disposition and ocular penetration of pilocarpine in rabbits. *Int. J. Pharm.*, 19 (1984) 215–227.

Ahmed, I., Ph.D. Thesis, The University of Kansas, Lawrence, KS, 1985.

Ahmed, I. and Patton, T.F., Importance of the non-corneal absorption route in topical ophthalmic drug delivery. *Invest. Ophthalmol.*, 26 (1985) 584–587.

Davson, H., The intraocular fluids. In Davson, H. (Ed.), *The Eye*, Academic Press, London, 1969, pp. 67–186.

Ehlers, N., On the size of the conjunctival sac. *Acta Ophthalmol. (Kbh.)*, 43 (1965) 205–210.

Forresen, E.A. and Tokes, Z.A., In vitro and in vivo studies with adriamycin liposomes. *Biochem. Biophys. Res. Commun.*, 91 (1979) 1295–1301.

Lee, V.H.L., Takemoto, K.A. and Iimoto, D.S., Precorneal factors influencing the ocular distribution of topically applied liposomal inulin. *Curr. Eye Res.*, 3 (1984) 585–591.

Maurice, D.M. and Mishima, S., Ocular pharmacokinetics. In Sears, M.L. (Ed.), *Handbook of Experimental Pharmacology, Vol. 69*, Springer-Verlag, Berlin-Heidelberg, 1984, pp. 19–116.

Mezei, M. and Gulasekharam, V., Liposomes – a selective drug delivery system for the topical route of administration. 1. Lotion dosage form. *Life Sci.*, 26 (1980) 1473–1477.

Mezei, M. and Gulasekharam, V., Liposomes – a selective drug delivery system for the topical route of administration. 2. Gel dosage form. *J. Pharm. Pharmacol.*, 34 (1982) 473–474.

Schaeffer, H.E. and Krohn, D.L., Liposomes in topical drug delivery. *Invest. Ophthalmol.*, 21 (1982) 220–227.

Singh, K. and Mezei, M., Liposomal ophthalmic drug delivery system I. Triamcinolone acetonide. *Int. J. Pharm.*, 16 (1983) 339–344.

Smolin, G., Okumoto, M., Feiler, S. and Condon, D., Idoxuri-

dineliposome therapy for *herpes simplex* keratitis. *Am. J. Ophthalmol.*, 91 (1981) 220–225.

Stratford, R.E., Jr., Yang, D.C., Redell, M.A. and Lee, V.H.L., Effects of topically applied liposomes and disposition of epinephrine and inulin in the albino rabbit eye. *Int. J. Pharm.*, 13 (1983a) 263–272.

Stratford, R.E., Jr., Yang, D.C., Redell, M.A. and Lee, V.H.L., Ocular distribution of liposome-encapsulated epinephrine and inulin in the albino rabbit. *Curr. Eye Res.*, 2 (1983b) 377–386.

Thombre, A.G. and Himmelstein, K.J., Quantitative evaluation of topically applied pilocarpine in the precorneal area. *J. Pharm. Sci.*, 73 (1983) 216–222.

Wilson, C.G., Olejnik, O. and Hardy, J.G., Precorneal drainage of polyvinyl alcohol solutions in the rabbit assessed by gamma scintigraphy. *J. Pharm. Pharmacol.*, 35 (1983) 451–454.