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Liposomal ophthalmic drug delivery system I. Triamcinolone acetonide

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Summary

The potential of liposomes as an ophthalmic drug delivery system was investigated in the rabbit. Triamcinolone acetonide as a model compound for lipophilic drugs was encapsulated into large multilamellar vesicles and instilled into the eye. A suspension form served as a control preparation. Drug distribution was determined at various time intervals. Compared to the suspension, the liposomal form produced significantly higher drug levels in the ocular tissues up to 5 h after the drug administration. The results suggest that liposomal encapsulation of the drug may be a superior drug delivery system for ocular therapy.

Introduction

One of the major problems of ophthalmic therapy is to provide and maintain an adequate concentration of the drug at the site of action for a prolonged period of time. Many of the topically applied drugs do not penetrate well into various parts of the eye (Bloomfield et al., 1978; Hanna, 1980). The commonly used opthalmic dosage form, eye drops, provide a "pulse entry" of the drug (Shell, 1980), and generally results only in a short duration of action (Massey et al., 1976; Hanna et al., 1978). To overcome some of these shortcomings, other dosage forms, like ointments and Ocusert, and routes of administration, like intraocular injections, are employed to treat ocular affections.

A new approach to achieve controlled and selective drug delivery is to use liposomes as drug carriers (Gregoriadis, 1978; Alving et al., 1978; Mezei and

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Gulasekharam, 1980; Juliano, 1981). A recent report (Smolin et al., 1981) indicated the advantage of liposomal-idoxuridine in the treatment of herpes simplex keratitis. Schaeffer and Krohn (1982) have reported that liposomal encapsulation increases the corneal penetration of both lipophilic and hydrophilic drugs. This report deals with the relative ocular disposition of a model drug, triamcinolone acetonide, instilled in the rabbit eye in liposomal and in suspension forms.

Materials and Methods

Multilamellar liposomes composed of DL-α-dipalmitoyl-phosphatidyl choline, cholesterol and triamcinolone acetonide (Sigma Chemicals, St. Louis, MO) and [^{3}H]triamcinolone acetonide (New England Nuclear, Boston, MA) in the molar ratio of 11 : 5 : 0.25 were prepared by essentially the same method as described by Bangham et al. (1965). Briefly, the lipids and triamcinolone acetonide were dissolved in chloroform in a conical flask. The solvent was evaporated under vacuum on a rotary evaporator until a thin, smooth and dry film of lipids was deposited on the walls of the flask. The flask was immersed in a water bath at 60°C for 15 min. Normal saline solution heated to the same temperature was added (1 ml/17 mg phospholipid) to the flask which was mechanically shaken for 1 h in a water bath kept at 60°C. The flask was allowed to equilibrate at room temperature for 1 h. To separate the liposomes from the unencapsulated triamcinolone acetonide crystals, the dispersion was filtered through a polycarbonate filter having a pore size of 8 μm. The radioactivity of the liposomal fraction was measured to determine the amount of encapsulated triamcinolone acetonide. The volume of liposomal fraction was adjusted so that each ml contained 0.15 mg triamcinolone acetonide with a radioactivity of 15 μCi.

A suspension of an equal strength of triamcinolone acetonide was prepared. Aliquots of triamcinolone acetonide (powder) and [^{3}H]triamcinolone acetonide (37.0 Ci/mM in benzene : alcohol) were mixed in a mortar. The solvent was allowed to evaporate. The residue was triturated with normal saline solution containing 0.05% polysorbate 80. Final volume adjustment was done with normal saline so that the preparation contained the same concentration of triamcinolone acetonide as the liposomal preparation with the same radioactivity. To ensure homogeneity and appropriate particle size, the suspension was sonicated for approximately 5 min in a bath type sonicator (Bransonic 220) before each animal experiment. The average particle size of both the liposomes and the sonicated suspension was around 5 μm, as determined by optical microscope.

Animal studies

New Zealand white rabbits of both sexes, weighing 3.0–3.4 kg were used. The rabbits were randomly divided into 3 groups, each group composed of two rabbits. Each rabbit of group A received liposomal-triamcinolone acetonide in both eyes. The rabbits of group B received the suspension in both eyes. Each rabbit of group C received liposomal preparation in one eye and the suspension form in the other eye.

A single 50 μl dose of the appropriate preparation was administered to the eye.

The lower eyelid was pulled out gently and the drop was allowed to fall on the cornea with the excess collecting in the conjunctival sac. The eyelid was gradually returned to its normal position.

The rabbits were sacrificed by an injection of pentobarbitone sodium into the marginal ear vein at 0.5, 1, 3 and 5 h intervals. About 0.1–0.2 ml of aqueous humor samples were transferred from the anterior chamber to preweighed counting vials with the aid of a 25-gauge needle attached to a 1-ml tuberculin syringe. The eyes and the optic nerves were enucleated and frozen in liquid nitrogen. Blood samples were withdrawn from the heart just before the pentobarbitone was injected.

The frozen eye globe was bisected (Abel and Boyle, 1976) past through the back of the lens, and a sample of vitreous humor was transferred to preweighed vials. Lens, iris, ciliary body, cornea, conjunctiva and sclera were separated, dipped in normal saline and blotted dry. Each tissue was placed in separate preweighed vials. The vials were reweighed. The tissues were digested by the addition of 1 ml of NCS tissue solubilizer (Amersham). Ten ml of Biofluor (New England Nuclear) was added to each vial and the samples were counted in the liquid scintillation counter (Beckman LS 3133T), utilizing a quench curve prepared by external standardization. Ten ml of blood was extracted with 30 ml methanol 3 times. The solvent was evaporated. The residue was re-extracted with acetone into counting vials and evaporated before adding 10 ml Biofluor.

Results and Discussion

The drug concentration in various ocular tissues at different time intervals after a single application of liposomal or suspension preparations is shown in Table 1. The liposomal preparation provided significantly higher drug levels ($P < 0.05$) than the suspension in the ocular tissues for up to 3 h. A more than 2-fold drug concentration was obtained and maintained in the aqueous humor for up to 5 h with the liposome-encapsulated triamcinolone acetonide when compared with that achieved with the control preparation.

At 0.5 h after administration the corneal and aqueous humor levels were 2.5–2.9 times higher with the liposomal preparation than those with the control. The external ocular tissues, conjunctiva and sclera showed approximately 4-fold higher drug concentrations, which may suggest a stronger liposomal attraction for these tissues.

It is possible that the peak concentration occurred before the 0.5 h measurement in the cornea, conjunctiva and sclera in case of the treatment with both the test and control preparations. Comparing the drug levels measured in the aqueous humor, lens and iris, however, the values are the same or higher at 1.0 h than at 0.5 h with the control preparation, but the pattern is reversed with the liposomal product. This indicates that the liposomal form provides not only higher drug concentration in most ocular tissues, but also a higher rate of drug delivery, and drug absorption.

A small amount of the drug apparently entered systemic circulation on application of the control preparation and was detected in the blood for up to 3 h. No

TABLE 1
CONCENTRATION OF TRIAMCINOLONE ACETONIDE IN ng/g OF THE TISSUE [a]

	0.5 h		1 h		3 h		5 h	
Tissue	Suspension	Liposomes	Suspension	Liposomes	Suspension	Liposomes	Suspension	Liposomes
Cornea	462.0 ± 46.5	1159.5 ± 299.2	285.0 ± 72.0	491.2 ± 189.0	177.7 ± 88.5	312.0 ± 92.2	39.5 ± 20.2	89.2 ± 25.5
Aqueous humor	51.0 ± 11.2	149.2 ± 18.0	51.0 ± 19.5	117.7 ± 36.7	19.5 ± 9.7	66.0 ± 20.2	6.0 ± 3.0	15.7 ± 3.7
Lens	2.2 ± 0.5	8.25 ± 2.2	3.7 ± 0.7	6.7 ± 2.2	3.7 ± 0.7	6.7 ± 3.0	3.7 ± 1.5	4.5 ± 1.5
Iris	62.2 ± 12.7	143.2 ± 39.7	83.2 ± 12.0	135.0 ± 38.2	39.7 ± 8.2	80.2 ± 20.2	–	trace
Ciliary body	33.0 ± 6.7	84.0 ± 6.0	61.5 ± 20.2	96.0 ± 30.7	–	60.7 ± 12.0	–	trace
Conjunctiva	80.2 ± 34.5	319.5 ± 126.7	65.2 ± 28.5	136.5 ± 64.5	68.2 ± 4.5	38.2 ± 13.5	30.7 ± 19.5	23.2 ± 10.5
Sclera	57.7 ± 18.5	239.2 ± 111.7	37.5 ± 8.2	70.5 ± 24.7	17.2 ± 6.0	25.5 ± 5.2	7.5 ± 3.7	12.0 ± 5.2
Blood	1.1	–	3.2	–	2.6	–	–	–

[a] Mean ± standard deviation (n = 6).

radioactivity was detected in the blood of rabbits treated with the liposomal form. This indicates that the systemic side-effects of steroids on topical application in ocular therapy may be reduced by liposomal encapsulation.

Analysis of brain, liver, spleen and the optic nerve of both treated and control rabbits, showed no radioactivity during the time intervals studied. Vitreous body in some cases contained trace, but not quantifiable, amounts of drug.

The possible mechanisms by which liposomes can interact with the cells (i.e. deliver their content) can be divided into 4 categories: lipid exchange, stable adsorption, endocytosis and fusion (Poste and Papahadjopoulos, 1978). It is not possible to assign a single mechanism in a given actual in vivo system, as a combination of any one of these may be responsible in that experimental situation. It is difficult to ascertain whether intact liposomes penetrate through the cornea, or fuse with the corneal cells, or remain in the conjunctival sac and interact with tear components leading to gradual release of the entrapped drug which is then absorbed by the cornea. The elimination profiles of the drug in the eye are similar in both the liposomal and suspension dosage forms. If liposomes were absorbed intact, they might prefer one or the other intraocular tissue, accumulating in that tissue. But no such trend is seen in the rabbits treated with liposomes. We can only speculate that the drug may be released from the liposomes at the corneal surface, and then enter the aqueous humor and intraocular tissues. This supposition would not hold true if the intraocular distribution pattern of the liposomal and the "free" drug are identical.

On the basis of this study and other reports (Smolin et al., 1981; Schaeffer and Krohn, 1982), it seems that liposomes have a good potential as a drug delivery system in the ocular therapy; the liposomal form provided a higher drug concentration than did the suspension dosage form.

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