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Liposomal ophthalmic drug delivery system. II. Dihydrostreptomycin sulfate

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Summary

The carrier ability of liposomes for a model hydrophilic compound was investigated in the rabbit eye. Dihydrostreptomycin sulfate was encapsulated in various types of liposomes, i.e. large and small uni- and multilamellar vesicles having either positive or neutral surface charge. An aqueous solution served as control preparation. Results indicated that liposomal encapsulation reduced the ocular drug concentration. Addition of empty liposomes to the control solution did not alter drug levels in most of the ocular tissues. Among the liposomal preparations the large multi- and unilamellar vesicles provided higher drug concentration in all ocular tissue than the small unilamellar ones. Introduction of a positive charge on liposome surface enhanced liposome–conjunctiva interactions. The results suggest that liposomal encapsulation alters drug disposition in the eye depending on the type of liposomes and the physicochemical properties of the encapsulated drug. In the case of the dihydrostreptomycin sulfate and possibly other hydrophilic drugs the liposomal encapsulation provides no advantages as far as drug delivery is concerned.

Introduction

The most common ophthalmic dosage form, the eyedrop, does not provide an optimal drug delivery system to the intraocular tissues. Less than 3% of a drug applied topically in solution form penetrates the cornea (Benson, 1974; Patton and Francoeur, 1978). The other disadvantages of the eyedrop are the pulse-entry and

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most of the time a short duration of action (Hanna et al., 1978). Research to develop new drug delivery systems for ophthalmic drug preparations have recently been intensified (Ruben and Trodd, 1978; Hanna, 1980; Gurny, 1981; Chiou and Watanabe, 1982).

The potential of liposomes as drug carriers in medicine has been widely studied (Gregoriadis, 1979; Juliano, 1981). Liposomal preparations, when administered by various routes including intravenous (New et al., 1981), intramuscular (Arrowsmith et al., 1981), oral (Patel and Ryman, 1976) and topical (Mezei and Gulasekharam, 1982) have been shown to enhance the bioavailability of the entrapped drug. Their potential usefulness in ocular therapy was first reported by Smolin et al. (1981), who described the advantage of liposome associated idoxuridine over a solution of the drug in the treatment of herpes simplex keratitis in the rabbit. Schaefer and Krohn (1982) observed that the corneal penetration of penicillin G, a water-soluble antibiotic, and the ocular bioavailability of indoxole, a lipophilic compound, were increased by entrapping them in liposomes. Stratford et al. (1983) found that liposomal entrapment reduced the corneal and conjunctival absorption of epinephrine by 50%, but it greatly (10 times) increased the absorption of inulin. We have recently reported (Singh and Mezei, 1983) that the liposomal form of triamcinolone acetonide produced higher drug levels in the ocular tissues than the suspension form. This report deals with the study of the ocular disposition of a water-soluble antibiotic, dihydrostreptomycin sulfate (DHSS), in solution and in various liposomal forms in the rabbit. The tritiated form of DHSS was used in order to have a sensitive assay technique for the determination of the drug in the ocular tissues.

Materials and Methods

Dipalmitoylphosphatidyl choline (DPPC) cholesterol (CHOL), stearylamine (SA) and dihydrostreptomycin sulfate (DHSS) were purchased from Sigma Chemicals, St. Louis, MO. Tritiated dihydrostreptomycin sulfate ([^{3}H]DHSS) was obtained from Amersham, Oakville, Ontario.

Multilamellar vesicles (MLV), large unilamellar vesicles (LUV) and small unilamellar vesicles (SUV) were prepared by the methods described by Bangham et al. (1965), Szoka and Papahadjopoulos (1978) and Shaefer and Krohn (1982), respectively. Neutral liposomes were prepared from DPPC and CHOL in the mole ratio of 7 : 2. Liposomes with a positive charge were composed of DPPC:CHOL:SA in the mole ratio of 7 : 2 : 1. The aqueous swelling solvent consisted of normal saline containing a mixture of DHSS and [^{3}H]DHSS. The unencapsulated drug was separated from liposomal DHSS by different procedures. MLV and LUV were centrifuged at 20,000 *g* for 45 min, the supernatant was removed, and the pellet was resuspended in normal saline. This process was repeated three times. The unentrapped drug was separated from the SUV by passing the liposomes through a Sephadex G-150 column. The liposomal fraction was then ultracentrifuged at 160,000 *g* for an hour. The supernatant was removed and the pellet was resuspended in normal saline to give the desired concentration. The entrapment efficiency was

found to be 8% for MLV, 17% for LUV and 2.5% for SUV. Two control preparations were used: an aqueous solution of DHSS/[^{3}H]DHSS in normal saline (Control I), and a DHSS/[^{3}H]DHSS solution mixed with preformed empty liposomes (Control II). All of the above preparations were adjusted to contain 0.1% DHSS. The pH in all preparations was 6.8 ± 0.2.

New Zealand white rabbits, of both sexes, weighing 2.5–3 kg were used for animal experiments. The lower eyelid was gently pulled away from the globe, and a single 20 μl dose of one preparation was instilled on the cornea, with the excess collecting in the conjuctival sac. Forty minutes after drug administration, the animals were sacrificed by an intravenous injection of pentobarbitone sodium. Aqueous humor was withdrawn from the anterior chamber with the aid of a 25-gauge needle fitted to a tuberculin syringe. The eyes were enucleated and immediately frozen in liquid nitrogen to minimize post-mortem redistribution of the drug. Frozen eyes were dissected to separate cornea, lens, iris, ciliary body, vitreous humor, conjunctiva and sclera. Each tissue was rinsed with normal saline, blotted dry, and transferred to preweighed counting vials. The vials were reweighed, and the weight of the tissues was calculated. The tissues were digested at 50 °C for 24 h in 1 ml of NCS tissue solubilizer (Amersham). The colored samples were decolorized by adding 200 μl of hydrogen peroxide and warming at 50 °C for 30 min. Ten ml Bioflour, (New England Nuclear) and 100 μl of glacial acetic acid were added to each vial. The samples were dark-adapted for 24 h before counting in a liquid scintillation counter (Beckman, LS 3133 T). A quench curve prepared by external standardization method, was utilized to calculate DHSS concentrations.

Results and Discussion

Drug concentration in various ocular tissues, 40 min after the topical application of the control and liposomal preparations is shown in Table 1. The control preparations provided higher drug levels than the liposomal preparations in all ocular tissues. The addition of empty liposomes to DHSS solution (Control II) did not alter the drug concentration in most of the ocular tissues ($P > 0.05$), except in the cornea ($P < 0.01$) and sclera ($P < 0.02$). The relative drug distribution, however, was similar in intraocular tissues in both cases.

All the liposomal preparations, neutral MLV, LUV, SUV, and positively charged LUV, SUV, each containing an equivalent amount of the drug per dose, showed markedly lower levels than was observed in the controls. The aqueous humor levels were 15–20 times below those produced by the control solutions. The intraocular tissues such as iris, ciliary body and vitreous humor showed no detectable radioactivity.

Statistical analysis (t-test) of the data obtained with the neutral and positively charged liposomes of the same type showed no difference ($P > 0.2$) in drug levels of aqueous humor. The corneal drug levels were, however, higher ($P < 0.001$) in the eyes treated with neutral LUV than in those treated with positively charged LUV. The conjunctival drug levels were higher ($P < 0.05$) as a result of treatment with

TABLE 1

CONCENTRATION OF DIHYDROSTREPTOMYCIN SULFATE [a] (ng/g of the tissue) [b]

Tissue	Control [c]	Control [d]	MLV [e]	LUV [f]	LUV+ [g]	SUV [h]	SUV+ [i]
Aqueous humor	147.2 ± 28.0	151.8 ± 28.6	12.2 ± 1.5	10.6 ± 2.5	11.0 ± 2.1	6.5 ± 1.1	7.3 ± 1.9
Vitreous humor	6.6 ± 2.7	11.2 ± 2.3	–	–	–	–	–
Lens	18.7 ± 5.3	30.3 ± 7.5	2.7 ± 1.5	1.4 ± 0.3	1.6 ± 0.3	2.0 ± 0.3	3.1 ± 0.6
Iris	55.6 ± 8.3	49.9 ± 6.0	–	–	–	–	–
Ciliary body	40.1 ± 4.8	40.7 ± 11.6	–	–	–	–	–
Cornea	114.9 ± 22.2	74.7 ± 20.2	18.3 ± 3.4	20.8 ± 5.8	10.0 ± 3.2	13.2 ± 3.5	15.4 ± 5.1
Conjunctiva	98.6 ± 32.9	75.5 ± 32.8	32.3 ± 6.3	20.8 ± 5.2	32.6 ± 13.8	14.5 ± 8.4	34.2 ± 6.0
Sclera	50.1 ± 17.0	29.5 ± 10.8	6.8 ± 1.2	5.6 ± 1.7	8.6 ± 3.2	3.2 ± 1.1	4.8 ± 1.6

[a] The concentration of the drug was measured 40 min after administration of a 20 μl dose

[b] Mean ± S.D. ($n = 8$).

[c] DHSS solution in normal saline.

[d] DHSS solution mixed with empty liposomes.

[e] Multilamellar vesicles.

[f] Large unilamellar vesicles.

[g] Large unilamellar vesicles with positive surface charge.

[h] Small unilamellar vesicles.

[i] Small unilamellar vesicles with positive surface charge.

positively charged SUV as compared to the treatment with neutral SUV. However, the introduction of a positive charge to SUV did not increase the drug penetration through the cornea into the eye. The initial electrostatic interaction between the SUV and the corneal surface as shown by in vitro experiments (Schaefer and Krohn, 1982) was probably upset in the in vivo experiments due to the precorneal tear fluid. Introduction of a positive charge, though, tended to enhance conjunctival absorption of the drug.

The penetration of drug molecules into the eye from a topically applied preparation is a complex phenomenon. The rate of drug penetration depends not only on the physicochemical properties of the drug itself, such as its solubility (Hanna et al., 1980) and particle size, in case of the suspensions (Schoenwald and Stewart, 1980), but also on those of its vehicle (Kupferman et al., 1981). The conventional ophthalmic dosage forms such as ointments, are assumed to release the drug from its vehicle into the tear film for subsequent absorption into the cornea. In the liposomal dosage form the drug is encapsulated in lipid vesicles, which can cross cell membranes. The liposomes, therefore, can be viewed as drug carriers, and as such, they can change the rate and extent of absorption, as well as the disposition of the drug. As yet there is not much known about the mechanism by which liposomes interact with the cornea.

Various possible mechanisms by which liposomes can interact with the cells include lipid exchange, stable adsorption, endocytosis and fusion (Huang et al., 1978). Endocytosis usually occurs in the cells capable of phagocytosis (Onaga and Baillie, 1980). Consequently this mechanism cannot be a dominant one here. Fusion of the liposomes with the cells requires special conditions of lipid fluidity (Martin and MacDonald, 1976), temperature (Ekerdt et al., 1981) and is more prominent in the presence of certain chemical agents (Szoka et al., 1981). Fusion of liposomes with the cornea may exist to some extent, but the major mechanism might be the adsorption and/or surface lipid exchange. This could explain why the water-soluble drug, DHSS, which is completely enclosed with the liposomal bilayers, produced lower ocular drug concentration in liposomal form than in its solution form. On the other hand, the ocular drug concentration of lipophilic drugs has been increased by liposomal encapsulation (Stratford et al., 1983; Singh and Mezei, 1983). If the liposomes were taken up as such, absorbed as intact vesicles containing their drug, then the ocular drug concentration would be similar irrespective of the nature of the drug entrapped; the absorption would only depend on the type of liposomes, their size and surface charge. If the mere presence of liposomes, ('empty' lipid vesicles) added to drug solutions had enhanced the permeability of the cornea, then Control II should have shown higher drug levels in cornea and aqueous humor; this was not found in this investigation. Similar observations were reported by Stratford et al. (1983).

Conclusion

Any explanation related to the mechanisms by which liposome-encapsulation alters ocular drug disposition can be only speculative at the present. On the basis of

this and previous investigations (Stratford et al., 1983; Singh and Mezei, 1983), it could be concluded, however, that the ocular drug concentration achieved by the use of liposomal preparations seems to depend on the type of association between the drug molecules and the lipid vesicles. The lipophilic drugs, located within or bound to the lipid bilayers may have a different fate as far as drug disposition is concerned, than the hydrophilic drugs that are within the aqueous compartments of the uni- or multilamellar liposomes. One should realize, however, that this conclusion is based on limited data. Although DHSS was used as a model for hydrophilic drugs, it is possible that another hydrophilic drug encapsulated in these types of liposomes may behave differently. It is also possible that the same types of liposomes may provide different 'delivery systems' if their lipid compositions are different than those used in this investigation.

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