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LIPOSOMAL ENCAPSULATION AND STABILITY OF DIDEOXYINOSINE TRIPHOSPHATE

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ABSTRACT

Dideoxyinosine triphosphate (ddITP) was encapsulated in multilamellar liposomes prepared with various lipid composition. The stability of liposomes in terms of retention of ddITP was measured at 4°, 25° and 37° C. The encapsulation of ddITP was 7.5 times greater in liposomes prepared with dipalmitoylphosphatidylcholine (DPPC) compared to dimyristoylphosphatidylcholine (DMPC). When equimolar cholesterol (CHOL) was added to DMPC liposomes the encapsulation of ddITP was increased by 4.5 times. The leakage of ddITP was 60% from DMPC liposomes stored at 4° and 25° C after a month and 100% leakage after 16 days when stored at 37° C. The leakage of ddITP from DMPC:CHOL liposomes was only 20% after a month at 4° C, 50% at 25° and 90% at 37° C. These results suggest that the encapsulation of hydrophilic compound such as ddITP can be increased either by increasing the fatty acid chain length (DPPC) or by inclusion of CHOL. However, the optimum encapsulation and retention of ddITP was achieved using DMPC:CHOL liposomes. Retention of ddITP in these liposomes was maximum when stored at 4° C.

INTRODUCTION

Liposomal encapsulation of drugs can dramatically alter the distribution of drugs in the body and their rate of clearance[1]. These pharmacokinetic

differences and other, less well understood effects can result in "targeting" a drug to particular organs or sites of disease, prolonged levels in serum or tissue, reduced toxicity, and/or enhanced efficacy of the encapsulated drug. Multilamellar liposomes (MLVs) are readily prepared by hydration of thin lipid films and subsequent agitation[2]. The size distribution and entrapment of aqueous phase are governed by the hydration time, method of lipid dispersion, thickness of the lipid film, and the concentration and composition of the lipid phase[3]. Sonication of MLVs produces a more homogeneous population of small unilamellar vesicles (SUVs). Liposomes of intermediate size between MLVs and SUVs and entrapping a high percentage of aqueous phase can be produced by the techniques of ether infusion[4] or reverse phase evaporation[5].

To formulate drugs in liposomes it is necessary to reduce the leakage of an entrapped drug. The rate of leakage of a molecule from liposomes is governed by the physico-chemical properties of a molecule. Liposomes are freely permeable to water, but cations are released at a slower rate than anions[2], whereas, aqueous hydrogen bonding may determine the leakage rate of non-electrolytes[6]. The degree of disorder of the lipid bilayer determines the permeability of liposomes. Phospholipids in the liquid crystalline state are more permeable to entrapped material than when they are in the gel state. Thus, loss of entrapped material is temperature dependent, generally being greatest around the phospholipid phase transition temperature (T_c)[7]. At T_c rapid efflux of material has been attributed to passage through regions of high bilayer disorder, where gel and liquid crystalline states temporarily coexist. The incorporation of cholesterol into liposomal bilayers decreases the rotational freedom of the phospholipid hydrocarbon chains. At 50 mol% cholesterol the phase transition is lost, the efflux rate of cations is decreased, and the leakage is independent of temperature[8]. At temperatures below the T_c of the

phospholipids incorporation of cholesterol into liposomes decreases the release rate of hydrophilic materials[9,10], while producing a much smaller effect on the loss of lipophilic materials[11]. It is important to determine the lipid composition in the preparation of liposomes that are stable in terms of retention of the encapsulated drug. In this study an anti-AIDS drug dideoxyinosine triphosphate (ddITP), an active metabolite of ddI was encapsulated in liposomes of various lipid composition, efficiency of encapsulation and stability of these formulations were studied.

EXPERIMENTAL

MATERIALS

Dimyristoyl-L-α-phosphatidylcholine (DMPC), and dipalmitoyl-L-α-phospha-tidylcholine (DPPC), were obtained from Avanti polar lipids, Birmingham, AL. Cholesterol (CHOL), dicetyl phosphate (DCP), Dideoxyinosine triphosphate (ddITP) was purchased from Sigma Chemical Co., St. Louis, MO. ^{3}H-ddITP was purchased from Moravek Biochemicals (Brea, CA). Glass triple distilled water was used in the preparation of all aqueous solutions. All other chemicals and solvents were reagent grade.

METHODS

Preparation of liposomes: Phospholipids with or without cholesterol were utilized in the preparation of liposomes. Bangham method was used to prepare liposomes[2]. Briefly, the lipids were dissolved in chloroform and the chloroform was evaporated using rotary evaporator. ddITP was dissolved in phosphate buffer saline (PBS, pH 7.4) and added to the flask containing lipid film. The flask was vortex mixed until all the lipid was dispersed in the aqueous phase. ^{3}H-ddITP was used as tracer to measure the encapsulation and leakage.

TABLE 1

Phase transition temperature (T_c), Particle size distribution, and Encapsulation of ddITP in liposomes of various lipid composition.

Lipid Composition	T_c	Particle size (Mean±SD)	Encapsulation (%)
DMPC	23°	788±28	1.53
DPPC	41°	1370±37	11.64
DMPC:CHOL (1:1 mole ratio)	---	820±29	7.10

DMPC = Dimyristoyl phosphatidylcholine

DPPC = Dipalmitoyl phosphatidylcholine

CHOL = Cholesterol

Measurement of ddITP leakage: 100 μl samples of liposomes were transferred to Eppendorf tubes and stored at 4°, 25° and 27° C. Every 48 hrs, the samples from each temperature and formulations were centrifuged in microcentrifuge (10,000 rpm) for 10 minutes. Supernatants (50 μl) were transferred to scintillation vials, scintillation fluid was added and the radioactivity was measured using liquid scintillation counter.

Particle Size Analysis: Light scattering measurements were performed with a Coulter submicron particle size analyzer (Model N4MD). The liposome preparations were diluted 1:20 with filtered PBS (pH 7.4). The instrument settings used were as follows: temperature 20° C; viscosity 0.01 P; refractive index, 1.333; scattering angle, 90°; run time 300 sec; range 0 - 3000 nm.

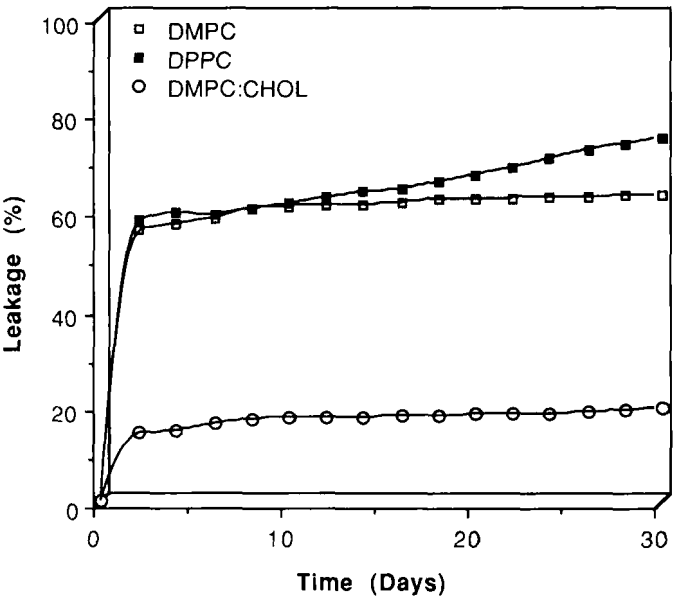

FIGURE 1

Leakage of ddITP from multilamellar liposomes prepared with various lipid composition at 4° C.

RESULTS AND DISCUSSION

The phase transition temperature (T_c), particle size distribution and encapsulation of ddITP in various lipid composition is shown in table 1. The mean particle size increased with an increase in fatty acid chain length (DPPC). The liposomes prepared with dipalmitoylphosphatidylcholine (DPPC) showed the maximum encapsulation of ddITP. The addition of cholesterol (CHOL) increased the encapsulation of ddITP in dimyristolphosphatidylcholine (DMPC) liposomes by 4.5 times. Inclusion of cholesterol and increase in fatty acid chain length increases the ordered structure lipid bilayers. This effect reduces the immediate leakage of an entrapped drug from liposomes and results into higher encapsulation.

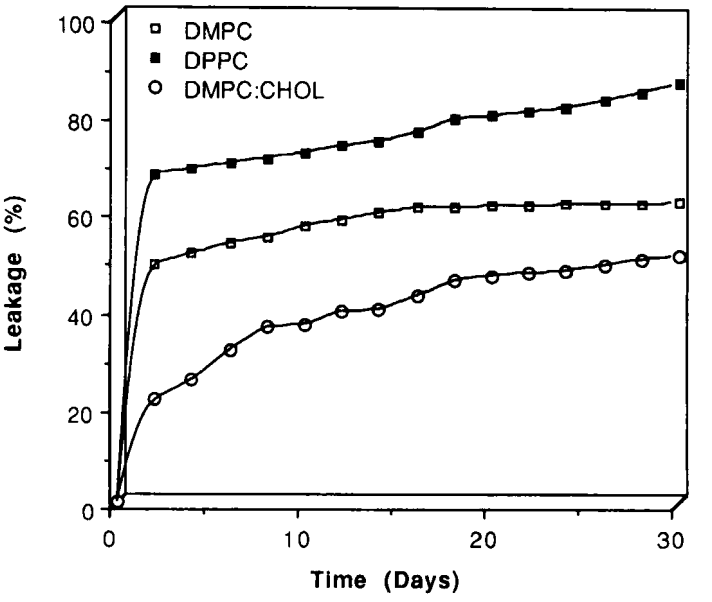

FIGURE 2

Leakage of ddITP from multilamellar liposomes prepared with various lipid composition at 25° C.

The leakage of ddITP from liposomes at 4°, 25° and 37° C are shown in Fig. 1, 2 and 3 respectively. The leakage at 4° and 25° is biphasic in liposomes prepared with all the lipid compositions. The rate of leakage is faster within first two days followed by slower rate of leakage.The leakage was least with liposomes prepared with DMPC:CHOL (1:1 mole ratio) at 4° C. The leakage from DMPC and DPPC was higher and similar at 4° C. After 30 days 72% of ddITP was leaked from DPPC liposomes 60% from DMPC liposomes and <20% from DMPC:CHOL liposomes at 4° C. Leakage of ddITP from DMPC:CHOL liposomes increased drastically with an increase in temperature from <20% at 4° to 50% at 25° and 90% at 37° C after 30 days. However, the leakage of ddITP was maximum in DMPC liposomes at 37° C. The leakage was 100% from DMPC liposomes within 16 days and 26 days from DPPC liposomes.

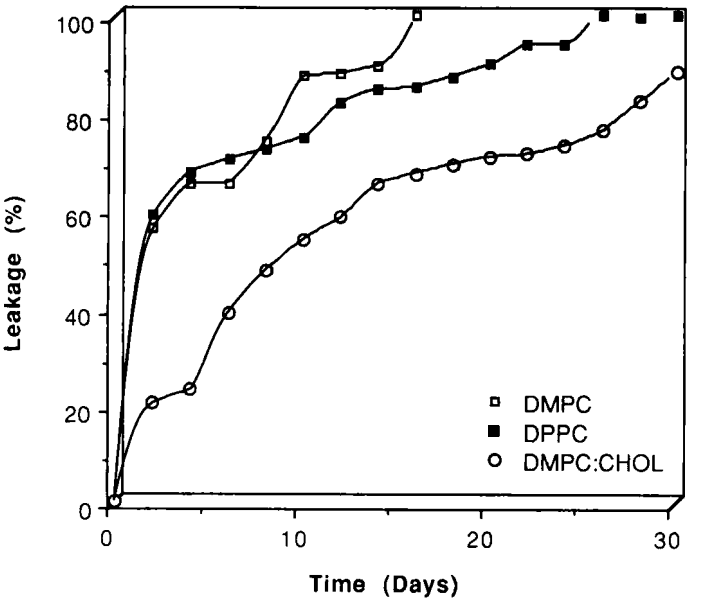

FIGURE 3

Leakage of ddITP from multilamellar liposomes prepared with various lipid composition at 37° C.

The leakage process is also influenced by the physical state of the molecules in the liposomal bilayers. The T_c of DMPC is 23° C, and therefore except liposomes stored at 4° C are in fluid state. Hence, reduced leakage of ddITP was observed at 4° C. The T_c of DPPC is 41° C, and therefore liposomes stored at 4°, 25° and 37° C are in gel state. Hence, the temperature did not play significant role in leakage of ddITP from DPPC liposomes. Addition of equimolar concentration of cholesterol to DMPC eliminates T_c and alters the fluidity of the membrane both below and above T_c. However, by affecting the fluidity of DMPC liposomes the encapsulation of ddITP was increased and leakage was reduced significantly at 4° C.

These results suggest that the encapsulation of hydrophilic compound such as ddITP can be increased either by increasing the fatty acid chain length

(DPPC) or by inclusion of CHOL in DMPC. However, the optimum encapsulation and retention of ddITP was achieved using DMPC:CHOL liposomes. Retention of ddITP in these liposomes was maximum when stored at 4° C.

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REFERENCES

1. K. J. Hwang, in "Liposomes: from biophysics to therapeutics", Marcel dekker, Inc., New York.1987. p. 109.

2. A. D. Bangham, M. M. Standish, and J. C. Watkins, J. Mol. Biol. 13, 238 (1965).

3. F. Olson, C. A. Hunt, F. C. Szoka, W. J. Vail, and D. Papahadjopoulos, Biochim. Biophys. Acta, 557, 9 (1979).

4. D. Deamer, and A. D. Bangham, Biochim. Biophys. Acta, 443, 629 (1976).

5. F. Szoka, and D. Papahadjopoulos, Proc. Natl. Acad. Sci. U.S.A., 75, 4194 (1978).

6. B. E. Cohen, J. Membr. Biol. 20, 205 (1975).

7. D. Papahadjopoulos, K. Jacobson, S. Nir, and T. Isac, Biochim. Biophys. Acta, 311, 330 (1973).

8. J. De Gier, J. G. Mandersloot, and L. L. M. Van Deenen, Biochim. Biophys. Acta, 150, 666 (1968).

9. R. Ganapathi, A. Krishnan, I. Woodinsky, C. G. Zubord, and L. J. Lesko, Cancer Res. 40, 633 (1980).

10. J. Senior, and G. Gregoriadis, Life Sci. 30, 2123 (1982).

11. R. Ganapathi, and A. Krishnan, Biochem. Pharmacol. 33, 698 (1984).