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COMMUNICATION

Nonionic Surfactant Vesicles (Niosomes) of Cytarabine Hydrochloride for Effective Treatment of Leukemias: Encapsulation, Storage, and In Vitro Release

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ABSTRACT

Niosome vesicles of cytarabine hydrochloride were prepared by a lipid hydration method that excluded dicetylphosphate. The sizes of the vesicles obtained ranged from 600 to 1000 nm, with the objective of producing more blood levels in vivo. The study of the release of drug from niosomes exhibited a prolonged release profile as studied over a period of 16 hr. The drug entrapment efficiency was about 80% with Tween 80, Span 60 and Tween 20; for Span 80, it was 67.5%. The physical stability profile of vesicular suspension was good as studied over a period of 4 weeks.

Key Words: *Cytarabine hydrochloride; Niosomes.*

INTRODUCTION

One way of achieving the transport of drug directly by a delivery device is by means of liposomes, which can deliver drugs to the desired location in the body and reach a high local concentration (1). This kind of drug delivery system could be used successfully in the treatment of malignancy by means of cytotoxic drugs (2–4). Nonionic surfactant vesicles (niosomes) are now widely studied as an alternative to liposomes, and an increasing number of nonionic surfactants have been found to form vesicles capable of entrapping hydrophilic and hydropho-

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bic drugs (5). Niosomes are biodegradable, biocompatible, and nontoxic and are capable of encapsulating large quantities of material in a relatively small volume of vesicles (6). Inclusion of cholesterol in the preparation of niosomes has been demonstrated to alter the properties of niosomes and markedly decrease efflux–entrapped solute (7). Niosomes behave in vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability (8–10).

Being an important antimetabolite, cytarabine hydrochloride (i.e., cytosine arabinoside hydrochloride [CTH]) provides an effective treatment for acute lymphocytic and granulocytic leukemias (11,12). The low lipophilicity of CTH gives rise to various problems that result in the reduction of bioavailability, particularly when administered orally. Therefore, the alternative route of administration for obtaining optimum bioavailability is the parenteral route. For effective cancer chemotherapy, a high concentration of anticancer agent is required at the tumor site for a particular period of time. This minimizes the concentration of the drug in other tissue compartments of the body, thus minimizing their adverse reactions. Niosomes-1jyhave been studied by several groups for enhanced delivery of anticancer agents to regional lymphatics (13–15).

Niosomes can provide a drug delivery system that creates selectively high concentrations of anticancer agents in the lymphatic system to prevent metastasis and for the treatment of malignant lymphoma. However, the conventional method of incorporating dicetylphosphate yields niosomes of somewhat smaller size (100–200 nm) (16), which may be unsuitable for CTH, for reasons explained below.

The purpose of this investigation was to develop and characterize cytarabine-encapsulated niosomes without incorporating dicetylphosphate, which yields niosomes of somewhat large size. These larger niosomes are accumulated in the liver to a lesser extent and thus converts CTH to the inactive deaminated form, ensuring a higher plasma level (17). The entrapment efficiency and niosome size were determined to optimize the manufacturing conditions. In vitro release and storage stability studies were carried out to assess the suitability of the drug delivery system.

EXPERIMENTAL

Materials

Cytarabine hydrochloride (98.7%), Span 60, Span 80, Tween 20, Tween 80, and cholesterol were obtained from Sigma Chemical (St. Louis, MO). All solvents used were high-performance liquid chromatography (HPLC) grade, and other chemicals were analytical grade.

Preparation of Niosomes

Niosomes were prepared by a lipid hydration method (8). Surfactant and cholesterol (1:1; 75 mg each) were dissolved in diethyl ether, and the solvent was evaporated using a rotary flash evaporator (Yamato RE-67), under low pressure at 40°C–50°C for preparing niosomes. Niosomes were formed by adding 2 ml of 7.8 mM phosphate buffered saline (PBS) (pH 7.5) containing CTH (20 mg ml^{-1}) slowly to the dried thin film formed on the walls of the round-bottom flask, which was heated to about 40°C–50°C on a water bath with gentle agitation. The mixture was intermittently mixed on a vortex. Dispersion of the mixture was carried out at 25°C using a probe sonicator, 20-kHz, 500-W vibra cell (Sonics and Materials, Inc., Co., USA) for 30 sec at 1-min intervals for a period of 3 min. After sonication, the suspension was maintained for 2 hr to allow niosomes to form and seal.

Removal of Unentrapped Solute

The aqueous dispersions of CTH niosomes prepared were dialyzed exhaustively in Cuprophane dialysis tubing against PBS.

Size Characterization

The sizes of the vesicles were obtained from the magnification data of electron microscopy for 10 batches of vesicles at random. The average was calculated from the observed value of 10 batches.

Entrapment Efficiency of Cytosine Arabinoside Hydrochloride

To determine the entrapment efficiency of CTH, 0.1 ml of freshly prepared niosomal suspension was diluted to 2 ml with PBS, sedimented using an ultracentrifuge (Beckman 4C-90) at 200,000*g* for 30 min at 4°C. The supernatant liquid was separated. To the sediment, 2 ml of 10% Triton X-100 was added, and the mixture was shaken well to lyse the vesicles to release the encapsulated CTH (7). It was then diluted to 100 ml with PBS, filtered using a membrane filter (0.2-mm pore size), and measured using a spectrophotometer (Hitachi 220A) at 272 nm. In each case, niosomal suspension without drug

was treated likewise and regarded as a blank. The efficiency of CTH entrapment is defined as follows:

$$\text{Efficiency of CTH entrapment (\%)} = ([\text{Amount entrapped})/(\text{Total amount of CTH})] \times 100$$

In Vitro Release of Cytosine Arabinoside Hydrochloride from Niosomes into Phosphate Buffered Saline

After separation of the free drug, the niosome preparation was transferred to a dialysis tube and subjected to dialysis with the dialysis tube immersed in a receptor compartment containing PBS (100 ml). At different time intervals for 16 hr, 5-ml samples were withdrawn from the receptor compartment, and the drug content was determined spectrophotometrically at 272 nm. Each sample withdrawn was replaced by an equal volume of PBS.

CTH degradation was not observed during drug release. The lack of degradation of CTH was confirmed by HPLC (Spectra-Physics SP 8800 HPLC pump, SP 100 ultraviolet detector, and SP4400 computing integrator). The stationary phase was 5-mm Spherisorb ODS2 (25 cm × 0.46 cm); the mobile phase was 0.005 M monobasic sodium phosphate in distilled water containing 5% (v/v) methanol (18). The flow rate was set at 1.2 ml min^{-1}, and the detector wavelength was 272 nm. The

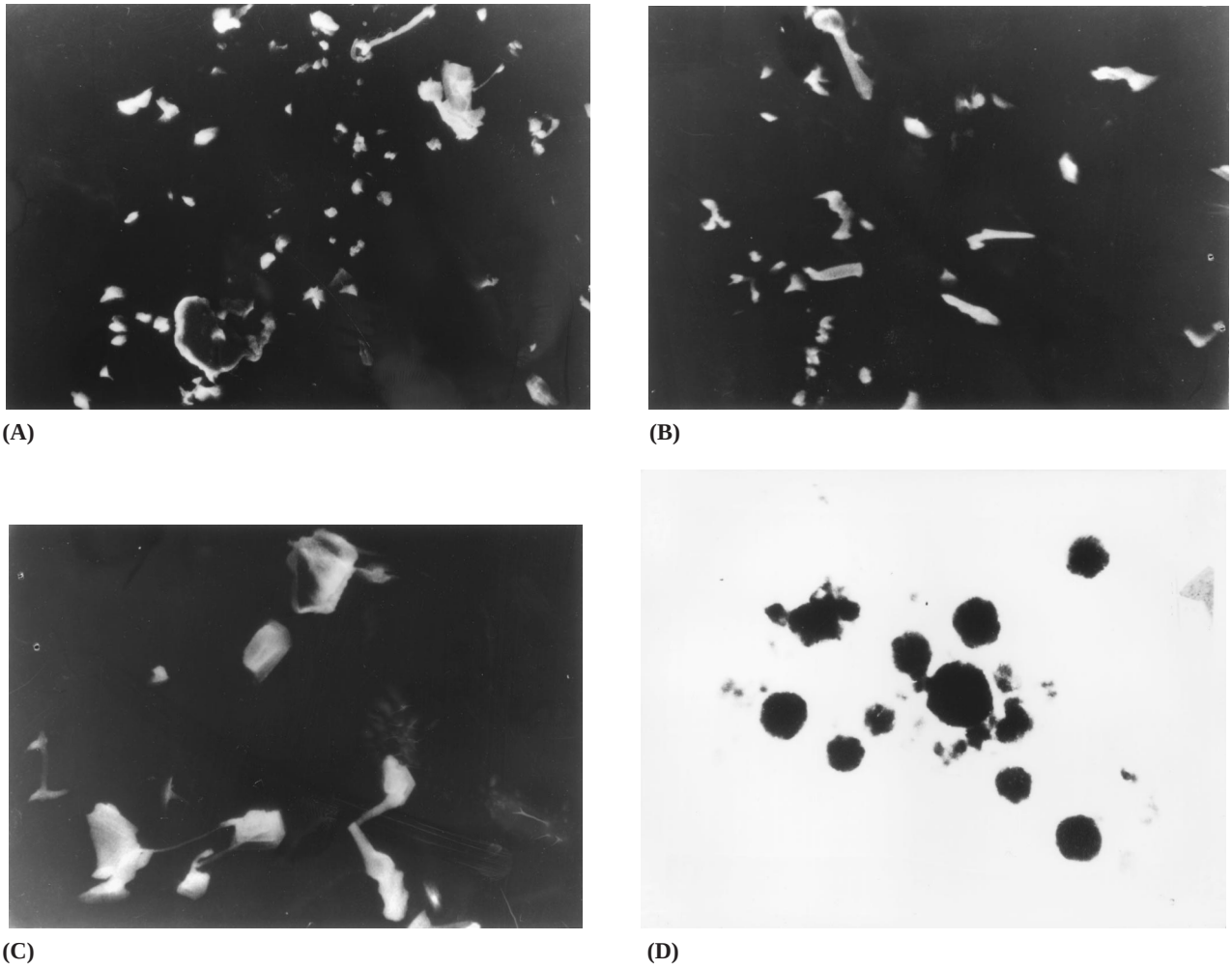

Figure 1. (A) Electron micrograph of Span 80 niosomes (magnification 3000×). (B) Electron micrograph of Span 60 niosomes (magnification 3000×). (C) Electron micrograph of Tween 20 niosomes (magnification 7000×). (D) Electron micrograph of Tween 80 niosomes (magnification 10,000×).

Table 1

Entrapment of Cytosine Arabinoside Hydrochloride and Mean Size of Niosomes

Type	HLB	% CTH	Size[a] (nm)
Span 80	4.3	67.5	633
Span 60	4.7	79.8	800
Tween 80	15.0	82.3	900
Tween 20	16.7	88.2	957

[a] Mean of 10 batches.

chromatogram of the samples contained a single peak (retention time 2.8 ± 0.4 min), which belonged to CTH (18,19).

Stability

The formulated niosomes were separated into three portions. One portion was kept at room temperature, the second at 37°C, and the third at 4°C for 1 month. At weekly intervals, these portions were then evaluated for their drug content and release characteristics.

RESULTS AND DISCUSSION

The transmission electron microscopy images of large unilamellar niosomes formulated using different surfactants are shown in Fig. 1. Most of the vesicles are discrete particles with sharp boundaries that range in size from 600 to 1000 nm. The mean size of the niosome increased with progressive increase in the HLB value from Span 80 (HLB 4.3) to Tween 20 (HLB 16.7) (Table 1) because surface free energy decreases on increasing hydrophobicity of surfactant (20).

Entrapment efficiency (Table 1) was determined by a centrifugation method as dialysis was reported to be unsuitable for the estimation of drug encapsulated due to slow equilibration of encapsulated drug as the free drug is dialyzed, leading to either over- or underestimation of encapsulation, depending on the duration of dialysis (21). Entrapment efficiency of the Span 60 formulation was higher than that of the Span 80 formulation. Although Span 60 and Span 80 have the same head group, Span 80 has an unsaturated alkyl chain. De Gier et al. (22) demonstrated that the introduction of double bonds into the paraffin chains causes a marked enhancement in the permeability of liposome, possibly explaining the lower entrapment efficiency of the Span 80 systems in this case.

Results of an in vitro study on the release of CTH from niosomes in PBS are shown in Fig. 2. As expected, the rate of release of CTH across the dialysis membrane from loaded vesicles was slower than that from free CTH solution used as a control.

The results showed that approximately 95% of the drug was released within 40 min from the control solution, whereas the release of CTH from the Span 60,

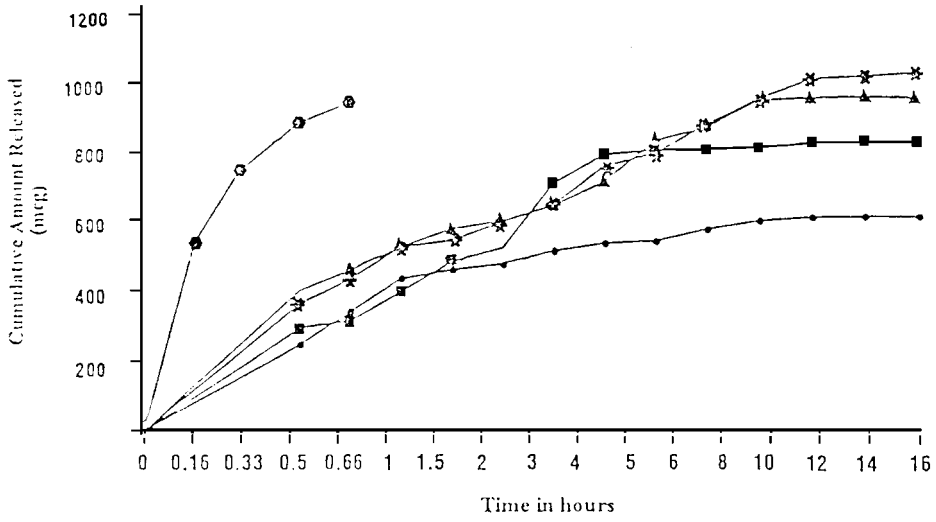

Figure 2. In vitro release profiles of CTH from niosomes in PBS. ○, free drug; △, Span 80 niosomes; *, Tween 20 niosomes; ■, Tween 80 niosomes; ●, Span 60 niosomes.

Table 2

Percentage Retention of Cytarabine Hydrochloride in Niosomes at Various Temperatures

	Amount of Drug Retained (%) After 30 days		
Formulation	4°C	RT	37°C
Span 60	90.40	68.08	36.21
Span 80	89.60	80.25	40.29
Tween 20	82.47	76.48	59.22
Tween 80	90.03	81.91	45.01

RT = room temperature, 27°C ± 2°C.

Tween 80, Span 80, and Tween 20 was about 6%, 8%, 10%, and 10%, respectively, during a period of 16 hr. Of these, the Span 60 formulation yielded the slowest rate of release. Release occurred in two phases, an initial burst release that lasted for 2–6 hr, followed by a sustained, but reduced, release that was maintained at least for 16 hr. The biphasic release pattern might be due to size heterogeneity of the vesicles. The comparative release data indicate that, by niosome encapsulation, it is possible to sustain and control the release of the drug for a longer duration.

Stability data, as shown in Table 2, indicate that niosome encapsulation gives good protection for CTH, at least under refrigerated conditions.

Thus, niosome encapsulation may be useful for improving the stability characteristics of cytarabine. The results indicate that it will be helpful for maintaining the therapeutic efficacy of cytarabine for longer periods of storage during therapy. However, it is also observed that, at higher temperatures, the rate of degradation is greater even in the niosome vesicular form; the increased degradation may be a consequence of the presence of lipid materials (e.g., cholesterol and fatty material such as Span 60 [sorbitan monostearate]) in the vesicular suspension. After storage for 1 month, there was no significant change in the release pattern of the niosome-encapsulated CTH.

CONCLUSION

These interesting findings reveal that vesicular niosomes may be very useful for designing a sustained-release delivery system of cytarabine hydrochloride. Particularly, the niosome formulation of this drug excluding dicetylphosphate, yielding larger sizes of vesicles, will be beneficial for obtaining higher blood levels for combating acute lymphocytic and granulocytic leukemias. The conventional technique provides smaller vesicles that are accumulated in the liver, is metabolized more and therefore provides lower blood levels of cytarabine.

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