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RESEARCH PAPER

Preparation, Characterization, and Evaluation of Liposomal Dispersions of Lidocaine

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ABSTRACT

Lidocaine, a local anesthetic agent, was encapsulated into liposomes employing the conventional lipid-film hydration technique. An attempt was made to freeze dry the aqueous liposomal dispersions. The prepared liposomal dispersions were investigated by differential scanning calorimetry (DSC), transmission electron microscopy (TEM), scanning electron microscopy (SEM), ^{31}P-nuclear magnetic resonance (NMR) spectroscopy, and laser counting studies for characterization. The skin partition coefficient for liposomal lidocaine was calculated. The results showed that lidocaine incorporated into the liposomes got selectively partitioned and localized in the skin to a great extent. A topical liposomal gel formulation containing 2% w/w lidocaine was prepared using Carbopol-934 as the gelling agent. The prepared formulation was tested for its local anesthetic efficacy employing the pinprick test. The liposomal preparation of lidocaine gave a much longer duration of action compared to the conventional topical formulation.

INTRODUCTION

Liposomes, first described by Bangham (1), are lyotropic liquid crystals consisting of one or more concentric spheres of amphiphilic lipid bilayers separated by aqueous phase compartments. An assortment of different drugs can be incorporated into liposomes which can then be administered by various routes. Mezei and Gulsekharam (2), using liposomes as drug carriers for topical administration of triamcinolone acetonide, proved that despite higher drug concentrations in the skin with the liposomal formulation, percutaneous absorption of

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the drug was greatly reduced. Thus, it would be possible to reduce the dose of the drug applied in the liposomal form and achieve a better value of therapeutic index.

Many attempts have been made to provide effective anesthesia of the skin in order to alleviate pain, burning, and itching associated with minor surgical operations, injections, and various dermatological disorders. However, to achieve sufficient anesthetic effect on intact skin, prolonged application and high concentration of drug (10–30%) are required (3–6). Recent studies indicated that tetracaine in liposome encapsulated form provides better topical anesthesia, tested by pin-prick, than a conventional form (7–8). Liposomes applied topically were proposed to penetrate into the skin (dermis) through the "lipid channels" of the epidermis and localize the drug within the skin (8). Due to multilamellar structure of liposomes, sustained release of the encapsulated drug is possible (9).

This paper describes the encapsulation of lidocaine into liposomes with emphasis on their characterization and evaluation of lidocaine gel as a potential selective drug delivery system for deep anesthesia of the skin.

MATERIALS AND METHODS

Materials

The phospholipids (Phospholipon 90 and Phospholipon 90 H) for liposome preparation were obtained as gift samples from Nattermann Phospholipid, Germany. Cholesterol was obtained from Loba Chemie. Lidocaine base was obtained as a gift sample from Smith Kline Beecham Pharmaceuticals, India. Carbopol-934 was obtained from Alfa Biochem. All other materials and solvents used were of analytical grade. Double-distilled water from an all-glass still was used.

Methods

Formulation of Liposomal Dispersions

Aqueous liposomal dispersions were prepared by the conventional lipid film hydration technique (1). Briefly, the lipid phase and the appropriate amount of lidocaine were dissolved in chloroform in a round-bottom flask containing glass beads. A thin lipid film was formed on the inner side of the flask and on the surface of the glass beads by evaporating the chloroform under vacuum using a rotary evaporator. The flask was kept overnight under high vacuum (10^{-1} torr) to remove traces of organic solvent. Once the thin lipid film was formed, the flask was heated to about 55–60°C in a water bath (5°C above the gel-to-liquid crystalline transition temperature of the phospholipid). The appropriate aqueous phase containing 0.45% sodium chloride and 7% propylene glycol of pH 5.5 was then added and the flask was hand-shaken vigorously for 5 min to hydrate the lipid film. The flask was maintained at 55°C for 5 min and then shaken for 4 hr on a mechanical shaker with intermittent sonication using a bath sonicator.

Freeze Drying of Liposomal Dispersions

The aqueous liposomal dispersions were freeze-dried in an Edwards freeze-drier (model EFO3) using mannitol as the cryoprotectant.

Investigation of the Liposomal Dispersions

The prepared liposomal dispersions were investigated by DSC, TEM, SEM, ^{31}P-NMR spectroscopy, and laser counting studies.

DSC was performed by heating the samples in sealed aluminum pans from 40 to 150°C at a heating rate of 10°C/min. on a Shimadzu DT-40 thermal analyzer. The shape and lamellarity of the liposomes were investigated by observing the liposomes, negatively stained using phosphotungstic acid, under a Zeiss TM-101 transmission electron microscope. The shape and surface topography of the freeze dried liposomal dispersions were investigated by observing them under a Jeal JSM-840 scanning electron microscope after they were coated with gold. The various liposomal dispersions were subjected to ^{31}P-NMR studies using a Bruker AMX-500 FT-NMR spectrometer. The mean particle size and size distribution of the liposomal dispersions were studied using a Shimadzu SALD-2001 laser counter.

Determination of Skin Partition Coefficient (K_m) for Liposomal Lidocaine (10)

The experiment was carried out in Erweka diffusion cells maintained at 37 ± 1°C with the receiver compartment empty. Full thickness abdominal skin excised from adult guinea pig was mounted with the stratum corneum facing the donor cell with a surface area of 5 cm^2 exposed to donor cell. Two milliliters of the liposomal dispersion containing 10 mg/ml of lidocaine in the donor compartment was measured at zero time (C_1) and at the end of 24 hr (C) by acid–base titration.

K_m was calculated from the equation

$$K_m = \frac{C_1 - C}{C_1}$$

Preparation of Topical Liposomal Gel Formulation

A quantity of aqueous liposomal dispersion containing 200 mg of lidocaine was concentrated sufficiently under high vacuum and then incorporated into a sufficient amount of Carbopol-934 gel (previously neutralized with sodium hydroxide solution) to obtain a gel containing 2% w/w of lidocaine base. The gel was filled in a lacquered aluminum collapsible tube.

Evaluation of Liposomal Gel Formulation (11)

A single dose of each of 0.4 g of the prepared formulation, 0.4 g of the conventional marketed gel, or 0.16 g of marketed ointment formulation (each containing 8 mg of lidocaine) was applied on a 10 cm^2 area and was covered with Parafilm® to provide occlusion for 30 min. The pin-prick test was used to assess the local anesthetic effect. A surgical pin pushed through a rubber stopper, which prevents the pin from penetrating the skin, was used to assess the local anesthetic effect. The advantage of the device over a needle is more uniform stimulus intensity and prevention of skin injury. Testing was done immediately after removal of the occluding tape and at 15, 30, 45, 60, 75, 90, and 105 min and 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, and 6 hr. Thereafter, the same guinea pig was used for testing of conventional gel, ointment, and liposomal formulation to avoid differences in the behavior of the animals. The guinea pig was allowed to recover after each experiment for at least 24 hr. Empty liposomes (no drug), served as control. All the preparations were tested twice on three different guinea pigs.

Stability of the Liposomal Dispersions

The lidocaine-loaded aqueous liposomal dispersion, freeze-dried lidocaine-loaded liposomal dispersion, and prepared gel formulation were stored at 4°C and at room temperature. They were observed visually as well as for microscopic appearance at the end of 15, 30, 45, 60, 90, and 120 days from the date of preparation.

RESULTS AND DISCUSSION

Methods for Characterization of Liposomal Dispersions

DSC

DSC thermogram of empty aqueous liposomal dispersion containing P-90, P-90 H, cholesterol, and stearic acid showed many small endotherms in the temperature range of 50–80°C, with major endotherms at 53.5°C and 83.9°C corresponding to the phase transition temperature of individual lecithins (Fig. 1a). The melting endotherm of cholesterol was found to be shifted from 150°C to 128°C, signifying that all the lipid components interact with each other to a great extent while forming the lipid bilayer.

DSC thermogram of the drug-loaded liposomal dispersion (Fig. 1b), interestingly showed complete disappearance of all the small endotherms in the range of 50–80°C and exhibited endotherms at 76.4°C and 118°C. The incorporated lidocaine got associated with the lipid bilayers, interacted to a large extent with them, and perturbed them. The cholesterol endotherm exhibited a shift from 151°C to 118°C while the endotherm at 76.4°C indicated the increase in phase transition temperature of liposomes upon loading with lidocaine. Absence of the melting endotherm of lidocaine suggested significant interaction of lidocaine with bilayer structure. The above observation is concordant with the finding of Ladbrooke et al. (12) that interaction of the encapsulated drug with the lipid components of liposomes may alter the physicochemical properties of liposomes, which in turn would influence the drug transfer from the liposomes. The transition temperature profile not only provides information as to the possible "fluidization" or "solidification" of the bilayer, but also allows for determination of critical thermodynamic properties.

^{31}P-NMR

The ^{31}P-NMR spectra obtained from the empty liposomal dispersion showed a very sharp peak at 0.8074 ppm (Fig. 2a) while that for the drug-loaded liposomal

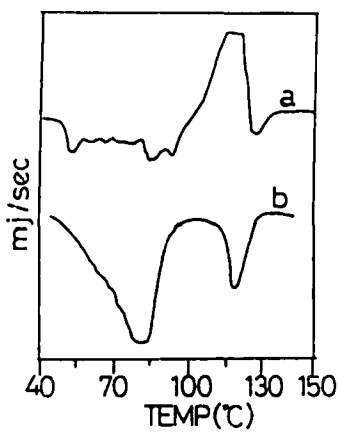

Figure 1. DSC thermograms of a) empty (no drug) liposomal dispersion; b) lidocaine-loaded liposomal dispersion.

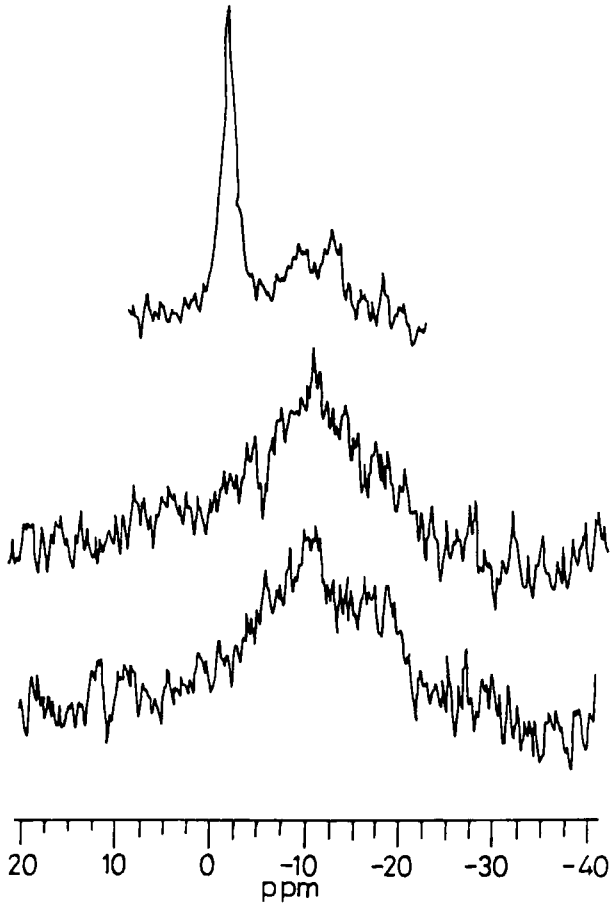

Figure 2. ^{31}P-NMR spectra of a) empty liposomal dispersion; b) lidocaine-loaded liposomal dispersion; c) lidocaine-loaded liposomal dispersion in presence of Mn^{++} ions.

dispersion showed a characteristic broadening and a notable shift of peak from 0.884 ppm to around -10.4 ppm (Fig. 2b). Cullise and Hope (13) using ^{31}P-NMR, showed that the bilayer phosphorous signals were obtained due to specific orientation of phosphate groups of phospholipids in the bilayers. When the bilayer structure is distorted or affected due to the interaction with other substances, it results in broadening of the bilayer peak in ^{31}P-NMR. However, TEM photographs of the prepared liposomal dispersions showed presence of spherical bilayered vesicles. On addition of a band broadening agent like Mn^{++} ions to the above dispersion, a slight broadening of the peak was observed, suggesting a minimal interaction of Mn^{++} ions with the bilayer (Fig. 2c). This could be attributed to the presence of cholesterol, which rigidifies the bilayer allowing only a minimal interaction.

TEM

TEM studies revealed that the empty liposomes were spherical with mean size of 7–8 μm and some of them aggregated to form a bee hive-like structure (Fig. 3a). Most of the vesicles showed the presence of two or

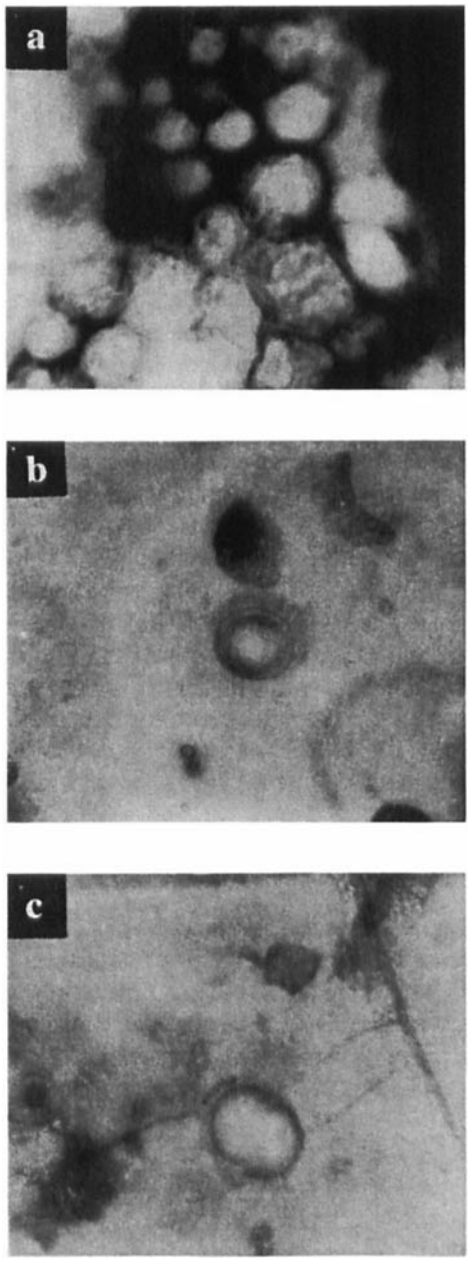

Figure 3. TEM photomicrographs of a) empty liposomal dispersion; b) Lidocaine-loaded liposomal dispersion; c) empty freeze-dried and reconstituted liposomal dispersion; d) lidocaine-loaded freeze-dried, and reconstituted liposomal dispersion

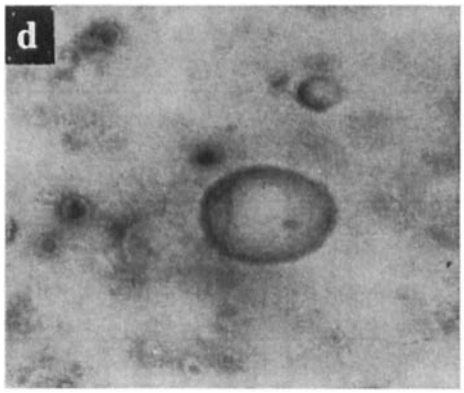

Figure 3. Continued

more bilayers. The drug-loaded liposomes were separate spherical entities with a vesicle size of 4–5 μm (Fig. 3b). The majority of the vesicles showed the presence of two bilayers.

The reconstituted freeze-dried liposomal dispersions showed a few aggregated spherical vesicles (Fig. 3c and 3d). There was a slight increase in the vesicle size which could be attributed to the aggregation of vesicles during freeze-drying.

SEM

The SEM photographs of the empty and drug-loaded freeze-dried dispersions exhibited complete absence of vesicular structure, which could be due to drying or breakdown of the vesicular structure to the phospholipid mixture during freeze-drying (Fig. 4a, 4b).

Estimation of Vesicle Size Distribution Using Laser Counter

The mean vesicle size and mean volume of the various liposomal dispersions are given in Table 1.

A marked decrease in mean vesicle size was observed after drug incorporation. For molecules that strongly interact with the bilayers, freeze drying does not pose a problem. If proper cryoprotectants are used, retention of drug and vesicle size does not change much upon rehydration (14). TEM photographs of the reconstituted freeze-dried dispersions revealed presence of properly formed spherical vesicles with a slight increase in the vesicle size. Thus, mannitol appeared to be a good cryoprotectant for freeze drying of the prepared liposomal dispersions.

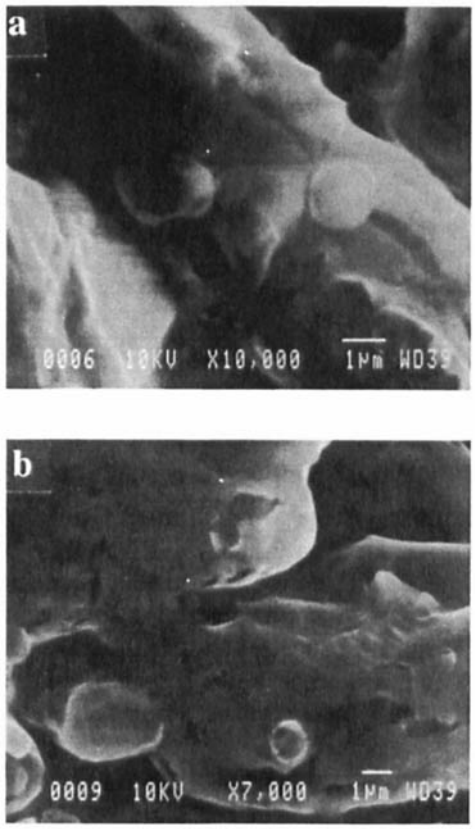

Figure 4. SEM photomicrographs of a) empty freeze-dried liposomal dispersion; b) lidocaine-loaded freeze-dried liposomal dispersion.

Evaluation of the Liposomal Dispersions and Gel Formulation

Determination of the Skin Partition Coefficient (K_m) of Liposomal Lidocaine

The K_m value was found to be 0.69305, which indicated that the drug loaded in liposomal dispersion had penetrated the skin and was retained in the skin to a large extent.

Preparation of Topical Liposomal Gel Formulation

The liposomal gel formulation was prepared by incorporating the liposomal dispersion into Carbopol-934 gel to obtain a white, opaque topical gel containing 2% w/w of lidocaine base and with excellent application and spreading properties.

Table 1

Mean Vesicle Size and Volume of Various Liposomal Dispersions

Parameters	Empty Liposomes	Loaded Liposomes	Empty Freeze-Dried Reconstituted	Loaded Freeze-Dried Reconstituted
Mean vesicle size	8.225 μm	4.823 μm	5.233 μm	7.059 μm
Mean vesicle volume	9.283 μm^3	2.827 μm^3	5.658 μm^3	3.277 μm^3

Evaluation of Local Anesthetic Efficacy of the Liposomal Formulation

The guinea pig response to the pin-prick test for various formulations is as shown in Table 2.

The guinea pigs with untreated skin responded very well to the test giving a shivering response on every prick. Treatment of the skin with blank liposomes did not cause much change in the responses compared to those by untreated skin. Thus, blank liposomes themselves did not contribute to local anesthetic action. The conventional ointment exhibited a slow onset of action and although the duration of action was around 150 min., the minimal painful scores never went below five. Lidocaine, being a highly lipophilic drug, has greater affinity for the oily ointment base and hence the delay in onset of action. Either the ointment did not release all the drug or the drug was slowly partitioning into the stratum corneum and simultaneously getting cleared from the skin, accounting for its lesser degree of local anesthesia.

The conventional gel formulation had a fast onset of action and the skin was totally anesthetized. However, it lasted for a very short time. This indicated that the ionized form of lidocaine penetrated much more rapidly than the free base but was rapidly absorbed and cleared off by the cutaneous capillaries. Thus the gel appeared to be of good use where a deep local anesthesia is re-

Table 2

Comparison of the Local Anesthetic Activity of Various Formulations as Assessed by the Pin-Prick Test

Time (min)	Average Number of Painful Pricks Out of 10 Pricks on Treatment with: Untreated Skin	Blank Liposomes	Lidocaine HCl gel	Lidocaine Ointment	Liposomal Lidocaine
0	10	10	8	10	8
15	10	10	5	10	7
30	9	9	2	10	7
45	10	10	0	10	4
60	9	10	3	8	2
75	10	9	5	7	1
90	10	9	7	7	0
105	10	10	9	6	0
120	10	10	9	6	1
150	10	10	10	5	2
180	9	9	10	6	2
210	10	10	10	5	3
240	10	10	10	6	4
270	10	8	10	5	5
300	10	10	10	8	6
330	10	10	10	8	6
360	10	10	10	9	7

quired for a short duration.

The prepared liposomal formulation had a moderate onset of action and the total anesthesia lasted for 1 hr, while the duration of action was 3.5 hr, in which the number of painful scores were four or less. This may be explained on the basis that initially the liposomes on the skin surface released some of the drug slowly and then the liposomes along with the drug penetrated the upper part of stratum corneum, got mixed with the skin lipids, and thus acted as a depot from which the drug was released slowly. Thus, the liposomal formulation appeared to be of good use in cases where a deep anesthesia is required over a long duration.

Physical Stability of the Liposomal Dispersion

Visual Appearance

The aqueous liposomal dispersion stored at 4°C showed no signs of sedimentation or creaming up to 90 days. Thereafter, a slight sedimentation was noticed which on slight shaking redispersed easily, and no perceptible change in color of the dispersion was observed even after 120 days. The dispersion stored at room temperature showed an early sedimentation and a slight yellowing after storage beyond 90 days.

The freeze-dried dispersions stored at 4°C and room temperature did not exhibit any visual changes in the color or appearance even after 120 days.

The liposomal gel formulation remained unaffected when stored at 4°C and room temperature for 120 days, and so did its application properties.

Microscopic Observations

The dispersion stored at 4°C did not show noticeable aggregation or increase in vesicle size at the end of 120 days, while the one stored at room temperature did exhibit aggregation and increase in vesicle size after 120 days. The size distribution of the reconstituted freeze-dried dispersions did not deviate much from that of freshly dried and reconstituted dispersions.

CONCLUSION

The process of lipid–film hydration resulted in the formation of bilamellar liposomes in the size range of 4–5 μm as evident from the TEM and laser counting studies. DSC studies indicated a significant interaction of lidocaine with liposomal bilayers. This was also confirmed by ^{31}P-NMR studies. Lidocaine loaded in liposomal form penetrated and was retained in the skin to a large extent as evidenced by the value of the skin partition coefficient of liposomal lidocaine.

The prepared liposomal formulation was found to have a moderate onset of action and significantly longer duration of action as compared to the conventional gel and ointment, as evidenced in the local anesthetic efficacy tests on guinea pigs.

The liposomal dispersions stored at 4°C kept very well compared to those stored at room temperature and their stability could be improved by freeze drying.

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