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Free versus liposome-encapsulated lignocaine hydrochloride topical applications

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The influence of encapsulating lignocaine hydrochloride, in a liposomal delivery system on its release from a topical formulation was studied. Liposomes were prepared from a mixture of L-α-dipalmitoylphosphatidyl choline (DPPC) and cholesterol in different molar ratios with or without charge inducing agents. The swelling time affording maximum entrappment was tested from 0–48 h at 53 °C. The percentage of drug entrapped in liposomes was found to be charge dependent and more pronounced for negatively charged liposomes. The amount of drug entrapped increased by increasing the ratio of cholesterol. The selected formulations were evaluated *in vivo* using the pin prick method. The results revealed localized and prolonged activity of lignocaine contained in liposomes when compared with an equivalent conventional topical application.

1. Introduction

Due to their physicochemical similarities with cellular membranes, liposomes are biocompatible, biodegradable and non-toxic vehicles for drug molecules [1]. Liposomes have proved to be efficient controlled delivery devices in extravascular regions when applied directly to the site of required action [2].

And were shown to provide a promising drug-delivery system for the skin [3–10]. *In vivo* experiments showed that liposome-encapsulated drugs produce several-fold higher concentrations in the epidermis and dermis and lower systemic concentrations, than conventional dosage forms. It is hypothesized that liposomes can act as micro-reservoirs from which drugs may be slowly released [10]. The aim of the present study was to investigate the therapeutic efficacy of lignocaine hydrochloride containing liposomes.

2. Investigations, results and discussion

Lignocaine hydrochloride liposomes of three different surface charges and two molar ratios were prepared. Dicetyl phosphate and stearylamine were used as the charge-inducing agents for negatively and positively charged liposomes, respectively. The liposomes were prepared using the vortex dispersion method. Saline phosphate buffer (SPB) was used as the hydrating agent. The liposomes were left to swell, in the hydrating solutions, at 53 °C for different periods of time.

Table 1 presents the effect of swelling time on the percentage of lignocaine entrapped in liposomes of the molar ratio 7 : 4. Increasing swelling time from 0 to 24 h at 53 °C increases the percentage of drug entrapped.

Table 2 depicts that negatively charged liposomes (DPPC/cholesterol/DCP 7 : 4 : 1) are characterized by the highest percentage drug entrapment. Similar observations were found by Sharma et al. [12] for lignocaine liposomes and by El-Ridy et al. [13] for oxamniquine liposomes. The liposomal formulations containing DPPC and cholesterol in a molar ratio of 7 : 6, have the highest capability of drug entrappment. Increasing drug entrappment by increasing the ratio of cholesterol in liposome formulations was reported for other drugs [14–16]. It was reported that inclusion of cholesterol increases the ordered structure lipid bilayers and that this effect reduces the immediate leakage of an entrapped drug from liposomes and results into higher encapsulation [17].

The Fig. shows four electron microscope photographs of some liposomal formulations viz., molar ratios 7 : 4, neutral and negative (A, B), and 7 : 6 neutral (C). The figure reveals the circular liposomal structure of these formulations exhibiting a particle size range of 1.6–8 μm. The neutral liposomes with a molar ratio of 7 : 6 showed a larger particle size than those of lower cholesterol content either neutral or charged.

Table 1: Effect of swelling time on the percentage of lignocaine hydrochloride entrapped (molar ratio 7 : 4)

Swelling time (h)	Lignocaine entrapped (%)
0.0	10.16 ± 2.94
24	16.97 ± 3.89
48	15.04 ± 5.15

Table 2: Effect of phospholipid molar ratio and surface charge on the percentage of lignocaine hydrochloride entrapped (swelling time 24 h)

Molar ratio	Surface charge	Lignocaine entrapped (%)
7 : 4 : 1	(−ve)	18.0 ± 4.17
7 : 4 : 1	(+ve)	15.41 ± 4.36
7 : 4	(uncharged)	16.97 ± 3.89
7 : 6	(uncharged)	20.15 ± 9.14

Table 3: Onset and duration of action of lignocaine hydrochloride after topical application of different formulations

Formulations	Onset of action (min)	Duration of action (min)
Control gel (2%)	9.0 ± 1.10	35.00 ± 2.83
7 : 4 n (1%)	13.17 ± 2.14	33.33 ± 8.76
7 : 4 −ve (1%)	13.14 ± 2.10	30.83 ± 5.85
7 : 6 n (1%)	17.0 ± 2.45	30.0 ± 3.16
7 : 4 n (2%)	15.5 ± 4.64	136.66 ± 24.22***
7 : 4 −ve (2%)	16.5 ± 2.07	148.33 ± 21.68***
7 : 6n (2%)	18.0 ± 2.45	158.33 ± 34.88***

All data are the mean ± SD of 6 experiments. Ranges are shown in parentheses
*** Very highly significant ($P < 0.001$)

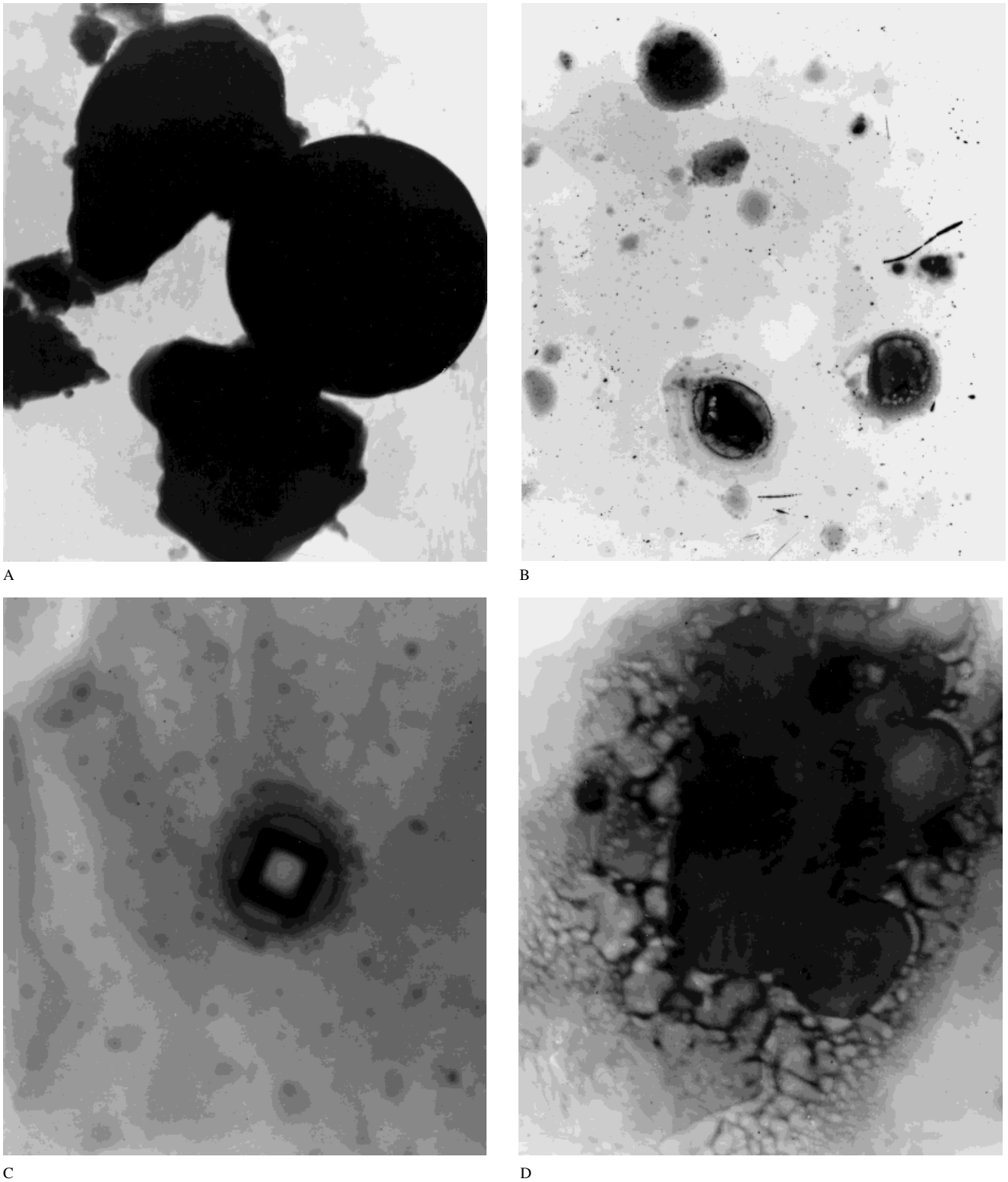

Fig.: Electron microscope photographes of some liposomal formulations (A) 7 : 4 n, (B) 7 : 4 − ve, (C) 7 : 6 n

The effect of liposomes on the delivery of lignocaine was assessed by measuring the onset and duration of drug action. The data presented in Table 3 reveal that all liposomal formulations incorporated into gel increase the onset of drug action; liposome-encapsulated lignocaine hydrochloride (7 : 6), showed maximum onset of action.

Considering the duration of drug action, there is no significant difference between liposome preparations containing 1% lignocaine and control preparations containing 2% of the drug.

Table 3 also shows that the liposomal gel product (2% lignocaine) increases the duration of action; this effect is very highly significant ($p < 0.001$). Liposome preparations of a molar ratio of 7 : 6 show maximum duration of action. This may be ascribed to the greater accumulation of liposomes in the epidermis. It was thus observed that prolonging the duration of action with liposomal products was at least 4–5 times more efficient compared to control preparations. This prolonged duration of action of the 2% drug content liposomes could be explained by the satura-

tion of the epidermis with half the drug content. Hence, the other half takes considerable time to be released.
In conclusion, it could be stated that the use of lignocaine liposomes form leads to an increase of therapeutic efficacy of topical dosage forms in terms of prolonging the drugs duration of action.

3. Experimental

3.1. Material

Lignocaine hydrochloride (El-Nasr Chemical Pharmaceuticals Co. Egypt). L-α-Dipalmitoyl phosphatidyl choline, crystalline, 99% (DPPC), dicetyl phosphate (DCP) stearylamine and cholesterol (Sigma Chemical Co., U.S.A.). Gel: hydroxy propyl methyl cellulose and water (Colorcon Co. England, UK). All other reagents used were of analytical grade.

3.2. Instruments

Büchi rotavapor HB-140 (Büchi, Switzerland). MLW, Cooling Centrifuge, Germany, LKB, UV spectrophotometer, Cambridge, England. Electronic Microscope (Zeiss, Germany) 80 KV (High Voltage).

3.3. Preparation of lignocaine liposomes

Multilamellar lignocaine liposomes were prepared by the chloroform film vortex dispersion method from a mixture of DPPC and cholesterol, with or without the charge − inducing agents DCP and stearylamine. Two different molar ratios of DPPC and cholesterol were used viz, 7 : 4 and 7 : 6. Hundred mg of the lipid mixture were dissolved in 10 ml chloroform in a round-bottom flask and the solvent was evaporated to dryness, using a rotary evaporator at a temperature of 50–54 °C.
Isotonic SPB (10 ml, pH 7.4) containing 100 mg of lignocaine hydrochloride, at the same temperature, were then added to the dried lipid film along with a sufficient number of 0.5 mm glass beads and then rotated at the same temperature for 10 min. After swelling, the liposomes were separated from the free drug by centrifugation at 20000 × g. The pellets thus produced were washed twice with 60 ml PSB each, and resuspended in the same buffer. The liposome preparations were kept freezed (−10 °C) in a refrigerator until needed. The washings were collected and assayed for lignocaine spectrophotometrically at 262 nm. The amount of entrapped drug was determined from the difference between the weight of drug used for preparation and the assayed amount in the washing.

3.4. Preparation of lignocaine gel

Lignocaine liposomes were incorporated into gels (hydroxy propyl methyl cellulose and water) in such a way that the final formulation contained 1 and 2% w/w drug. Similarly, formulations containing equivalent amounts of plain drug were prepared and used as control.

3.5. Characterization of liposomes

The particle size range of the liposomal formulation was determined using an electron microscope. The liposomes were stained using 2% phosphotungistic acid. The particle size determination was accomplished using the magnification powers 5000 and 20000.

3.6. In vivo study

The study was based on cross over design. The investigation was carried out on healthy human subjects, 6 volunteers, 30–45 years of age (mean 36.0 ± 3.2) weighing 60–80 kg (mean 70.5 ± 8.0). The products were applied topically on a cleaned 9 cm^2 area of the upper arm of each subject. The liposomal products were applied to the right upper arm of each subject. At the same time the same subject received topical application of preparation containing plain drug on the left upper arm as a control.
The onset and duration of action were noted by the pinprick method [18].

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