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Chemoprophylaxis of schistosomiasis using liposome-encapsulated tartar emetic

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Summary

In this work, tartar emetic was entrapped in liposomes of L-α-dipalmitoyl-phosphatidyl choline, cholesterol with or without a charge-inducing agent. A dose of drug equivalent to 25 mg/kg b. wt., entrapped in neutral or negatively charged liposomes, was injected 7 days before infection with 150 cercariae of *Schistosoma mansoni* per mouse. After 3 months, the results obtained indicated a 100% survival compared to 28% for the mice injected with drug free liposomes. A 100% survival was also observed for the mice injected with free tartar emetic. The worm count, conducted for the groups of mice injected with free or liposome encapsulated drug, reveals statistically schistosomicidal activity for the liposome encapsulated drug. The same data reveal that neutral liposomes are more effective than negatively charged ones.

Introduction

Tartar emetic, potassium antimony tartrate, is by far the most commonly used trivalent antimonial drug for the treatment of schistosomiasis (Atta and Mousa, 1961), with no reported chemoprophylactic activity (Standen, 1955; Stohler and Frey, 1963). Failure of mass treatment to control schistosomiasis has been reported and this failure was attributed to the fact that treatment was not sufficiently long-lasting (Polderman and Manshande, 1981). The use of liposomes to entrap tartar emetic would be expected to have some preventive schistosomicidal activity. The reasons for this are:

(1) the high affinity of the schistosomiasis parasite for phospholipids and the possibility that the liposomes encapsulated antimony will be ingested by the parasites;
(2) the ingested lipid was found to be incorporated into stable parasite structures rather than being utilized for degradative energy-yielding metabolism (Rumjanek and Simpson, 1980);
(3) it has been reported that the concentration of antimony in the liver, after 14 days of injecting tartar emetic liposomes, was nearly double that reached in the liver after the administration of the free drug (El-Ridy et al., 1984).

Schistosomiasis parasite resides in the sinuosides of the liver for 2–3 weeks following infection

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as it matures (Markell et al., 1986), and it is assumed that the slow release of antimony in the sinuosides of the liver will prevent the development of the parasite. The purpose of this work is to study the physical characteristics of the drug–liposome preparations and the impact of these characteristics on the schistosomicidal activity of tartar emetic–liposomes in mice.

Materials and Methods

Materials

Tartar emetic, antimony potassium tartrate (USP), L-α-dipalmitoyl phosphatidyl choline synthetic crystalline (DPPC), dicetyl phosphate crystalline (DCP), stearylamine crystalline and cholesterol (reagents grade), were all obtained from Sigma Chemical Co., U.S.A.

Methods

(a) In vitro. Tartar emetic liposomes were prepared from a mixture of (DPPC), cholesterol, with or without (neutral) a charge-inducing agent in the molar ratio of 7 : 4 : 1. The lipids were dissolved in 10 ml chloroform and dried in a pear-shaped flask on a rotary evaporator (Büchi Rotavapor RE 121, Büchi, Switzerland) under vacuum. 10 ml aqueous antimony potassium tartrate solution containing 80 mg/ml, at the same temperature as that of the lipids, were then added to the dried lipid film along with 0.5 mm glass beads. The resultant turbid solution was sonicated, at 30 W for 3 min (Braunsonic 1510 ultrasonic sonicator, B. Braun, AG, F.R.G.). The liposomes were allowed to remain in the tartar emetic solution at 53°C for a period of 24 h. The liposomes, after swelling, were separated from the free drug by centrifugation (Sorvall RC2-B super speed refrigerated centrifuge) at 20,000 *g*. The pellets, thus produced, were washed twice and resuspended in distilled water. The liposome preparation and antimony potassium tartrate standards were appropriately diluted with distilled water and assayed for total antimony concentration by atomic absorption spectrophotometry at a wavelength of 217.5 nm and slit width of 0.5 (Varian Atomic Absorption Spectrophotometer, Model 1000).

Particle size analysis of the sonicated liposomes was carried out by photon correlation spectroscopy. The liposome preparation, after thawing, was suitably diluted and the size distribution was determined using the Coulter sub-micron particle analyzer (Model N4SD, Coulter Electronics of Canada, Montreal).

Differential scanning calorimetry of the multilamellar liposomes was carried out using a sample weight equivalent to 3 mg. DPPC for all the liposome preparations (DSC, DuPont Instruments 9900, Willmington, DE).

A shaking incubator (Precision Scientific Co., Chicago) was used to study the release of tartar emetic from liposomes under hydrodynamic stress.

(b) In vivo: Swiss female mice, average weight 18–22 g, were injected with the tartar emetic preparation 7 days before infection with 150 cercariae of *Schistosoma mansoni*, Puertrican strain. Five groups of mice, 13–15 mice each, were used. Each group received a different pretreatment. The pretreatments were, liposome-encapsulated tartar emetic (negatively charged), liposome-encapsulated tartar emetic (neutral), drug-free (negatively charged and neutral) liposomes, and free tartar emetic solution. All treatments were given i.v. in the tail vein. The injection volume of tartar emetic was adjusted to administer a dose of 25 mg/kg body weight for all experiments. Seven days after the pretreatment, each mouse was infected with about 150 cercariae of *Schistosoma mansoni* as previously described (Smithers and Terry, 1965).

Mortality was recorded daily during 12 weeks. At the end of this period, surviving animals were sacrificed and a total worm count was performed according to the procedure described by Smithers and Terry (1965).

Results and Discussion

Tartar emetic liposomes of 3 different surface charges were used in the in vitro studies. Dicetyl phosphate (DCP) and stearylamine were used as the charge-inducing agents for negatively and positively charged liposomes, respectively. The combination of DPPC and a high ratio of cholesterol was used to obtain a liposome prepara-

TABLE 1

Effect of the tartar emetic liposome surface charge on the percentage of drug entrapped

Surface charge	Percentage of drug entrapped
Positive	2.14
Negative	1.77
Neutral	1.41

tion providing a slow and sustained release of the drug which could be effective in the preventive treatment of shistosomiasis. The results obtained can be summarized as follows.

(1) The entrapment efficiency was determined for different lots of the sonicated liposomes. The amount of tartar emetic entrapped was estimated and found to range from 1.41% to 2.14% of the initial amount of drug used for preparation of the liposomes depending on the surface charge of the liposomal vesicles. As seen in Table 1, positively charged liposomes appear to have the highest percentage entrapment, namely, 2.14%. The negative and neutral liposomes entrapped lower percentages, respectively.

(2) Particle size analysis of the sonicated liposomes was conducted by photon correlation spectroscopy. Table 2 demonstrates that the positively charged liposomes exhibit a higher mean particle diameter than the negatively charged or neutral liposomes.

(3) The release kinetics of tartar emetic from the drug–liposome systems of different surface charges was investigated, under hydrodynamic stress (Fig. 1). Table 3 shows the data obtained from the linear regression of the release kinetics

TABLE 2

The mean particle diameter for tartar emetic liposomes with different surface charges

Liposome composition	Liposome charge	Mean diameter (nm)
DPPC : Cholesterol (7.6 : 4.4)	Neutral	715
DPPC : Cholesterol : DCP (7 : 4 : 1)	Negative	767
(DPPC : Cholesterol : Stearylamine (7 : 4 : 1)	Positive	1225

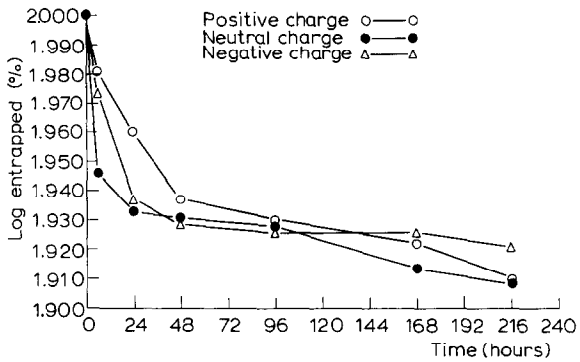

Fig. 1. Permeability of tartar emetic entrapped in liposomes of different surface charges.

over a period of 216 h at 37 °C. The results indicate a biphasic system which is valid for the 3 liposomes with different surface charges. The first phase represents desorption of the drug from the exterior surface of the lipid phase as well as a small amount diffused from inside of the liposomes. The release rate is nearly similar for the 3 liposome preparations. The second slower release phase, which occurred after 24 h, is solely due to diffusion of the entrapped drug through the lipid bilayer. The release rate, in that phase, is considerably different depending on the surface charge of the liposomal vesicles. For neutral and positively charged liposomes the half-life of the second phase is 16.8 and 9.8 times that of the first phase, respectively. The very slow second phase release rate of negatively charged liposomes could be

TABLE 3

Kinetics of the biphasic release of tartar emetic from liposomes

Liposome surface charge	First phase		Second phase	
	Release rate constant (k) (h^{-1})	Half-life (h)	Release rate constant (k) (h^{-1})	Half-life (h)
Negative	5.8×10^{-3} $r = 0.9851$	1.19×10^{2}	1.0×10^{-4} $r = 0.9191$	6.93×10^{3}
Positive	3.5×10^{-3} $r = 0.9648$	1.98×10^{2}	4.0×10^{-4} $r = 0.9817$	1.95×10^{3}
Neutral	5.4×10^{-3} $r = 0.8152$	1.29×10^{2}	3.0×10^{-4} $r = 0.9853$	2.11×10^{3}

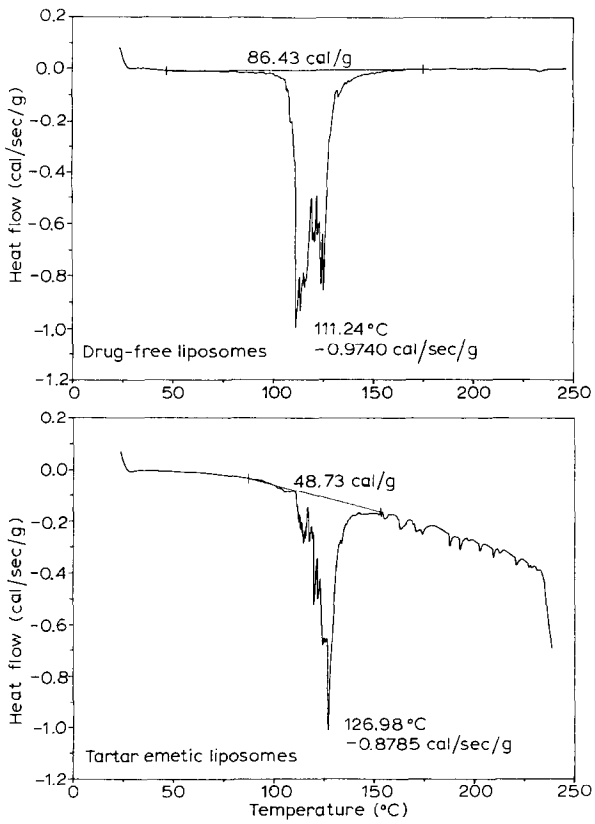

Fig. 2. DSC thermograms of neutral tartar emetic liposomes.

explained by its relatively higher cholesterol content.

(4) Characterisation of tartar emetic liposomes, by differential scanning calorimetry is shown in Fig. 2. Tartar emetic shifted the peak temperature to a higher values, i.e. 126.98°C compared to 111.24°C for drug-free liposomes, and decreased the enthalpy of melting by about 44%. This indicates a pronounced effect of the drug on the phospholipids phase transition.

The chemoprophylactic efficacy of sonicated tartar emetic liposomes was determined using mice as the experimental animal. Positively charged liposomes were not tested because of its toxicity (Juliano, 1983) and its aggregation, as indicated by a previous work from our laboratory (El-Ridy et al., 1988).

Tartar emetic was found completely inactive in mice when administered prophylactically or at an early stage of infection (Standen, 1955; Stohler and Frey, 1963). These studies were conducted by injecting the drug, 24 h or less, before infection. In this work, the injection of the free drug, 7 days before infection, surprisingly showed a 100% survival. This result has not been reported before with tartar emetic and it is identical to those obtained when injecting the liposome-encapsulated drug, at the same period before infection. It should be noted that the mice injected with drug-free liposomes, neutral and negatively charged, showed 28% survival. On the other hand, the worm count, conducted by the perfusion method, for the 3 groups of mice injected with free drug or liposome-encapsulated drug indicated a statistically significant difference on the schistosomicidal activity. The liposome-encapsulated drug gave a lower worm count than the free drug injection as seen in Table 4. In the same time, the data reveals that neutrally charged liposomes are more effective than the negatively charged based on the mean total of worm-count. If we consider in vitro data, the slow drug release for negatively charged liposomes should give higher biological activity than the neutral liposomes. It has been also reported (Senior et al., 1985) that neutral and positively charged liposomes are cleared less rapidly than negatively charged liposomes. However, the data obtained in this study shows the contrary (see Table 3). Further investigation is necessary to clarify the schistosomicidal effect of charged and uncharged liposomes encapsulated with tartar emetic. In these experiments a larger number of

TABLE 4

Total worm-count for mice injected with different tartar emetic preparations before infections with Schistosoma mansoni

Tartar emetic preparation	Number of mice investigated	Mean of total worms recovered *	Significance
Free drug	12	44 ± 7.47	$P < 0.05$
Negative liposome	10	25 ± 2.53	
Neutral liposome	8	15 ± 2.25	$P < 0.01$

* Four months after injection.

cercariae and a longer period of time between injection and infection are considered.

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