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RESEARCH PAPER

Chemoprophylaxis of Schistosomiasis Using Liposome Encapsulated Oxamniquine

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ABSTRACT

Oxamniquine liposomes with different compositions and surface charges were prepared by the chloroform-film method. The amount of oxamniquine entrapped was estimated and found to range from 1 to 23.09% of the initial amount of drug used for preparation of the liposomes depending on the surface charge of liposomal vesicles. Negatively charged liposomes exhibited the highest percentage entrapment viz., 23.09%. The maximum oxamniquine entrapment was achieved in liposomes prepared from phospholipids molar ratio (7:4.1). The liposome formulations were characterized by laser light scattering technique, particle size analysis and rheological characterization. In vitro release kinetics of oxainquine liposomes reveal that the percentage drug release is surface charge dependent and irrelevant to the molar ratio. The results of organ, liver and spleen, targetting of oxamniquine liposomes in mice reveal that after 7 days of (S.C.) injection the amount of oxamniquine retain more than three times (7:4:1) more than two times (7:6) and nearly double (7:6:1) the amount of drug retained by injection of the free drug. After 14 days of (S.C.) injection of oxamniquine liposomes, negatively charged liposomes of the highest cholesterol content exhibit better drug retention than neutral liposomes and no free drug detected in these organs, after this period of time. The chemoprophylactic effect of free and oxamniquine liposome formulations was estimated using female Swiss mice injected 7, 15, 30, and 60 days before larval infection. For 7 days chemoprophylactic study liposomes encapsulation show

more efficient prophylaxis. In the 15 days study the percentage worm count reduction is in the following order: 7:4:1 (-ve) (68.8%) > 7:2:1 (-ve) (66%) > 7:2 (34.4%) > free oxamniquine (0.00%). For the 30 day study the negatively charged liposome 7:4:1 exhibited a significant reduction of worm burden. For the 60 days, the liposomes 7:6 produced better chemoprophylaxis than 7:6:1 (-ve). The results of T-cell and B-cell responses against soluble adult warm proteins reveal that oxamniquine, when encapsulated in liposomes, stimulate the immune system of mice against the worms of S. mansoni.

INTRODUCTION

Oxamniquine is one of the two drugs approved by WHO for treatment of Schistosomiasis mansoni (1). A single oral dose is usually effective, immediate toxicity is generally low, and genetic effects are weak or absent (2). Liposome-encapsulated oxamniquine was used in this investigation for the chemoprophylaxis of Schistosomiasis. Schistosomiasis parasite resides in the sinusoid of the liver for 2–3 weeks following infection as it matures there (3). It is assumed that the slow release of oxamniquine in the sinusoid of the liver will prevent the development of the parasite. Schistosomicidal drugs were encapsulated in liposomes and used for protection against S. mansoni (4–7). These experiments resulted in a significant chemoprophylaxis but for only 7 days before the time of infection. In order to extend the chemoprophylactic period against S. mansoni, oxamniquine was selected in this investigation because it has its own prophylactic effect. This effect could be prolonged further by liposome encapsulation.

EXPERIMENTAL

Materials

Oxamniquine (Pfizer, USA), L-α-dipalmitoyl phosphatidyl choline synthetic, crystalline (99%) (DPPC), dicetyl phosphate crystalline (DCP), stearylamine crystalline, cholesterol, and heparin were all obtained from Sigma Chemical Co., USA. Polyethylene-glycol 600 (Hoechst Chemikalien, Burgkirchen, Germany), acetonitrile 190 (For UV) "HPLC Grade" Romi Chemicals Ltd., Leics, England, thiopental (Specia, Paris, France), other materials used were of analytical grade.

Methods

Preparation of Liposomes

Oxamniquine liposomes were prepared by the chloroform film method, from a mixture of (DPPC, cholesterol) with or without (neutral) a charge inducing agent. Liposomes were separated from the free drug by centrifugation (MLW, K23D refrigerated centrifuge) at 2000 Xg. The liposome pellets were washed twice and resuspended in saline-Kolthoff's borax phosphate buffer. The washings were assayed for their oxamniquine content by the (USP XXII, 1996) spectrophotometric method. The amount of oxamniquine entrapped was estimated by difference from the amount added at the start of preparation, and the oxamniquine content of the washings.

Liposomes Characterization

Laser-Characterization of Liposomes Composition and Interactions

The liposomes composition and phospholipid interaction was investigated by laser light scattering technique. The liposomes sample concentration investigated was 0.2 mg/ml for all the formulations studied. The normal temperature scan was conducted at Ivv (θ) and at a temperature range at 25–50°C. The instrument is working with helium neon laser lamp, oriel-USA, at $\lambda = 630$ nm at 0.5 mw.

Laser Characterization of Liposomes Particle Size

The particle size distribution, for six drug-free liposome-formulations was studied using the same instruments and conditions mentioned above.

Particle Size Analysis

Particle size analysis for oxamniquine liposomes was also conducted by the Coulter Counter TAII; Coulter Electronic Inc., Florida, USA.

Rheological Characterization

The rheological study was conducted on eight liposomal pellets and four liposomal suspensions having different molar ratios and surface charges using the Ferranti-Shirley viscometer.

In Vitro Release Kinetics Study

The liposomal pellets were suspended in a saline buffer forming a 10 ml suspension, then placed on the shaker in an incubator 37 ± 0.5, and moving at a rate of 165 strokes/min. A one ml aliquot sample was taken for analysis at six time intervals, viz., 0, 3, 6, 12, 24, and 48 hr. The one ml samples were separated by centrifugation and washed twice with saline buffer. The amount of drug encapsulated was determined by difference using the HPLC-method (8).

Oxamniquine Organ, Liver, and Spleen Targeting Study

12 groups of female Swiss mice eight-weeks old, each weighing ≅ 20 gm, were injected subcutaneoushy with one of the following five oxamniquine liposome formulations, five drug free liposome formulations, oxamniquine in saline buffer, and the same volume of saline buffer/mouse in a dose equivalent to 1.2 mg oxamniquine/mouse. One or two mice from each group were dissected at the following time intervals, zero, 1, 4, 7, and 14 days. The liver and spleen were extracted from each mouse and dried by an air current till constant weight. 200 mg from the liver and spleen were homogenized with tissue homogenizer at 24000 rpm, for 0.5 min, in the presence of 0.4 ml, 1 N sodium hydroxide, 0.5 ml saline buffer, and 5 ml ethyl acetate. All samples were analysed by HPLC-method (8).

Chemoprophylactic Effect of Free Oxamniquine (Choice of the Route of Injection)

A total of 125 outbred female Swiss mice were divided at random into five equal groups. The first was kept untreated and considered control. Mice of the other groups were injected each with 1.2 mg oxamniquine, in 0.36 ml PEG. subcutaneously (S.C., group 2), orally (group 3), intraperitoneally (i.p., groups), or intramuscularly (i.m., group 5). All mice were exposed, a week later, to 200 live unattenuated S. mansoni Cercariae, by the tail immersion method. After 6 weeks, worm burden was assessed by the portal vein perfusion method (9).

Chemoprophylactic Effect of Oxamniquine Liposomes

The same methods were adopted for evaluation of oxamniquine liposomes chemoprophylaxy for different periods of time viz., 7, 15, 30, and 60 days using subcutaneous route of injection.

Immunological Responses

Cellular and humoral, T-cell, and B-cell responses were studied against soluble adult S. mansoni worm proteins separated by "sodium dodecyl sulphate polyacrylamide gel electrophoresis" (SDSPAGE) and transferred into nitrocellulose paper. These studies were conducted using strain C 57 BL6 mice. C 57 BL6 mice were injected with oxamniquine liposomes of molar ratio 7:4, 7 days before infection, with 150 S. mansoni, cercariae via tail immersion. Sera and spleenocytes were collected 5, 10, and 15 days after infection and used for T-cell and B-cell tests.

RESULTS AND DISCUSSION

Studying the Effect of Phospholipids Surface Charge and Molar Ratio on the Percentage Oxamniquine Entrapped

The amount of oxamniquine entrapped was estimated and found to range from 1% to 23.09% 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, negatively charged liposome appear to have the highest percentage entrapment (23.09%). The neutral and positive liposomes entrapped lower percentages respectively.

The maximum oxamniquine entrapment was achieved in liposomes prepared from the phospholipid molar ratio (7: 4: 1) regardless of the vesicles surface charge Table 2.

Laser Characterization

The liposomes composition and phospholipid interactions, was investigated by laser light scattering technique. Figure 1 shows the normal temperature scan for

Table 1

Effect of Surface Charge on the Percentage Oxamniquine Entrapped for (7:4:1) Drug Liposomes

Surface Charge	Percentage Oxamniquine Entrapped* %	
Negative	Mean ± S.D.	23.09 ± 10.336
	S.E.	3.113
Positive	Mean ± S.D.	1.00
	S.E.	—
Neutral	Mean ± S.D.	12.42 ± 11.367
	S.E.	2.842

*Mean of 12 ± 1 experiment.

Table 2

Effect of Lipids Molar Ratio on Percentage Oxamniquine Entrapped

DPPC: Cholesterol: Charge Molar Ratio			7: 2: 1*	7: 4: 1**
Percentage Oxamniquine entrapped (%)	Negative	Mean ± S.D.	15.76 ± 7.8	23.09 ± 10.34
		S.E.	2.25	3.11
	Positive	Mean ± S.D.	—	1
		S.E.	—	—
	Neutral	Mean ± S.D.	8.52 ± 6.9	12.42 ± 11.377
		S.E.	2.30	2.84

*Mean of 9 experiment ± 1
**Mean of 12 experiment ± 1

six oxamniquine liposomes with different molar ratios and surface charges. The differentiated phase transition of these liposomes is presented, Table 3. It is concluded that, for the same DPPC:cholesterol molar ratio, the liposomes surface charge does not affect the main transition temperature. On the other hand, increasing cholesterol content results in a noticeable decrease of the main transition temperature for all different vesicles surface charges investigated. In addition, the transition width decreases with the increase of cholesterol concentration. The transition width for the positively charged liposome, 7:4:1, is the least compared with that of the neutral and negatively charged liposomes, respectively.

Figure 2 reveals the particle size distribution for six drug-free liposomes using the laser light scattering technique. All the liposome formulations investigated showed moderately wide distribution, for the 7:4:1 (+ve) a relatively wider distribution is exhibited. The mean particle diameters for these multilamellar liposome formulations ranged form ≈ 1.6 μm for neutral and negatively charged liposomes, to 3 μm for the positively charged liposome 7:4:1. A larger particle size is expected for positively charged liposomes (10–11).

Rheological Characterization

The Rheological Properties of Oxamniquine Liposomal Suspension

Liposome suspensions, viz., 7:2, 7:4 (neutral), and 7:2:1 and 7:4:1 (-ve) exhibit negligible viscosity. This indicates that these liposomes could be easily injected with high syringeability.

The Rheological Properties of Oxamniquine Liposomal Pellets

Table 4 reveals that negatively charged liposomes of the three molar ratio investigated exhibit the highest viscosity values compared to the other charges. Neutral liposomes exhibited less values, while positively charged liposomes showed different and intermediate positions. The use of the liposomal pellets is a simulation of the

Table 3

The Laser Light Scattering Phase Transition of Oxamniquine Liposomes

Liposomes	Main Transition Temperature TcC		Transition Width ΔTC	
Surface Charge	Molar ratio 7: 2: 1	7: 4: 1	Molar ratio 7: 2: 1	7: 4: 1
Negative	42	39.25	12	9.5
Positive	42.5	40.15	11	6.3
Neutral	42.2	39.5	7	9.6

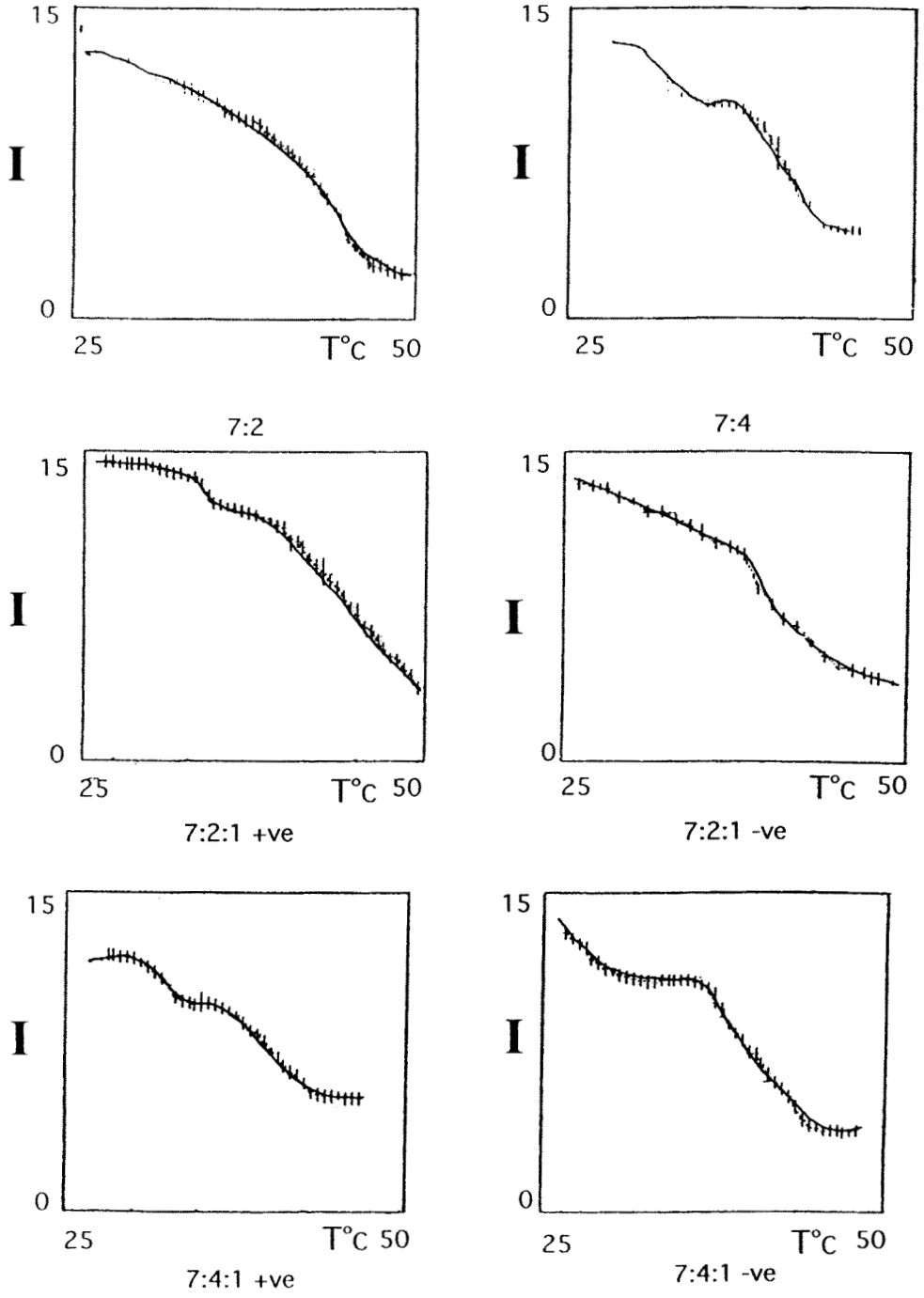

Figure 1. Normal temperature scan, using light scattering technique IVv (θ), for oxamniquine liposomes.

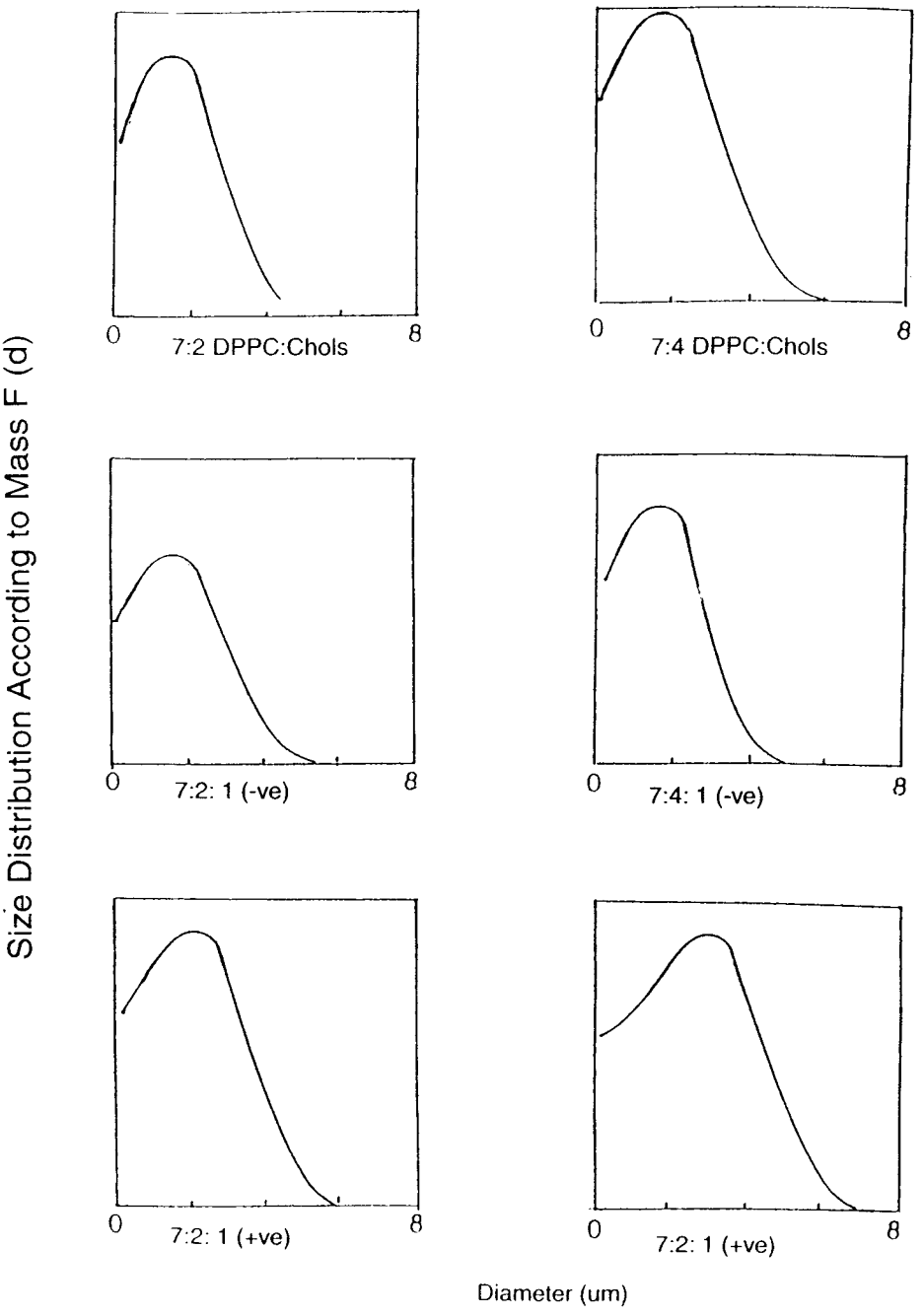

Figure 2. Particle size distribution (according to mass) for drug free liposomes, light scattering method.

Table 4

Visocity Values of Oxamniquine-Liposomal Pellets

Molar	Surface Charge	Viscosity (cp)
7:2	(N)	156.78
7:4	(N)	191.62
7:6	(N)	231.69
7:2:1	(-ve)	374.53
7:4:1	(-ve)	461.63
7:6:1	(-ve)	499.95
7:2:1	(+ve)	348.40
7:4:1	(+ve)	155.04

conditions encountered in vivo after injection of the liposome suspensions and subsequent absorption of the aqueous phase.

In Vitro Release Kinetics

The in vitro release kinetics study of oxamniquine liposomes was done as trial to anticipate the expected behavior of these liposomes under the hydrodynamic stress of the in vivo conditions. Table 5 illustrates that the percentage oxaminquine retained after 48 hr is surface charge dependent, and irrelevant to the molar ratio. At the same time, the molar ratio 7:4 exhibits a higher percentage retained compared to the molar ratios 7:2 and 7:6 for the neutral liposomes. Liposomes with molar ratio 7:6:1, negatively charged, exhibit an intermediate position. The graphical illustration of the logarithm of the percentage amount of retained oxaminiquine, as a function of time (Fig. 3) clearly demonstrates that the release kinetics of the liposomes exhibit more than one phase. The existence of more than one phase could be explained by the first fast release of the drug, intercalated with the surface of liposomes, followed by a slower release of the drug encapsulated in the aqueous compartments and inner lipid bilayers of the liposomal vesicles. The release rate constant values of the different liposomes during the first phase were calculated and presented in Table 5. From these results, it is clear that neutral liposomes exhibit the least release rate constant values followed by the negatively charged liposomes, 7:4:1 (-ve) exhibit an odd position. It can be concluded that neutral liposomes, with the least release rate constants, are expected to exhibit a marked sustained release of the drug. The same could by anticipated for the negatively charge liposomes 7:4:1. However, inspite of the better sustained release properties of the neutral liposomes, the negatively charged liposomes are more suitable for liver targeting as they are targeted more efficiently to the liver phagocytic cells compared to neutral liposomes (12–14).

Oxamniquine Organ, Liver, and Spleen Targeting Study

Liposomes, upon injection, have been shown to localize primarily in the liver and to a lesser extent in the spleen (15). While early studies suggested that liposomes might be taken up by hepatocytes, it now seems fairly clear that they are taken up primarily by Kupffer cells and possibly by other liver sinusoid cells, followed by slow redistribution of some material to hepatocytes (16).

Table 6 illustrates that after subcutaneous injection of free oxamniquine, negligible amount of the drug is re-

Table 5

In Vitro Release Kinetics of Oxamniquine Liposomes

Time Interval (Hr)	Molar ratio / Surface charge:	Oxamniquine Retained (%) 7:2 (N)	7:2:1 (-ve)	7:4 (N)	7:4:1 (-ve)	7:6 (N)	7:6:1 (-ve)
0		100	100	100	100	100	100
3		89.90	87.74	91.98	92.5	90.87	86.90
6		80.53	75.68	83.66	85.04	82.34	76.13
12		77.47	69.76	82.32	82.17	80.10	72.48
24		—	62.13	—	77.35	76.90	68.27
48		75.11	55.25	78.74	73.48	74.84	63.52
Release rate constant (hr^{-1})		0.0361	0.0465	0.0297	0.0270	0.0324	0.0455

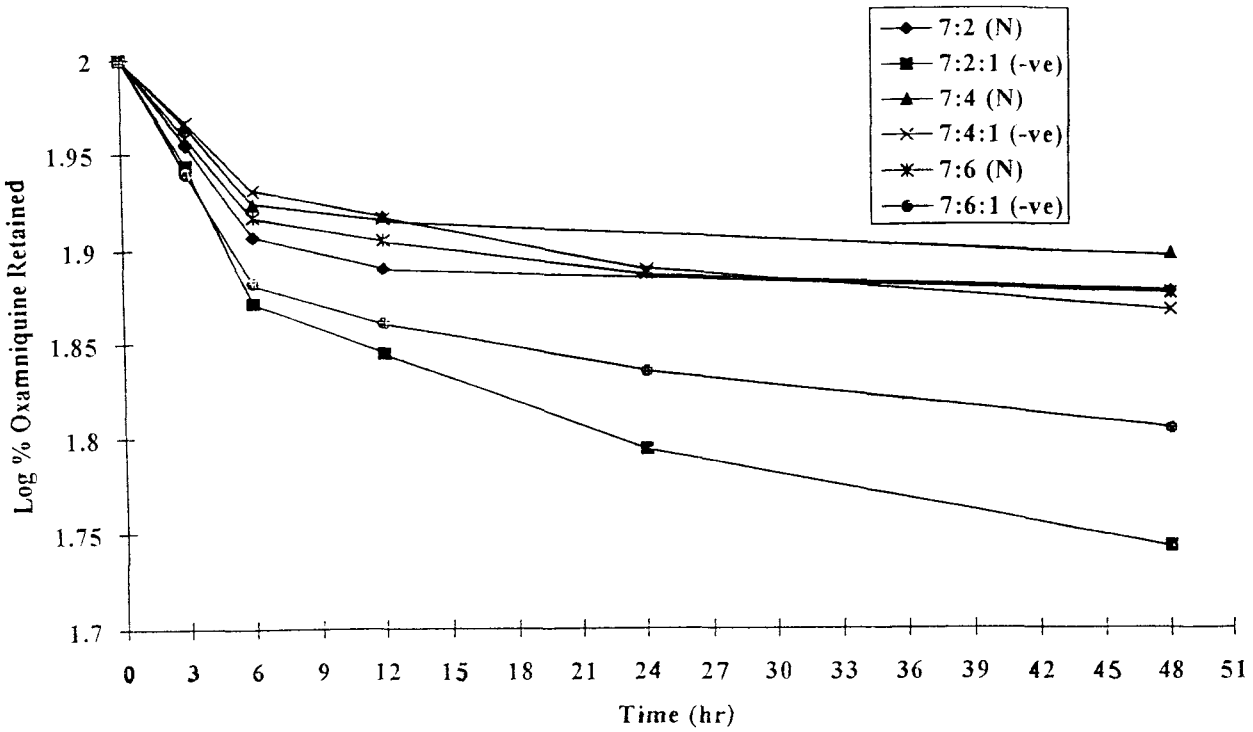

Figure 3. In vitro release kinetics of oxamniquine liposomes.

tained in the liver and spleen tissues after 7 days and no drug appeared to be retained in the liver beyond this time (14 days → zero μg). With regard to the oxamniquine liposomes, the results appear to be totally different: A considerable amount of the injection drug is retained in the liver and spleen tissues after 7 days and after 14 days, all the liposomes retained detectable amounts of the drug. The amount of oxamniquine retained after 14 days depends on the drug molar ratio and surface charge in the following manner; 7:6:1 > 7:4:1 > 7:4 > 7:6 > 7:2:1.

Table 6 shows that the weight of oxamniquine retained decreases with time reaching zero μg for the free drug compared to 0.116 μg for the liposome molar ratio 7:6:1. The amount of oxamniquine retained in the liver and spleen tissues after 7 days of injection of oxamniquine liposomes is more than three times (7:4:1), more than two times (7:6) and nearly double (7:6:1) the amount of drug retained by injection of the free drug. These results clearly indicate that encapsulation of oxamniquine in liposomes leads to prolongation of the half-life of the drug to a large extent.

Table 6

Organ Targeting Data of Oxamniquine Liposomes in Mice

Time Days	Molar Ratio Surface Charge	Oxamniquine Entrapped (μg/200 mg liver) 7:6:1 (-ve)	7:6 (N)	7:4:1 (-ve)	7:4 (N)	7:2:1 (-ve)	Free Oxamniquine
1		1.228	1.695	1.441	1.039	1.112	0.900
4		1.150	1.305	0.725	0.218	0.078	0.343
7		0.158	0.233	0.319	0.122	0.066	0.099
14		0.116	0.063	0.102	0.090	0.050	0.000

Choice of the Route of Injection

The data depicted in Table 7 indicated that oxamniquine given by the subcutaneous route leads to highly significant ($P < 0.001$, 48.2%) protection against S. mansoni. Injection through the oral, i.p., or i.m. route did not induce any significant protection. Accordingly, the subcuteaneous route of injection is considered in all the forthcoming chemoprophylactic studies against S. mansoni.

Chemoprophylactic Effect

Chemoprophylactic Effect of Oxamniquine Liposomes Injected 7 Days Before Larval Infection

The results presented in Table 8 indicated that the chemoprophylactic effect, against S. mansoni, of the negatively charged oxamniquine liposomes is molar ratio dependent. The molar ratio 7:2:1 (-ve) exhibits a highly significant protection against S. mansoni larval infection.

Injected 15 Days Before Larval Infection

It is obvious from the data complied in Table 9 that the free drug has no prophylactic effect. Also, it is clear that negatively charged oxamniquine liposomes, molar ratio 7:4:1 and 7:2:1, induce highly significant protection against S. mansoni larval infection. At the same time, it is depicted that oxamniquine liposomes, molar ratio 7:4:1, exhibit a relatively higher percentage reduction of the worm count, viz., 68.8 compared to 66 exhibited by the molar ratio 7:2:1. It should be mentioned that these two negatively charged liposomes exhibit a better protection when injected 15 days before larval infection compared to the protection obtained when they were injected 7 days before infection. It can be deduced from the data complied in Table 9 that the neutral oxamniquine liposomes, molar ratio 7:2, induce a significantly less protection against S. mansoni larval infection, compared to the negatively charged oxamniquine liposomes.

Injected 30 Days Before Larval Infection

Measurement of chemoprophylactic effect of oxamniquine liposomes 7:4:1 (-ve), 7:6 (N), and 7:6:1 (-ve) has been conducted. The results are evidenced in Table 10.

Table 7

Choice of the Route of Injection

Route of administration	Worm burden Mean ± S.D.	Protection (%)	p <
—	22.6 ± 11.3	—	—
s.c.	11.7 ± 5.1	48.20	0.001
oral	19.7 ± 10.4	12.80	NS
i.p.	19.4 ± 11.1	14.16	NS
i.m.	17.2 ± 9.7	23.80	NS

Table 8

Chemoprophylactic Effect of Oxamniquine Liposomes Injected 7 Days Before Larval Infection

Group	Worm Burden Mean ± S.D.	Protection (%)	p <
Free drug	9.6 ± 9.6	37.25	0.05
PEG 600	15.3 ± 9.3	—	—
Drug/Liposomes [7:4:1 (-ve)]	14.0 ± 14.0	48.18	0.001
Drug/Liposomes [7:2:1 (-ve)]	10.1 ± 10.1	62.40	0.001
Free Liposomes [7:4:1 (-ve)]	27.0 ± 19.0	—	—

Table 9

Chemoprophylactic Effect of Oxamniquine Liposomes Injected 15 Days Before Larval Infection

Group	(Mice No.)	Worm Burden Mean ± S.D.	Protection (%)	P[a] <
Control (PEG 600)	13	65.8 ± 17.3	—	—
Free drug	15	33.6 ± 8.6	—	—
7:4:1 (-ve)	19	10.5 ± 7.0	68.8	0.001
7:2:1 (-ve)	16	11.4 ± 8.0	66.0	0.001
7:2 (N)	15	43.2 ± 16.2	34.4	0.01*

*Two-tailed student's t-test.

Table 10

Chemoprophylaxis of Schistosomiasis by Oxamniquine Liposomes Injected 30 Days Before Infection

Group	(Mice No.)	Worm Burden Mean ± S.D.	Protection (%)	p <
Control (PEG 600)	12	40.3 ± 11.3	0.00	NS
Free drug	11	45.1 ± 11.1	0.00	NS
7:4:1 (-ve)	13	20.7 ± 7.09	42.42	0.0001
7:6 (N)	11	25.9 ± 17.4	35.80	0.01
7:6:1 (-ve)	11	25.4 ± 9.60	37.10	0.01

The selection of these three liposome formulations was based on the fact that these liposomes exhibit better organ targeting properties and rheological characteristics compared to the 7:2 oxamniquine liposomes (C.F. Table 7).

The percentage reduction of the number of worms clearly indicates that free oxamniquine and PEG 600 have no chemoprophylactic effect. As for oxamniquine liposomes, the results reveal that all these liposomes exhibit a significant reduction of the worm burden, in other words, a significant chemoprophylactic effect. As for oxamniquine liposomes, the results reveal that all these liposomes exhibit a significant reduction of the worm burden, in other words, a significant chemoprophylactic activity. This activity decreases in the following manner 7:4:1 (-ve) > 7:6:1 (-ve) > 7:6. Comparing this descending order with the order of the rheological characterization and organ targeting, it could be concluded that negatively charged liposomes exhibit better targeting (to the liver and spleen) properties compared to the neutral liposomes. At the same time the negatively charged liposomes exhibit higher viscosity values than the neutral ones. Accordingly, the more efficient chemorpophylactic effect could be explained by targeting more oxamniquine to the liver in a more viscous formulation. The presence of ultra minute amounts of oxamniquine (killing the very small schistosomules) may explain the chemoprophylactic effect of these formulations. But it should be mentioned that the immune dependence of chemotherapy plays an important role in the chemoprophylactic schistosomicidal effect of the investigated oxamniquine liposomes.

Chemoprophylactic Effect of Oxamniquine Liposomes Injected 60 Days Before Infection

Oxamniquine liposomes molar ratio 7:6 neutral and negatively charged, were used for their higher cholesterol content and viscosity values (C.F. Table 4). The results of oxamniquine liposomes Table 11 evidence a significant reduction of the worm burden compared to that of the free drug or PEG 600.

In this study, the neutral oxamniquine liposomes 7:6, evidence better chemoprophylaxis than the negatively charged liposomes, 7:6:1. A chemoprophylactic schistosomicidal effect for such a long period of time, 60 days,

Table 11

Chemoprophylaxis of Schistosomiasis by Oxamniquine Liposomes Injected 60 Days Before Infection

Group	(Mice No.)	Worm Burden Mean ± S.D.	Protection (%)	p <
Control (PEG 6000)	12	40.3 ± 11.3	0.00	NS
Free drug	11	45.1 ± 11.1	0.00	NS
7:6 (N)	11	17.5 ± 06.0	56.7	0.001
7:6:1 (-ve)	11	23.5 ± 12.6	41.6	0.01

could be explained only on the basis of immune dependence of chemotherapy. In other words, oxamniquine encapsulated in liposomes stimulates, by one way or the other, the immunological system of the mice against the schistosomules of S. mansoni.

Immunological Responses

Table 12 reveals that free liposomes stimulate, to a limited extent, the immune system of the mice. An explanation for this is that liposomes are made up of phospholipids having slight activity on the mice immune system. The T-cells have responded to the soluble adult worm proteins of molecular weight 108, 95, 45, and 40. The B-cells have responded only to the adult worm proteins of molecular weight 108 and 95.

Oxamniquine liposomes exhibit a more pronounced effect compared to the free liposomes. This is clear by comparing their action on the B- and T-cells response against the adult worm proteins of molecular weights 108, 95, 45, and 40. A more specific and efficient effect is visualized on the molecular weights 68–66 and 62–60 (Table 12). An explanation of this is that oxamniquine liposomes attenuate the worms and depress their capability of hiding form the immune system. In other words, they weaken the worms capability of

Table 12

Immunological Responses in C57 BL6 Mice Injected with Oxamniquine Liposomes 7:4 7 Days Before Infection

Molecular Wt of Soluble Proteins (Kilo Dalton)	T-Cell		B-Cell	
	Oxamniquine Liposomes	Free Liposomes	Oxamniquine Lipsomes	Free Liposomes
108	+	+	+	+
95	+	+	+	+
80				
72–70				
68–66	+++		+++	
62–60	++++		++++	
50				
45	+	+		
40	+	+		
30				
28				
24				
20–18				
14				

changing their skin every 3–6 hours. Free oxamniquine, of low molecular weight, is a weak immunogenic. Accordingly, we can conclude that oxamniquine liposomes stimulate the immune response against schistosomiasis worms.

It is evident from the afore mentioned results that this study has achieved its goal in efficiently prolonging the chemoprophylactic effect of oxamniquine through its encapsulation in liposomes. This accomplishment is based on both pharmacokinetic and immunological considerations. This could pave the way for future studies on immune dependence of chemotherapy specially for schistosomcidal and other orphan drugs. Liposome encapsulation of these drugs seems to be a must since liposomes are not only efficient and safe drug carriers but also immunological adjiuvants.

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