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Evaluation of the antischistosomal effect of praziquantel in a liposomal delivery system in mice

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Summary

Praziquantel liposomes were prepared from different mole fractions of L-α-dipalmitoylphosphatidylcholine and cholesterol according to the thin film method. The liposomes having optimum loading capacity for the drug were evaluated regarding their targeting properties towards the liver in mice. The results revealed pronounced targeting to the liver as well as sustained release properties which reflect its prophylactic action of the encapsulated drug towards schistosomiasis. This was clearly demonstrated by an increase in the survival rate of mice as well as a statistically significant decrease in the hepatic worm count.

Introduction

During the last few years, increasing efforts have been devoted to target sustained release systems to specific sites by systemic administration. Liposomal delivery systems can provide a relatively constant and sustained release formulation and also offer a convenient means of depositing the drug within selected sites or selected cell types.

Praziquantel is a broad-spectrum anthelmintic drug that has been demonstrated to be highly effective against all known species of schistosoma. However, failure of mass treatment to control schistosomiasis has been reported and attributed to the fact that treatment was not sufficiently long-lasting (Polderman and Manshande, 1981).

Targeting of praziquantel in a liposomal delivery system can protect uptake of the drug by non-diseased tissues as well as facilitate its uptake by the parasite over a longer period of time. On the other hand, it has been well established that the schistosoma parasite resides in the sinuosides of the liver for 2–3 weeks following infection to mature. Due to the slow release of the drug in the liver sinuosides, a chemoprophylactic effect of the encapsulated drug is expected.

The objective of the presented work was to evaluate the targeting properties as well as the chemoprophylactic action of praziquantel liposomes.

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Materials and Methods

Praziquantel was obtained from Bayer A.G., Germany. L-α-Dipalmitoylphosphatidylcholine, cholesterol, dicetyl phosphate and stearylamine were obtained from Sigma Chemical Co., St. Louis, U.S.A.

All solvents were supplied by Fisher Scientific, Montreal, Canada.

Preparation of praziquantel liposomes

Liposomes were prepared according to the chloroform film method. The liposome pellets were analyzed for entrapped praziquantel according to a high-performance liquid chromatographic method, at a wavelength of 210 nm., using a Hitachi 100-300 UV detector, a constametric III pump, at a speed of 1.5 ml/min, and a C8 column. The mobile phase consisted of 40% acetonitrile in phosphate buffer (0.05 M at pH 3.5). The results were recorded using a Perkin Elmer 56 recorder at a sensitivity of 50 mV.

Study of the liver targeting properties of praziquantel liposomes in mice

Two groups each of 10 Swiss female mice, average weight 18–22 g, were utilized in this study. The first group was injected subcutaneously with free drug and the second group with praziquantel liposomes (McDougall et al., 1975). 1 mg of free drug or its equivalent of praziquantel liposomes was injected to each mouse. After 1, 3, 5 and 7 days, two mice from each group were killed and the liver of each animal was removed, weighed and homogenized. The content of praziquantel in each homogenized liver was determined by the HPLC method.

Evaluation of the chemoprophylactic effect of praziquantel liposomes in mice

This was achieved by evaluating two main parameters, namely, the survival rate and hepatic worm count.

Survival rate

Six groups each of 15 Swiss female mice, average weight 18–22 g, were used in this study. The first and second groups were injected with 2 mg free drug per mouse; the third and fourth groups were injected with praziquantel liposomes (equivalent to 2 mg praziquantel per mouse); the fifth and sixth groups were injected with drug-free liposomes. 1 week later, each mouse from the first, third and fifth groups was infected with 100 cercariae of *Schistosoma mansoni* (Egyptian strain). 2 weeks from the date of liposome injection, each mouse from the second, fourth and sixth groups was also infected with 100 cercariae of *S. mansoni* (Egyptian strain). All the six groups were kept under standardized conditions and the number of mice surviving in each group was recorded every week up to 14 weeks.

Hepatic worm count Two groups each of 10 Swiss female mice, average weight 18–22 g were used in this study. The first group was injected with free drug (2 mg/mouse) and the second group was injected with praziquantel liposomes equivalent to 2 mg praziquantel per mouse. 2 weeks later, each mouse from both groups was infected with 40 cercariae of *S. mansoni* (Egyptian strain). On the 38th day after infection, the hepatic worm count for five mice from each group was conducted according to the method of Smithers and Terry (1965).

Results and Discussion

Preliminary investigations showed that maximum entrapment of praziquantel (19.1%) was achieved in liposomes prepared from a phospholipid mixture (DPPC and cholesterol) of molar ratio 7:6.

Hepatic targeting properties of praziquantel liposomes in mice

The results shown in Table 1 reveal that after subcutaneous injection of free drug, a very small amount of the drug was retained in the liver at 24 h and no appreciable amount of drug was retained beyond that time. However, regarding the encapsulated drug in liposomes, the picture appears to be totally different; about 50% of the administered drug was retained in the liver after 24 h. This retained amount decreased with time, reaching about 0.25% after 7 days. However, it can be seen from Table 1 that the amount of drug

TABLE 1

Targeting properties of praziquantel liposomes in mice after subcutaneous injection of praziquantel liposomes (1 mg per mouse)

Time (days)	Praziquantel retained in mice liver after injection of									
	Free drug					Praziquantel liposomes				
	μg/g liver	Weight of liver (g)	μg/liver	% drug retained in liver	Mean (%)	μg/g liver	Weight of liver (g)	μg/liver	% drug retained in liver	Mean (%)
1	0.1	2.001	0.2	0.02	0.02	255.0	1.970	502.4	50.24	46.78
	0.1	1.994	0.2	0.02		213.0	2.034	433.2	43.32	
3	0.0		0.0	0.00	0.00	31.2	1.897	59.2	5.92	4.28
						13.5	1.954	26.4	2.64	
5	0.0		0.0	0.00	0.00	2.7	1.867	5.0	0.50	0.48
						2.2	2.033	4.5	0.45	
7	0.0		0.0	0.00	0.00	1.2	2.120	2.5	0.25	0.25
						1.2	2.035	2.4	0.24	

TABLE 2

Chemoprophylactic effect of praziquantel liposomes (2 mg per mouse) administered 1 week before infection

Preparation	Mice surviving after (weeks)															
	0		2		4		6		8		10		12		14	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Praziquantel liposomes	15	100	14	93	14	93	14	93	14	93	12	80	10	67	8	53
Drug-free liposomes	15	100	13	87	12	80	12	80	8	53	4	27	4	27	2	13
Free drug	15	100	14	93	14	93	12	80	8	53	5	33	5	33	2	13

TABLE 3

Chemoprophylactic effect of praziquantel liposomes (2 mg per mouse) administered 2 weeks before infection

Preparation	Mice surviving after (weeks)															
	0		2		4		6		8		10		12		14	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Praziquantel liposomes	15	100	14	93	14	93	14	93	14	93	12	80	9	60	6	40
Drug-free liposomes	15	100	15	100	12	80	11	73	10	67	10	67	7	47	3	20
Free drug	15	100	13	87	13	87	10	67	10	67	10	67	8	53	3	20

retained in the liver after 1 week of injection of praziquantel liposomes is 10-fold in excess of the corresponding amount retained in the liver after 24 h of injection of free drug. This result clearly indicates that encapsulation of praziquantel in liposomes leads to prolongation of the half-life of the drug which has been previously reported to be 4 min in mice (Andrews, 1985). Administration of praziquantel in the form of liposomes appears to slow down the process of metabolism of the drug which would have a pronounced implication in its in-vivo performance. For an antischistosomal drug like praziquantel, its targeting to the liver not only plays a considerable role for the therapeutic effect of the drug, but may also throw some light on an expected prophylac-

TABLE 4

Statistical analysis (t-test) of the data of hepatic worm count in mice injected with praziquantel liposomes vs that of mice injected with free drug 2 weeks before infection

Preparation	Hepatic worm count in mice					Mean	*s*	*t*	*P*	Mean percentage of cercariae recovered as adult worms
	1	2	3	4	5					
Free drug	4	12	4	8	7	7				17.5
Praziquantel liposomes	2	–	1	1	1	1	2.3979	3.9563	0.01	2.5

s, overall standard deviation; *p*, probability.

tic action of praziquantel liposomes. This is due to the fact that the schistosoma parasite resides in the sinuosides of the liver for 2–3 weeks following infection as it matures (Markell et al., 1986) and it is assumed that the slow release of praziquantel in the sinuosides of the liver will prevent the development of the parasite. Also, the high affinity of schistosoma parasite for phospholipids (Markell et al., 1986) increases the possibility that liposome-encapsulated praziquantel will be ingested by the parasites.

Chemoprophylactic effect of praziquantel liposomes

The data presented in Table 2 regarding the survival rate of mice after administration of liposomes 1 week before infection clearly demonstrate that the survival rate of mice after administration of praziquantel liposomes is much higher than the corresponding rate for mice which had been administered free drug or drug-free liposomes. The percentage of mice surviving 8 weeks after praziquantel liposome administration exceeds 90% compared to about 50% for free drug or drug-free liposomes. On the other hand, the number of mice surviving after 14 weeks of administration of praziquantel liposomes is 4-fold greater than the corresponding number of mice surviving after administration of free drug or drug-free liposomes. It is clear from Table 3 that the chemoprophylactic effect of praziquantel liposomes administered 2 weeks before infection is also very evident after 8 weeks of administration; the percentage of mice surviving exceeds 90%. After 14 weeks of administration, the survival rate of mice after administration of praziquantel liposomes is double that of mice which had been administered free drug or drug-free liposomes.

Hepatic worm count

Many workers judge the success or failure of their attempts to cure or immunize the hosts from the ratio of the total number of living adult worms recorded to the number of infecting cercariae. The results of hepatic worm counting after 38 days from infection are listed in Table 4. It is evident from Table 4 that the hepatic worm count for mice injected with free drug greatly exceeds that for mice injected with praziquantel liposomes. This is clearly evident on comparison of the percentage worm recovery in the two groups. The result of the statistical analysis reveals that the difference in hepatic worm count for mice injected with free drug and those injected with the liposomes is highly significant ($P < 0.01$).

Conclusion

Encapsulation of praziquantel in a liposomal delivery system leads to the site-specific delivery of the drug towards the liver as well as ensuring sustained release properties. This has been shown to impart a prophylactic action to the drug towards schistosomiasis, demonstrated in mice by a marked increase in their survival rate as well as a statistically significant decrease in the hepatic worm count.

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