Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 295 (2005) 157–162
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Improvement of antischistosomal activity of praziquantel by incorporation into phosphatidylcholine-containing liposomes

Samanta C. Mourão [a,b], Paulo I. Costa [c], Hérida R.N. Salgado [a], Maria Palmira D. Gremião [a,*]

[a] *Programa de Pós-graduação em Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Rua Rodovia Araraquara, Jaú km 01, CEP 14801-902, Araraquara, SP, Brazil*
[b] *Nucleo de Investigacoes Quimico-Farmaceuticas (NIQFAR), Curso de Farmácia, Universidade do Vale do Itajaí, Rua Uruguai, 458, CEP 88302-202, Itajaí, SC, Brazil*
[c] *Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Rodovia Araraquara, Jaú km 01, CEP 14801-902, Araraquara, SP, Brazil*

Received 25 June 2004; received in revised form 8 November 2004; accepted 10 February 2005

Abstract

Praziquantel (PZQ) is effective against all known species of Schistosomes that infect humans. The failure of mass treatment of schistosomiasis has been attributed to the fact that therapy is not sufficiently long-lasting. This effect may be due to the low bioavailability of PZQ that has a low hydrosolubility and fast metabolism. Liposomes have been used to prolong drug levels, reduce the side effects, direct drugs to specific sites and increase bioavailability after administration. The aim of this work was to study the effect of phosphatidylcholine (PC)-containing liposomes to vehiculate PZQ to improve the treatment of schistosomiasis. The in vitro study was carried out using *Schistosoma mansoni* parasites recovered by perfusion from the hepatic portal system of infected mice. Suspensions of liposomes with PZQ and free PZQ were administered p.o. in mice after 14 days of infection. The effect of both preparations in vitro on *S. mansoni* culture was similar. In the in vivo test, PZQ-liposomes caused a decrease in amounts of eggs and parasites. Liposomes improve the antischistosomal activity of praziquantel. This can be used as a starting point to investigate alternative administration routes or dosage forms and to examine the mechanism of intestinal absorption of PRZ.

Keywords: Schistosomiasis; Praziquantel; Liposomes; Colloidal delivery systems; Controlled drug delivery

1. Introduction

Schistosomiasis is a serious public health problem in tropical countries. More than 600 million people are at risk and about 200 million actually infected in 74 countries (Chitsulo et al., 2000). An alternative to

* Corresponding author. Tel.: +55 16 33 01 69 75; fax: +55 16 2322022.
E-mail address: pgremiao@fcfar.unesp.br (M.P.D. Gremião).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.02.009

treatment of this disease is chemotherapy especially with praziquantel (PZQ), the effective drug against all important adult schistosome species and their immature forms (Harder, 2002; Xiaonong et al., 2002). Failures in mass treatment have occurred and have been attributed to the resistance of the parasite to PZQ (Xiaonong et al., 2002). One point that should be considered is drug bioavailability. This may be attributed to its fast metabolism and low hydrosolubility that can decrease the dissolution rate.

The effect of first passage can be evidenced by the low effectiveness in vivo against younger forms that are in the circulation. After oral administration, PZQ is extensively converted into inactive or considerably less potent compound. The younger forms are exposed to lower concentrations of unchanged PZQ in the systematic circulation than more mature forms located in the liver (Xiao et al., 1985).

Some researches have focused on methods to optimize the PZQ effect. The administration of PZQ concomitant with cimetidine or food increases the levels of PZQ in the plasma, with an improvement in the treatment outcome (Jung et al., 1997; Castro et al., 2000; Castro et al., 2002). Other studies have described the improvement of dissolution rate using the adjuvant such as β-cyclodextrin and polyvinylpyrrolidone (El-Arini and Leuenberg, 1998; Becket et al., 1999).

Another attempt of improving the effectiveness of PZQ is the use of liposomes. The use of PZQ in liposomes also decreased the infection of *Mesocestoides vogae tetrathyridia* in mice (Hrcková and Velebny, 1997). Using the arteether, an antimalarial agent as lipophilic as PZQ, Bayomi et al. (1998) showed an improvement of bioavailability by using an oral liposomal formulation when compared with the suspension formulation.

Taking into account the potential of liposomes to improve the bioavailability of PZQ, the aim of this work was to prepare phosphatidylcholine (PC) liposomes containing PZQ and also to study the effect of this system in vitro and in vivo.

2. Materials and methods

2.1. Materials

Praziquantel (Henrifarma), phosphatidylcholine (Epikuron-Luca Mayer), chloroform and ethanol (Merck), tris(hydroxymethyl) aminomethane (Aldrich), Hepes (Sigma Chemical), Pyrene (Sigma Chemical), RPMI (Sigma Chemical), and fetal calf serum.

2.2. Methods

2.2.1. Liposomal preparations

The drug was dissolved in chloroform solution containing PC. The solvent was evaporated under nitrogen flow and the dried film was kept under vacuum for 40 min. The dried film was hydrated with 20 mM Tris–HCl buffer pH 7.5 and kept in an ice bath for 45 min. The dispersion was then sonicated (Sonicator—Ultrasonic Processor, Heat Systems—model XL 2020).

In the solubility test, PC-containing liposomes with several concentrations of PZQ were prepared. After being stored at 25 °C overnight, the liposomal dispersions were centrifuged at 352 g for 10 min to separate the precipitated drug. The precipitate was dissolved in ethanol and was analyzed by spectrophotometer at 262 nm. The mean diameter of the liposomes was determined by dynamic light scattering spectrophotometer (Brookhaven-Laser He–Ne 10 mW, 514 and 532).

2.2.2. In vitro study

The in vitro study was carried out using *Schistosoma mansoni* parasites (strain LE.) recovered by perfusion from the hepatic portal system of infected mice (Balb-c) after 14 days of inoculation. The parasites were kept in RPMI medium (Sigma) with fetal calf serum, Hepes and penicillin and incubated with liposomes with PZQ (PZQ-L) or without it (PC-L) and PZQ in buffer solution (PZQ-F) (Table 1). Various concentrations of these samples were employed. The parasites were kept in an incubator at 37 °C under 95% air and 5% CO_2. The general aspect of the culture was monitored by optical inverted microscope at intervals (0, 2, 4, 20 and 40 h) to the death of all parasites. The death of parasites was visually observed by the absence of movement (Mourão,

Table 1
Concentration of PZQ and PC used in the in vivo test

Group	PZQ (mmol/L)	PC (mmol/L)
PZQ-F	8.6	–
PZQ-L	8.6	50
Control	–	–

Table 2
Concentration of the PZQ and PC used in vitro study

Sample	PZQ (μmol/L)	PC (mmol/L)
PC-L	–	0.05
	–	0.25
	–	0.5
PZQ-L	32	0.2
	3.2	0.02
	0.32	0.002
	0.0032	2×10^{-5}
PZQ-F	32	–
	3.2	–
	0.32	–
	0.0032	–

2001). After this procedure, the parasites were taken out of the culture media and the general aspect of the culture and the morphological alterations were analyzed by optical microscope with an image analyzer (Jenamed 2 Cal Zeiss-Jena).

2.2.3. In vivo study

The in vivo study was accomplished using Balb-c mice ($n = 10$) infected with *S. mansoni* strain LE. On the 14th day of infection, the mice were administered orally, using an appropriate syringe with 0.5 mL, PZQ suspended in buffer solution (PZQ-F group), PZQ in liposomes (PZQ-L group) or buffer Tris 20 mM pH 7.5 solution (control) (Table 2). On the 40th day of incubation, the feces were collected and the number of eggs per gram of the feces and the worms were counted (Zuim, 2003).

The feces pool was used to carry out the analysis of viable eggs. The mice were left for 1 h in a box with railing in bottom and their feces were collected. The sediments of the feces were rinsed and exposed to the light to allow the eggs to open. The animals were sacrificed and perfusion of the portal system was performed and worms recovery counted.

3. Results

3.1. Effect of liposomes on PZQ solubility and characterization of the liposomes

PC-liposomes 20 mmol/L with various concentrations of PZQ were prepared. Fig. 1 shows that using this concentration of PC there is a limit of solubilization of PZQ which was 3.7 ± 0.041 mmol/L. Fig. 2 shows that when 20 mM of PC was used, it was possible to incorporate, without precipitation, 4.2 mmol/L of PZQ. When we used 40 mM of PC, it was possible, in the same condition, to incorporate 8.69 mmol/L. So, the loading capacity of PZQ in PC-liposomes is around 1:5 PZQ:PC (molar ratio). From these data, an improvement of PZQ solubility correlated with lipid concentration was observed. The average diameters of PC-liposomes empty or with PZQ 3.2 mmol/L were 70.87 nm and 49.65 nm, respectively, indicating the formation of small unillamelar vesicles.

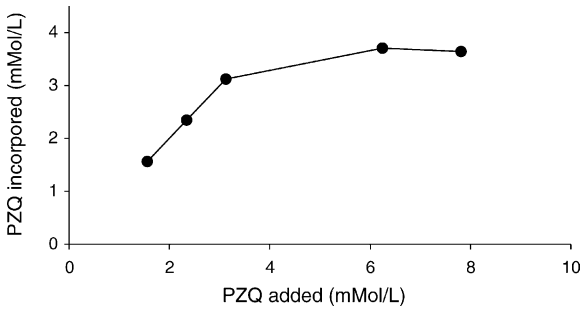

Fig. 1. Incorporation of PZQ using PC-containing liposomes (20 mmol/L).

3.2. Effect of liposomes and PZQ on culture of S. mansoni

The liposomal dispersion containing PZQ (PZQ-L) was used without separation. The parameters analyzed to verify the effect of the preparations in culture of *S.*

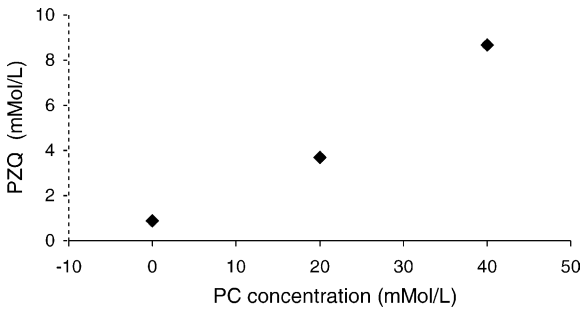

Fig. 2. Effect of PC concentration on PZQ incorporation in liposomes.

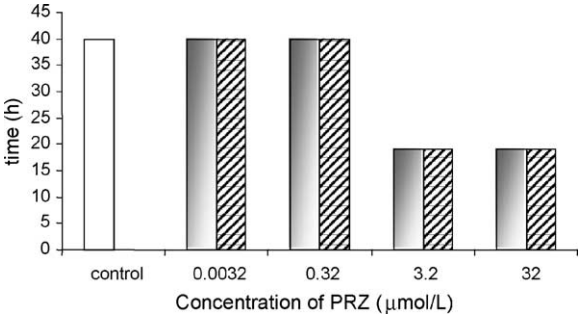

Fig. 3. Time of death for 100% of the parasites incubated with buffer Tris/pH 7.5 (control group) (□), PZQ-L (▣) and PZQ-F (▨).

mansoni were the general aspect of the culture observing the separation of couples and the motor activity of parasites. When compared with the control, higher concentration of liposomes without drug presented effect in culture of *S. mansoni*. Therefore, this was more significant in a lipid concentration. The concentration 0.5 mmol/L of the lipid caused the death of the parasites within 35 h. In the samples with concentrations lower than 0.25 mM, the aspect of the culture was almost similar to control. The PZQ-containing liposomal dispersions presented an effect similar to the one observed with the free drug (Fig. 3).

After incubation the parasites were analyzed under an optical microscope. Fig. 4 shows a parasite incubated with control solution (Tris buffer 20 mM pH 7.5). The presence of the female is observed in the ginecophoric channel indicating the couple's non-separation. There were no noticeable alterations in the external structure of the parasite. In the case of incubation with free PZQ (Fig. 5), a contraction of the parasite

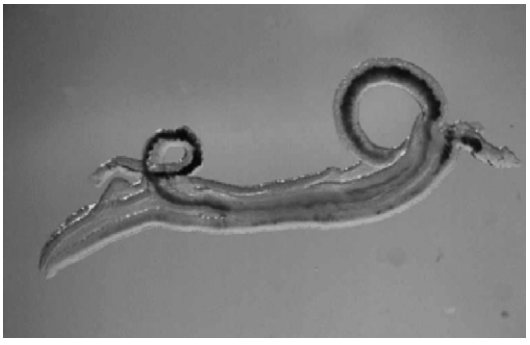

Fig. 4. Parasite incubated with control (Tris pH 7.5 20 mM); increase of 320×.

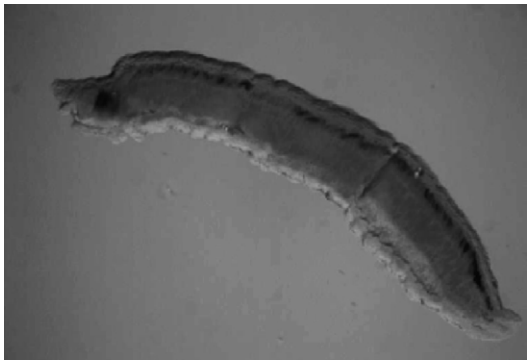

Fig. 5. Parasite incubated with PZQ-F (3.2 mM); increase of 500×.

and tegumental alterations was observed. Similar modifications were also observed when the parasites were incubated with liposomes of PC (Fig. 6) and liposomes of PC containing PZQ (Fig. 7).

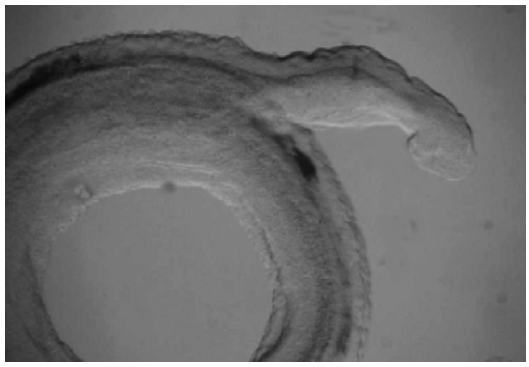

Fig. 6. Parasite incubated with liposomes of PC-L (0.5 mM); increase of 800×.

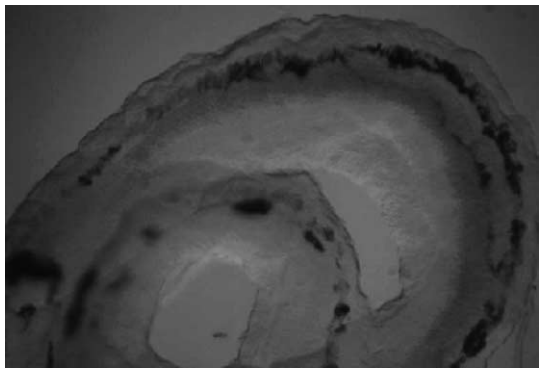

Fig. 7. Parasite incubated with PZQ-L (3.2 mM); increase of 800×.

Table 3
Effect of a single oral dose of PZQ-liposomes or PZQ-suspension in mice infected with *S. mansoni*

Group	No. of mice analyzed/no. of mice treated	No. of viable eggs/g of feces	Egg reduction (%)	No. of worms/mice	Worm reduction (%)
Control	6/10	103.23	–	39.83	–
PZQ-L	6/10	50	51.56	22.5	43.51
PZQ-F	9/10	85.56	17.18	40.44	0

3.3. Effect in vivo

Table 3 shows the effect of the single oral dose of PZQ in liposomes (PZQ-L) or PZQ-free (PZQ-F) in mice infected *S. mansoni*. PZQ-L was more effective than PZQ-free, since it was able to deliver a higher concentration of PZQ to reduce the amount of parasite eggs and to kill the worms. The intensive reduction can be calculated from the equation $[(C - \chi/C) \times 100]$, where C and χ are the number of the viable eggs per gram feces or worms recovery per mice of the control group and treated group, respectively.

4. Discussion

PZQ is the main drug for the treatment of schistosomiasis. However, new studies are still necessary to understand the mechanism of action, as well as to develop pharmaceutical forms to improve its bioavailability.

The incorporation of PZQ in small unilamellar liposomes composed of PC can allow the drug administration in an aqueous media without decreasing the effect of *S. mansoni* of the drug, since it is related with the lipophilic profile of the drug due to the interaction between the drug and the parasite membrane. The incorporation of PZQ in liposomes showed similar effect of the effect on *S. mansoni* cultures.

High concentration of the lipid in liposomes without the drug caused alteration in the motility and general aspect of *S. mansoni* culture. Similar results in culture of *Mesocestoides vogae tetrathyridia* have been observed (Hrcková and Velebny, 1997). *S. mansoni* is able to capture external lipids through an active process of absorption and metabolism (Browers et al., 1998).

The in vivo study demonstrated the possibility of the liposomes to improve the PZQ activity. This can be used as a starting point to investigate alternative administration routes in order to improve PZQ efficacy and to examine the mechanism of intestinal absorption of PRZ.

Acknowledgements

The authors are grateful to Prof Leila Oliveira for her assistance in reading and correcting the manuscript. Financial support provided by FAPESP, CNPq and CAPES is acknowledged.

References

Bayomi, M.A., Al-Angary, A.A., Al-Meshal, M.A., Al-Dardiri, M.M., 1998. In vivo evaluation of arteether liposomes. Int. J. Pharm. 175, 1–7.

Becket, G., Schep, L.J., Tan, M.Y., 1999. Improvement of the in vitro dissolution of praziquantel by complexation with α- , β- and γ-cyclodextrins. Int. J. Pharm. 179, 65–71.

Browers, J.F.H.M., Hellemond, J.J.V., Van Golde, L.M.G., Tielens, A.G.M., 1998. Ether lipids and their possible physiological function in adult *Schistosoma mansoni*. Mol. Biochem. Parasitol. 96, 49–58.

Castro, N., Jung, H., Medina, R., Gonzalez-Esquivel, D., Lopez, M., Sotelo, J., 2002. Interaction between grapefruit juice and praziquantel in humans. Antimicrob. Agents Chemother. 46, 1614–1616.

Castro, N., Medina, R., Sotelo, J., Jung, H., 2000. Bioavaibility of praziquantel increases with concomitant administration of food. Antimicrob. Agents Chemother. 44, 2903–2904.

Chitsulo, L., Engels, D., Montrsor, A., Savioli, L., 2000. The global status of schistosomiaisis and its control. Acta Trop. 77, 41–51.

El-Arini, S.K., Leuenberg, H., 1998. Dissolution proprieties of praziquantel-PVP systems. Pharm. Acta Helv. 73, 89–94.

Harder, A., 2002. Chemotherapeutical approaches to schistosomes: current knowledge and outlook. Parasitol. Res. 88, 395–397.

Hrcková, G., Velebny, S., 1997. Effect of praziquantel and liposome-incorporated praziquantel on peritoneal macrophage activation in mice infected with *Mesocestoides corti tetrathydria* (Cestoda). Parasitology 114, 475–482.

Jung, H., Medina, R., Castro, N., Corona, T., Sotelo, J., 1997. Pharmacokinetic study of praziquantel administered alone and in

combination with cimetidine in a single-day therapeutic regimen. Antimicrob. Agents Chemother. 41, 1256–1259.

Mourão, S.C., 2001. Preparação e caracterização de lipossomas contendo praziquantel. MSc thesis. Universidade Estadual Paulista, Araraquara-SP, Brazil.

Xiao, S., Catto, B.A., Webster Jr., L.T., 1985. Effect of praziquantel on different developmental stages of *Schistosoma mansoni* in vitro and in vivo. J. Infect. Dis. 151, 1130–1137.

Xiaonong, Z., Minggag, C., Mcmanus, D., Bergquist, R., 2002. Schistosomiasis control in the 21st century. Proceedings of the International Symposium on Schistosomiasis, Shangai, July 4–6, 2001. Acta Trop. 82, 95–114.

Zuim, N. R. B., 2003. Características morfológicas e biológicas do *Schistosoma mansoni* oriundo de população de moluscos selecionados geneticamente, MSc thesis. Universidade Estadual de Campinas-Campinas, Brazil.