Acta Tropica 128 (2013) 70–75

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Liposomal-praziquantel: Efficacy against *Schistosoma mansoni* in a preclinical assay

Tarsila Ferraz Frezza[a,*], Maria Palmira Daflon Gremião[b], Eliana Maria Zanotti-Magalhães[a], Luiz Augusto Magalhães[a], Ana Luiza Ribeiro de Souza[b], Silmara Marques Allegretti[a]

[a] Departamento de Biologia Animal, Laboratório de Helmintologia, Instituto de Biologia, UNICAMP, Campinas, Brazil
[b] Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, UNESP-Universidade Estadual Paulista, Araraquara, Brazil

ARTICLE INFO

Article history:
Received 9 May 2012
Received in revised form 14 June 2013
Accepted 15 June 2013
Available online 26 June 2013

Keywords:
Praziquantel
Encapsulation
Liposomes
Schistosoma mansoni
In vivo assay

ABSTRACT

Currently, schistosomiasis mansoni is treated clinically with praziquantel (PZQ). Nevertheless, cases of tolerance and resistance to this drug have been reported, creating the need to develop new drugs or to improve existing drugs. Considering the small number of new drugs against *Schistosoma mansoni*, the design of nanotechnology-based drug delivery systems is an important strategy in combating this disease. The aim of this study was to evaluate the activity of PZQ containing liposome (lip.PZQ) on *S. mansoni*, BH strain. Mice were treated orally with different concentrations of PZQ and lip.PZQ 30 and 45 days following infection. The number of worms, recovered by perfusion of the hepatic portal system, and the number of eggs found in the intestine and liver were analysed. Parasite egg counts were also performed. The most active formulation for all parameters was 300 mg/kg of lip.PZQ, since as it decreased the total number of worms by 68.8%, the number of eggs in the intestine by 79%, and the number of hepatic granulomas by 98.4% compared to untreated controls. In addition, this concentration decreased egg counts by 55.5%. The improved efficacy of the treatment with lip.PZQ, especially when administered 45 days following infection, compared with the positive-control group (untreated) and the groups that received free PZQ, can be explained by greater bioavailability in the host organism; the preferred target of lip.PZQ is the liver, and lip.PZQ is better absorbed by the tegument of *S. mansoni*, which has an affinity for phospholipids.

1. Introduction

Human schistosomiasis is a debilitating parasitic disease caused by digenetic trematodes of the genus *Schistosoma*. It is estimated that more than 207 million people worldwide are infected, and 500,000 people die every year due to this disease (Steinmann et al., 2006; King, 2009). It is considered one of the most prevalent and important neglected parasitic diseases in the developing world (Parise-Filho and Silveira, 2001; Utzinger and Keiser, 2004). *S. mansoni* occurs in 52 countries and territories. In Brazil, approximately 43 million people are at risk of infection and 7 million are infected, making Brazil the country most affected by this type of schistosomiasis in the Americas (Lambertucci, 2010; World Health Organisation, 2010).

Oxamniquine and praziquantel (PZQ) are currently the only drugs used to treat schistosomiasis mansoni in Africa and the Americas (Ferrari et al., 2003). The distribution of oxamniquine is not adequate because the only pharmaceutical company that produces and sells it has a limited production. PZQ is the only widely available form of treatment, and, despite its broad activity spectrum and low toxicity, it has failed in many treatments, as proven by the detection of eggs in stool examinations post-treatment–PZQ treatment fails because its dependence on the age of the parasite (the best results are achieved during the 5th and 6th weeks post infection; Gönnert and Andrews, 1977; Sabah et al., 1986; Stelma et al., 1995; Pica-Matoccia and Cioli, 2004); its potential to favour resistance (Doenhoff et al., 2002); its rapid absorption into the blood 1–3 h after ingestion (Leopold et al., 1978; Valencia et al., 1994); and its low and erratic bioavailability due to its poor water solubility and extensive first-pass metabolism (Yang et al., 2009). The many strategies currently being investigated to overcome these obstacles include the design and development of drug-delivery systems. Lipid-based delivery systems, such as liposomes, are finding increasing application in the oral delivery of poorly water-soluble drugs (Porter et al., 2008). Liposomes have received considerable attention as drug-delivery systems due their ability to incorporate hydrophilic and hydrophobic drugs, their good biocompatibility and their low toxicity (Mufamadi et al., 2011). Liposomes are

* Corresponding author at: Departamento de Biologia Animal, Laboratório de Helmintologia, Instituto de Biologia, Cidade Universitária Zeferino Vaz s/n°, Barão Geraldo, CEP: 13083-970, Campinas, SP, Brazil. Tel.: +55 19 35216303.
E-mail address: taferraz@gmail.com (T.F. Frezza).

0001-706X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.actatropica.2013.06.011

microscopic vesicles consisting of one or more concentric spheres of lipid bilayers separated by aqueous compartments. These spherical structures can have diameters ranging from 80 nm to 100 μm (Mourão et al., 2005).

Clinical and preclinical studies have proved that encapsulation of drugs into liposomes decreases their side effects, targets them to specific sites in the organism, reduces their toxicity, improves their bioavailability, changes their pharmacokinetics, increases their solubility in aqueous systems, and contributes to controlling drug release (Ranson et al., 1996; Siler-Marinkovic et al., 1997). Understanding the behaviour of liposomes in biological systems and the physical and chemical mechanisms involved in their interaction with the drug and the site of action is essential so that liposome-encapsulated drugs may become available in the future.

This research evaluated the activity of liposome-encapsulated PZQ (lip.PZQ) on mice infected with a Brazilian strain of *S. mansoni*; more specifically, this study evaluated the effects on the adult worms, egg laying, and formation of hepatic granulomas.

2. Materials and methods

2.1. Preparation of liposome-encapsulated PZQ

Soybean phosphatidylcholine (PC) liposomes were prepared using the sonication method (Cinto et al., 2009). A lipid film was obtained from a PC chloroform solution by evaporating the solvent under vacuum in a rotary evaporator until a thin lipid film formed. Final traces of solvent were eliminated in a vacuum desiccator, overnight. Next, 5 mL of Tris–HCl buffer (20 mM, pH 7.5) was added to the lipid film for hydration and then left at 25 °C for 40 min. After hydration, the PC liposomes were obtained by sonicating the suspension in an ice bath for 25 min. To prepare the PZQ-loaded liposomes, the drug was dissolved in the PC-chloroform solution, in a PZQ/PC molar ratio equal to 1:6. This ratio was used because the loading capacity of PZQ, without precipitation, in PC liposomes is approximately 1:5 PZQ/PC (molar ratio; Mourão et al., 2005).

2.2. S. mansoni strain

The BH strain (from Belo Horizonte, Minas Gerais, Brazil), kept at the Department of Animal Biology of the Institute of Biology of Unicamp, was propagated in *Biomphalaria glabrata* molluscs (Pellegrino and Katz, 1968).

2.3. Host animals

B. glabrata specimens sympatric with the *S. mansoni* strain were infected with miracidia collected from faeces of mice infected 45 days previously (such mice were used only for the infection of the intermediate host). After 40 days the molluscs were subjected to illumination at 28 °C to expose the cercariae. The cercarie were then used to infect 30-day-old females of *Mus musculus*, *Swiss* strain, by inserting their tails into the cercarial suspension at 70 cercariae per animal (Olivier and Stirewalt, 1952; Pellegrino and Katz, 1968). The protocol for these infection experiments (1117-1) was approved by the Ethics Commission for Animal Experimentation (CEEA) of the Institute of Biology of Unicamp, in accordance with the ethical principles adopted by the Brazilian Association of Animal Experimentation (COBEA).

2.4. Experimental groups

Eight groups with 30 animals each were used. Four groups were treated 30 days after infection so that the activity of the drugs on young adult worms could be tested. The remaining four groups were treated on the 45th day to test the activity of the drugs on adult worms. Four concentrations of both free and encapsulated PZQ (lip.PZQ) were used: 47, 60, 250, and 300 mg/kg. Ten animals in each group received PZQ; another ten animals received the same concentration of lip.PZQ; finally, the remaining ten animals (the positive-control group) received Tris–HCl buffer (20 mM, pH 7.5). These treatments were administered orally in a single dose (0.3 mL) by the intragastric route.

2.5. Treatment analysis

Fifteen days after the treatments, the animals were euthanised by cervical dislocation. Worms were collected by perfusion of the hepatic portal system (Yolles et al., 1947), and males, females and couples were counted. The liver was removed and compressed between glass plates so that worms could be observed. By removing 1-cm^2 fragments from the ascending colon, parasite egg counts were performed at the various maturity stages: 1st to 4th stage (immature eggs), 5th stage (mature eggs), and dead eggs. Any egg count was considered changed when one or more stages of immature eggs were missing (Pellegrino et al., 1962).

2.6. Histological observations

The dorsal lobe of the liver was removed and fixed in 10% formaldehyde. Then, 5-μm-thick histological sections were stained with haematoxylin and eosin, and their granulomas were photographed and counted in 10 random fields using Leica Image Manager 50/4.0 software.

2.7. Statistical analyses

The Kruskal–Wallis and Student-Newman-Keuls tests were carried out using the BioEstat 5.0 software. Statistical relevance was assumed at $p < 0.05$. The efficiency rates for worms and eggs, the relative sex ratio and the hepatic shift were calculated by the following formulas: Efficiency = (Positive Control Group − Treated Group/Positive-Control Group) × 100; Relative Sex Ratio = (Adult Male/Female Worms in the Treated Group)/(Adult Male/Female Worms in the Positive-Control Group); Hepatic Shift = (Number of Worms in the Liver/Total of Worms) × 100 (Delgado et al., 1992).

3. Results

3.1. Effect of free PZQ and lip.PZQ on the population of adult S. mansoni worms

The lowest concentrations (47 and 60 mg/kg) of lip.PZQ, on both days of treatment (30th and 45th), showed low activity on the adult worms found in the hepatic portal system of the necropsied mice, causing little or no decrease in their number (Tables 1 and 2). When 250 mg/kg of lip.PZQ was administered, the decrease in the number of worms was greater. The treatment efficiency was 68.8% at 45 days after infection. In addition, the highest concentrations of lip.PZQ (250 and 300 mg/kg), administered on both days of treatment, resulted in fewer mated worms in comparison with the other groups, including the control. The most efficient treatment for adult *S. mansoni* worms used 300 mg/kg of lip.PZQ 45 days following infection. This dose had higher efficiency and resulted in fewer worms within the hepatic portal system in comparison with the other treatments and positive-control groups. Additionally, the results from this dose showed a significant difference ($p = 0.00635$) compared with free PZQ administered on the same day of treatment. 300 mg/kg lip.PZQ at 45 days resulted in the lowest number of mated couples, a difference that proved significant even compared to the positive control group given the corresponding concentration ($p = 0.0007$). When lip.PZQ and PZQ were administered 45 days