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Enhanced schistosomicidal efficacy of tartar emetic encapsulated in pegylated liposomes

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Abstract

The aim of the present study was to evaluate the ability of liposomes to improve the efficacy of tartar emetic (TA) against established *Schistosoma mansoni* infection. TA was used as a schistosomicidal drug model and both conventional liposomes (CL) and long-circulating pegylated liposomes (LCL) were evaluated. In the first experiment, TA, either free or encapsulated within CL or LCL, was given intraperitoneally (i.p.) as a single dose of 11 mg Sb/kg to mice experimentally infected with *S. mansoni*. Only the group treated with LCL showed a significant (55%) reduction in the worm burden, compared to the control groups (untreated or treated with empty LCL). In the second experiment, the efficacy of TA-containing LCL was evaluated at a higher dose (27 mg Sb/kg) by both subcutaneous (s.c.) and i.p. routes. Reduction levels of 67 and 82% were achieved by s.c. and i.p. routes, respectively. Strikingly, all mice survived to this high dose of antimony. This is in contrast with free TA that was lethal in 100% of mice at the same dose. The present work demonstrates that LCL reduce the acute toxicity of TA and effectively deliver this drug to *S. mansoni* during the late stages of parasite infection.

Keywords: Liposomes; Tartar emetic; Schistosomiasis; Antimony; *Schistosoma mansoni*

1. Introduction

It is estimated that about 200 million people in the world are currently affected by schistosomiasis, a chronic and debilitating disease caused by flat worms belonging to the genus *Schistosoma* (Sturrock, 2001). Trivalent antimonials, including tartar emetic (TA), were the first class of compounds to be employed in the clinical treatment of schistosomiasis (Cioli et al., 1995). However, their use was discontinued because of their low therapeutic index and the appearance of less toxic single-dose drugs, oxamniquine and praziquantel. Oxamniquine is now effectively unobtainable and resistance to praziquantel is seen as an emerging

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problem (Appleton and Mbaye, 2001; Sturrock, 2001). In this context, new drugs need to be developed or existing ones improved.

Previous works have evidenced the potential of liposomes for enhancing the efficacy of schistosomicidal drugs (Ammar et al., 1994; El-Ridy et al., 1989; Frézard and Melo, 1997; Melo et al., 2001), however, improvements were reported only when liposome preparations were given before or at a time close to the infection.

In the present study, TA was used as a schistosomicidal drug model and both conventional liposomes (CLs) and long-circulating pegylated liposomes (LCL) were evaluated against late stages of *Schistosoma mansoni* infection.

2. Materials and methods

2.1. Preparation and characterization of liposomes

L-α-Distearoylphosphatidylcholine (DSPC), cholesterol (CHOL) and PEG(2000)-distearoylphosphatidylethanolamine (DSPE-PEG), obtained from Avanti Polar Lipids (Alabaster, AL, USA), were used to prepare CLs (DSPC/CHOL, 5:4 molar ratio) and LCLs (DSPC/CHOL/DSPE-PEG, 5:4:0.3 molar ratio). The encapsulation of TA (80 g/l in water) or PBS (150 mM NaCl, 10 mM phosphate, pH 7.2) was carried out within freeze–thawed multilamellar vesicles (FATMLVs), as described by Mayer et al. (1985). FATMLVs (lipid concentration of 120 g/l) were then repeatedly extruded through two stacked polycarbonate membranes of 100 nm pore size (Nayar et al., 1989) and finally submitted to dialysis against saline (150 mM NaCl) to remove non-encapsulated TA. The mean hydrodynamic diameter of the vesicles, as determined by photon correlation spectroscopy (Malvern Instruments type 4700), was found equal to 120 nm (polydispersity 0.088) and 130 nm (polydispersity 0.085) for LCL and CL, respectively.

To determine the concentration of encapsulated antimony, liposomes were first solubilized by mixing an aliquot of the suspension with 0.5 volume of a 20% (w/v) triton X-100 solution. Antimony was determined photometrically, using the chromogen bromopyrogallol red (BPR) (Frézard et al., 2001). The absorbance of BPR at 560 nm decreases proportionally to the amount of antimony, as a consequence of the formation of the 1:1 BPR–Sb(III) complex. A calibration curve was established using TA as the source of antimony.

2.2. Release of antimony from LCL liposomes in mice serum

The time-course of release of antimony from liposomes was determined in mice serum, following the addition of 50 μl of liposome suspension to 450 μl of the serum and incubation of the mixture at 37 °C (final antimony concentration of 1.35 mM). The concentration of released antimony was then determined photometrically at different intervals by adding a 20 μl aliquot of this mixture to 1 ml of the BPR analyte solution (Frézard et al., 2001). We checked that, in these conditions, the presence of serum components did not interfere significantly with the colorimetric assay.

2.3. Evaluation of antischistosomal activity

SWISS mice (male, adult, weighing about 30 g) were infected with about 80 *S. mansoni* (LE strain) cercariae. Treatment was carried out 35 days after the infection, by intraperitoneal (i.p.) or subcutaneous (s.c.) route, using TA, either free or encapsulated in CL or LCL. Control groups consisted of a group injected with empty LCL and of an untreated group. Two weeks after the treatment, animals were sacrificed and perfusion of the portal system was performed for worm recovery and count.

3. Results and discussion

Encapsulation of TA was achieved in both CLs and LCLs with a trapping efficiency of 6.5% and a final antimony/lipid ratio of 0.015 (w/w). These liposomes were made from CHOL and the high-phase transition temperature phospholipid, DSPC, a lipid composition known to promote high liposome stability in the blood (Frézard, 1999). When LCL were incubated in mice serum at 37 °C, about 7% of encapsulated antimony was found to be released within 24 h. As expected from the first Fick's law, the kinetic of release followed a monoexponential decay (data not shown). From this data, a half-time of drug release (time at which 50%

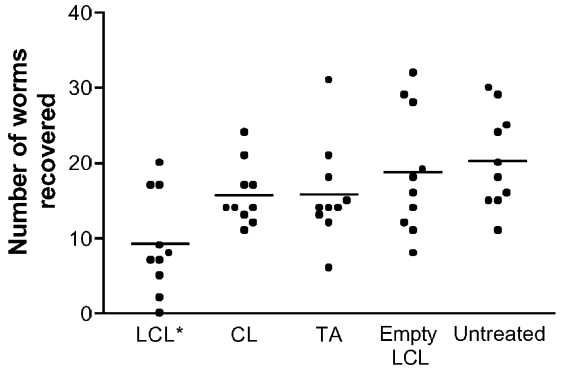

Fig. 1. Effect of tartar emetic (TA), either free or encapsulated within conventional (CL) or long-circulating (LCL) liposomes, and of empty liposomes, on the worm burden in mice infected with *S. mansoni*. Drugs were given to infected mice intraperitoneally (i.p.) at 11 mg Sb/kg. Each point represents the number of worms recovered from each animal, 2 weeks after treatment. Bars represent the means of worms. (*) $P < 0.01$ for comparison between LCL and untreated, and $P < 0.05$ for comparison between LCL and empty LCL (one-way ANOVA with Tukey post test).

of encapsulated antimony is released) of 9 days could be estimated.

TA, either free or encapsulated within CL or LCL, was given by i.p. route to infected mice as a single dose of 11 mg Sb/kg. The number of worms recovered from each animal after treatment is displayed in Fig. 1. Strikingly, only the group treated with TA-containing LCL showed a significant (55%) reduction in the worm burden compared to the control groups, either untreated or treated with empty LCL.

In LCL group, 7 out of 10 animals showed less than 11 worms. This is in contrast with the other groups, in which at best one animal showed less than 11 worms. Statistical analysis of this data (χ^2 test) established the higher effectiveness of LCL over CL and free TA ($P < 0.05$).

The present data represents the first evidence that LCL can enhance the effectiveness of an antischistosomal drug against the late stages of parasite infection. The fact that LCL were more effective than CL and free TA suggests an improvement of the bioavailability of antimony. It is well known that LCL, given by i.p. or intravenous routes, exhibit prolonged blood circulation times, owing to their surface modification with poly(ethylene glycol) polymer. In mice, LCL-encapsulated drugs showed prolonged blood levels, typically for a duration of 3 days (Woodle and Lasic, 1992). In contrast, CL are rapidly cleared from the blood circulation by fixed macrophages of the liver and spleen, and drug is released back into the blood after liposome degradation within phagolysosomes (Frézard, 1999). Finally, free TA, given i.p. to mice, was found to be rapidly absorbed and eliminated from the blood through excretion in both urine and feces. Typically, antimony blood level dropped to 10% of maximum level within 24 h (Ness et al., 1947). Considering that *S. mansoni* worms are located within the blood stream (Melo and Pereira, 1985) and feed on blood components at this late stage of parasite infection, the superiority of LCL may be attributed not only to the prolonged presence of TA within the host organism but also to their ability to be captured by the worms within the circulation.

In a second experiment, TA was administered at 27 mg Sb/kg by i.p. or s.c. route, either in the free form or in the encapsulated form in LCL. All mice that received the free drug died within 12 h (12 animals in each group). In contrast, all mice that received the liposome preparation survived (12 in each group). The reduced toxicity of this liposome preparation can be attributed to its slow release properties, as evidenced by the high half-time of TA release within serum.

Mice that received TA-containing LCL by i.p. and s.c. routes showed 5.5 and 3 times less worms, respectively, than mice from untreated group, indicating reduction levels of 82 and 67% (Fig. 2). Comparison

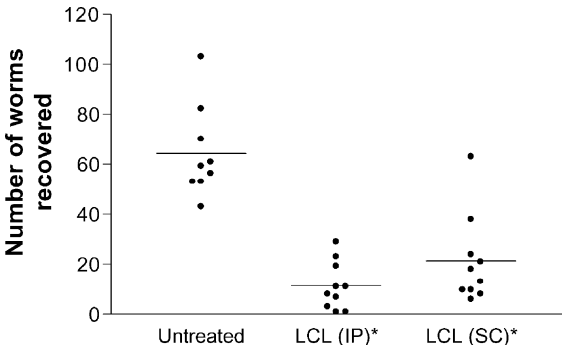

Fig. 2. Efficacy of TA-containing LCL by i.p. and s.c. routes against *S. mansoni* infection. Drug was given to mice at 27 mg Sb/kg 5 weeks after the infection. Each point represents the number of worms recovered from each animal, 2 weeks after treatment. Bars represent the means of worms. (*) $P < 0.001$ for comparisons between LCL (s.c.) or LCL (i.p.) and untreated (one-way ANOVA with Tukey post test). No significant difference between LCL (s.c.) or LCL (i.p.).

between i.p. and s.c. routes did not show statistically significant difference (one-way ANOVA, $P > 0.05$). A similar efficacy was indeed expected following i.p. and s.c. injections, as a large fraction of small-sized LCL (diameter inferior to 120 nm) was found to reach the blood circulation in both cases, even though absorption was slower by s.c. route (Allen et al., 1993).

4. Conclusions

The present work demonstrates that LCLs reduce the toxicity and effectively deliver TA to *S. mansoni* during the late stages of parasite infection. The use of this type of liposomes represents a promising strategy to improve the effectiveness of antischistosomal drugs. In future studies, strategies to further improve the selectivity of liposomes for the parasites should be investigated.

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