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Reduced cardiovascular alterations of tartar emetic administered in long-circulating liposomes in rats

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ABSTRACT

Trivalent antimonial drugs, including tartar emetic (TA), are known to induce important cardiotoxicity observed by electrocardiographic abnormalities. Liposome encapsulation was found to reduce the overall acute toxicity of TA. The present work investigated the cardiovascular parameters alterations of rats submitted to the treatment with free and encapsulated TA in long-circulating liposomes. Liposomes were made using lipids DSPC, DSPE-PEG and cholesterol. The cardiovascular signals, electrocardiogram (ECG) and arterial blood pressure (AP), were recorded from anaesthetized Wistar rats after intravenous (IV) administration of a single specially high dose (17 mg/kg) of TA in liposomes and in free form. The IV administration of TA solution caused significant increase of QT interval of ECG and significant reduction of AP when compared to the control group. These alterations were not observed when liposomes TA were administered and the profile of ECG and AP data was quite similar to the control groups. In conclusion, a liposomal formulation of TA showed a reduced cardiotoxic profile for TA when compared to the free form.

1. Introduction

Toxic effects on the cardiovascular system are routinely described for many drugs, including anti-parasitic ones (Crumb and Cavero, 1999; Batey and Coker, 2002). Non-cardiac drugs inducing rhythm disturbances, like QT interval prolongation, which have the electrocardiogram (ECG) features of 'torsade de points', were described in the 1960s (Shah, 2007). The determination of cardiac toxicity using QT interval prolongation continued to be studied (Morganroth, 1993) and it is a necessity for decision-making during drug development (EMEA, 1997, 2005; Haverkamp et al., 2000).

The trivalent antimonial drugs, including tartar emetic (TA), were the first class of compounds employed in the clinical treatment of schistosomiasis (Cioli et al., 1995), and TA was also used to treat leishmaniasis in the 1912–1960 (Frézard et al., 2005). However, their use was discontinued because of their low therapeutic index and side effects like trombocytopenia, other dyscrasias, and electrocardiographic disturbances. The cardiovascular alterations induced by antimonial compounds include ECG alterations such as ST segment inversion and QT interval prolongation, and, consequently, 'torsade de points' arrhythmias and sudden cardiac arrest (Lacerda-Junior et al., 1965; Chulay et al., 1985).

Brazil is considered one of the main area of schistosomiasis distribution in the world (Seubert et al., 1977), and the chemotherapy plays an important role in reducing schistosomiasis morbidity (WHO, 1993). However, it has always been limited due to the difficulty of discovering drugs presenting high efficacy and reasonable tolerance (Seubert et al., 1977; Andrews et al., 1983). In this context, efforts have been devoted to the improvement of old drugs by development of novel formulations. Importantly, TA encapsulated in long-circulating pegylated liposomes was more effective against established Shistosoma mansoni infection during the late stages of infection, than free TA or TA encapsulated in conventional liposomes (De-Melo et al., 2003). Furthermore, liposome encapsulation was found to reduce the overall acute toxicity of TA (De-Melo et al., 2003). However, the impact of liposome encapsulation, specifically in reducing cardiotoxicity of TA has not yet been determined. Thus, the aim of the present work was to compare the cardiovascular parameters, mainly the cardiotoxic markers, in rats submitted to the treatment with free TA and TA encapsulated in long-circulating liposomes.

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2. Materials and methods

2.1. Drugs and reagents

TA was purchased from Sigma (USA), L-α-distearoylphosphatidylcholine (DSPC) and PEG (2000)-distearoylphosphatidyl-ethanolamine (DSPE-PEG) were supplied by Lipoid GmBh (Ludwigshafen, Germany), and cholesterol was purchased from Sigma (USA). The solvents were of analytical grade and all other chemicals were commercially available. Water was purified by reverse osmosis (Symplicity System 185, Millipore, USA).

2.2. Preparation of tartar emetic containing pegylated liposomes

Liposomes were made from DSPC, cholesterol and DSPE-PEG at a molar ratio of 5:4:0.3. The encapsulation of TA (80 g/l in water) or PBS (150 mM NaCl, 10 mM phosphate, pH 7.2) was carried out in freeze–thawed multilamellar vesicles (Mayer et al., 1985). The multilamellar vesicles (lipid concentration of 120 g/l) were repeatedly extruded through two stacked polycarbonate membranes of 200 nm pore size (Nayar et al., 1989), and finally submitted to dialysis against saline (150 mM NaCl) to purify TA containing liposomes and to remove non-encapsulated TA.

2.3. Liposome characterization

The concentration of encapsulated antimony was determined after mixing an aliquot of the suspension with 0.5 ml of a 20% (w/v) triton X-100 solution to solubilize the liposomes. Antimony was determined photometrically, using the chromogen bromopyrogallol red (BPR) (Frézard et al., 2001). According to this method, 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. The mean hydrodynamic diameter and polydispersity index of the vesicles suspensions were measured using the 3000 HS Zetasizer equipment (Malvern Instruments, Worcestershire, England).

2.4. Experimental animals

Male Wistar rats (250 ± 30 g) were randomly distributed into four experimental groups: the first received 17 mg of Sb/kg (3.4 mg Sb/ml in PBS) of TA intravenously (IV); a second received 17 mg of Sb/kg (4.36 mg Sb/ml) of liposomal TA IV. The other two groups received control solutions containing only vehicles: PBS or empty liposomes. The final volume administered was 0.8 ± 0.2 ml. The animals were anaesthetized with 60 mg/kg of sodium thiopental administered intraperitoneally. When the anesthesia reached the appropriate depth, the animals were tracheotomized to facilitate breathing. The femoral artery and vein were catheterized to recording of arterial blood pressure (AP) and IV drug administration, respectively. The catheters were previously filled with 1% heparin in 0.9% NaCl sterile solution. Stainless steel needle electrodes were inserted subcutaneously to record ECG.

All procedures related to the use of animals in these studies were reviewed and conform to the Ethical Principles of Animal Experimentation (Brazilian College of Animal Experimentation) and were approved by the UFOP Ethics Committee under number 99/2007.

2.5. Determination of cardiovascular parameters and protocols

AP was continuously recorded using a disposable pressure transducer (TruWave, Edwards Life Sciences) connected to a signal conditioning system. Limb lead II ECG was continuously recorded using subcutaneous stainless steel needle electrodes connected by a shielded cable to a biopotential amplifier. The AP signal conditioning system and the biopotential amplifier were designed and built in our laboratory, and all the care related with the frequency response of amplifiers (0.5–100 Hz to the biopotential amplifier and 0–30 Hz to AP amplifier) and the non-utilization of 60 Hz notch filters was taken to avoid distortion on recorded signals (Vale-Cardoso and Guimarães, 2010). The output signals of these systems were sampled at 1200 Hz by a 16-bits A/D conversion board (DaqBoard/2000, IOtech, USA) and stored on a PC hard disk.

The ECG and AP signals were recorded for 5 and 20 min before and after injection of the different formulations, respectively. Thereafter, segmented data records of 30 s were performed every 10 min up to 1 h after the injection of the different formulations. The stored records were analyzed off-line. From the stored records were extracted 2 s segments (raw data), containing 6 to 12 heart beats depending on the heart rate, and all the cardiovascular parameters were calculate as a mean value of these segments (filtered data). The cardiac parameters extracted from ECG records were QT (interval between the beginning of the Q-wave and the end of the T-wave), RR (interval between two successive R-waves and used to obtain the heart rate: HR = 60/RR), PR (interval between the beginning of the P-wave and the end of the R-wave) and QRS (interval from the beginning of the Q-wave to the end of the S-wave) intervals. Several mathematical formulae have been proposed to minimize the QT interval dependence on heart rate (Simonson et al., 1962), deriving a heart rate corrected QT interval (QTc). The best known are Bazett's ($QTc = QT/RR^{1/2}$) (1920) and Fridericia's ($QTc = QT/RR^{1/3}$) (1920) formulae. We choose the Fridericia's formula, based on the work of Abernethy et al. (2001), who suggest this correction criteria for the QTc for the cases of RR < 500 ms, as was consistently observed in our experiments (Vidal et al., 2010). The cardiovascular parameters extracted from AP records were systolic (SAP) and diastolic blood pressure (DAP) signals.

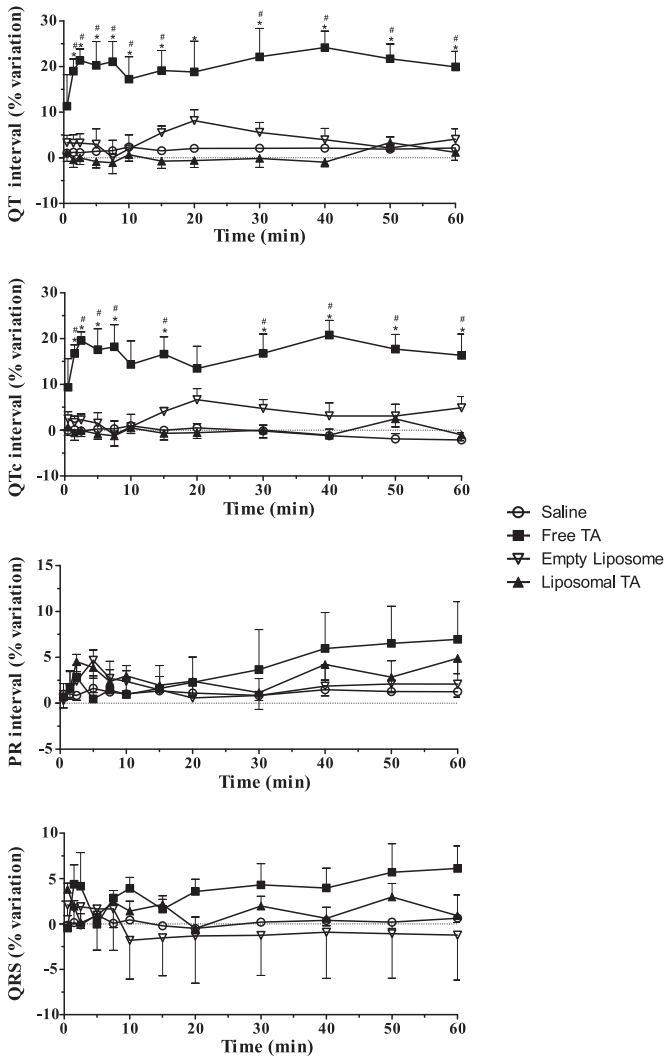

Fig. 1. Percentual variation of lead II ECG parameters obtained in anaesthetized rats after IV administration of free TA or in liposomes (17 mg Sb/kg) or control solutions. $^{\#}P$ value < 0.05 related to the saline group and $^{*}P$ value < 0.05 related to the TA in liposome group.

The percentage variations of each parameter, used in Figs. 1 and 2, were expressed as the mean value after drug injection minus the mean value before drug injection, divided by the mean value before drug injection and multiplied by 100.

2.6. Sb(III) determination in blood

Two other groups, that also received 17 mg of Sb/kg of free or of liposomal TA, were performed in order to collect blood samples at 10, 30 and 60 min after IV administration. The blood sample was storaged at −20 °C until the dosage procedure. Antimony was determined in serum and total blood by electrothermal atomic absorption spectrometry (ETAAS) using a Perkin-Elmer AA600 graphite furnace atomic absorption spectrometer, as previously described (Costantini et al., 1985). Samples were diluted 40 times in water containing 0.2% (v/v) HNO_3 and Sb was determined using pyrolise and atomization temperatures of 1300 °C and 2100 °C and $Pd(NO_3)_2$ and $Mg(NO_3)_2$ as matrix modifiers.

2.7. Statistics

Values are expressed as mean ± standard error of mean (S.E.M.). The percentual variation, calculated relative to the time before administration of TA, observed at each time was compared using the one-way ANOVA and Tukey post-test. Differences were considered statistically significant when the P values were lower than 0.05. Mann Whitney test was used to analyse the Sb blood determination.

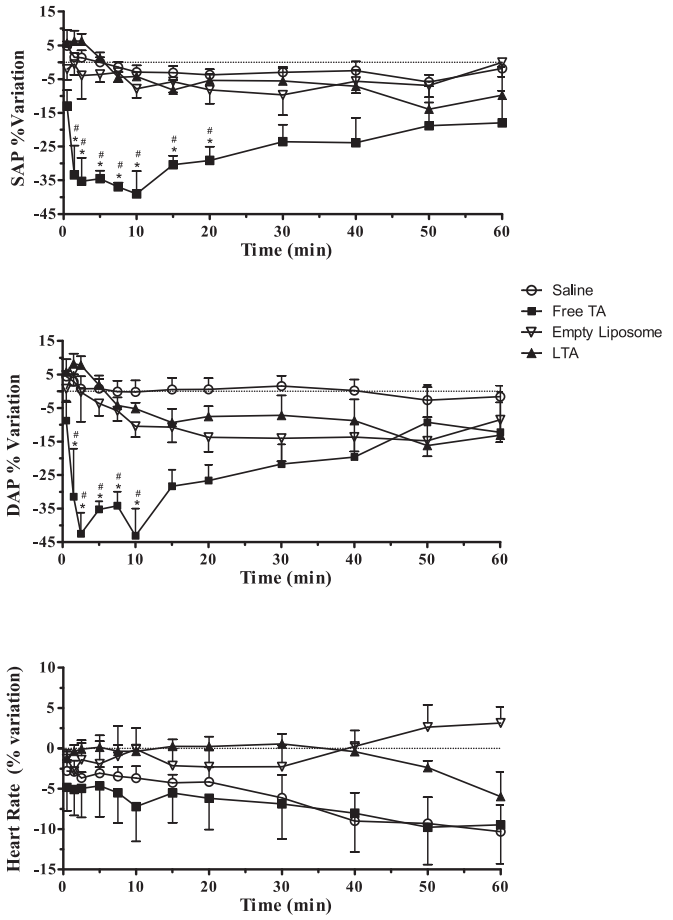

Fig. 2. Percentual variation of arterial pressure and heart rate obtained in anaesthetized rats, after IV administration of free TA or in liposomes (17 mg Sb/kg) or control solutions. $^{\#}P$ value < 0.05 related to the saline group and $^{*}P$ value < 0.05 related to the TA in liposome group.

3. Results

3.1. Liposome characterization

The mean hydrodynamics diameter of the vesicles was equal to 149 nm (polydispersity 0.014) for liposomal TA and 199 nm (polydispersity 0.016) for empty liposomes. Encapsulation of TA was achieved with a trapping efficiency of 14.5% and a final antimony/lipid ratio of 0.054 (w/w). The encapsulation of TA was found to be stable since trapping efficiency was similar after 10 days storage at 4 °C compared to the initial value (Table 1).

Table 1
Percentual of encapsulated antimony.

	Concentration of Sb(III) (mg/ml)	% of encapsulation
Soon after preparation	4.36	14.6
After 10 days of preparation	4.15	13.8

3.2. Determination of cardiovascular parameters

The IV administration of 17 mg of Sb/kg of TA solution in Wistar rats caused significant increase of QT and QTc intervals of ECG, that was observed since 1 min after its administration (Fig. 1), as it was expected to occur (Chulay et al., 1985; Thakur, 1998), and was maintained until the end of the experiment (1 h after administration). The increase of QT and QTc intervals was not observed when the liposomal TA was administered, neither soon after its IV administration nor until the end of the experiment, and this protective effect was similar among all the animals evaluated. No significant alterations were observed in QRS and PR intervals after TA in free ou liposomal forms. The free form of TA was also able to significantly reduce the blood pressure (SAP and DAP), mainly between 1 and 20 min after its administration (Fig. 2). The filtered values presented in Table 2 show that very low levels of AP were reached after administration of the free form of TA. These alterations were not observed when TA in liposomes was injected (Fig. 2), indicating that liposome encapsulation can prevent cardiovascular toxicity. In resume, the results obtained from the animals that received TA in liposomes were quite similar to those observed in control groups (saline and empty liposomes), all along the time of the experiment (Figs. 1 and 2, and Table 2). Also, there was no significant alteration of ECG parameters in control groups which received either empty liposomes or PBS (Figs. 1 and 2). Table 2 reports in details the filtered values of cardiovascular parameters measured before and after the administration of each formulation of TA.

3.3. Sb determination in blood

The Sb(III) determination showed that a large amount of Sb is maintained in blood compartment until 1 h after liposomal TA administration. In contrary, when free TA was administered the concentration of Sb(III) in blood, since 10 min after, was lower than Sb from liposomal TA (Fig. 3).

4. Discussion

Tartar emetic, as other antimony compounds, is a drug that can induce cardiotoxicity (Honey, 1960; Thakur, 1998; Kuryshev et al., 2006). In this case, the most important ECG abnormalities are ST segment inversion, QT interval prolongation, and therefore

Table 2
Filtered values of arterial blood pressure, heart rate and electrocardiographic parameters, measured before and after IV injection of 17 mg/kg free TA or liposomal TA at different times. The values represent the mean ± S.E.M; $^{*}P$ value < 0.05 compared to the time 0 (control period).

		Heart rate (bpm)	Systolic AP (mm Hg)	Diastolic AP (mm Hg)	QT interval (ms)	QTc interval (ms)	PR interval (ms)	QRS interval (ms)
TA								
Time (min)	0	334 ± 17.7	114 ± 9.7	86 ± 9.8	65 ± 1.6	116 ± 1.8	57 ± 2.1	21 ± 0.8
	5	324 ± 12.9	75 ± 7.0*	55 ± 6.0*	80 ± 3.1*	136 ± 4.9*	57 ± 1.9	21 ± 0.8
	15	322 ± 13.5	79 ± 7.4*	61 ± 6.8*	79 ± 3.4*	135 ± 5.4*	58 ± 2.3	21 ± 0.9
	30	316 ± 10.9	88 ± 10.2	66 ± 7.9	81 ± 3.8*	135 ± 4.1*	59 ± 2.4	22 ± 0.9
	60	306 ± 14.2	93 ± 12.5	74 ± 9.0	80 ± 2.9*	135 ± 5.8*	62 ± 2.6	22 ± 0.8
Liposomal TA								
Time (min)	0	396 ± 5.0	127 ± 5.6	98 ± 3.5	65 ± 1.4	122 ± 2.7	49 ± 0.5	25 ± 0.8
	5	402 ± 5.6	126 ± 5.7	100 ± 5.7	62 ± 1.5	121 ± 2.6	51 ± 0.4	25 ± 0.7
	15	403 ± 6.2	113 ± 4.0	89 ± 6.5	64 ± 1.1	121 ± 2.1	50 ± 0.3	26 ± 1.0
	30	404 ± 7.0	117 ± 6.3	91 ± 8.2	64 ± 0.8	122 ± 1.5	49 ± 0.6	26 ± 0.7
	60	375 ± 15.8	111 ± 12.8	86 ± 11.1	66 ± 2.0	121 ± 2.9	52 ± 0.7	26 ± 0.4

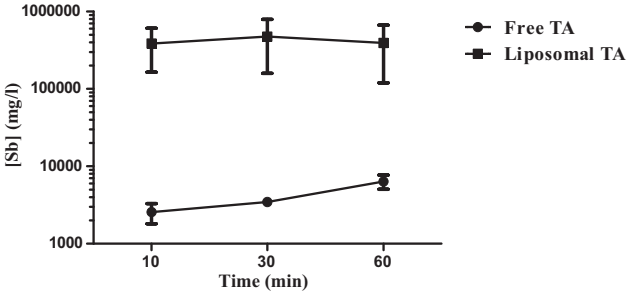

Fig. 3. Sb (III) blood concentration 10, 30 and 60 min after 17 mg of Sb/kg of free or liposomal IV administration.

'torsades de points' and sudden heart attack (Lacerda-Junior et al., 1965; Chulay et al., 1985). Its ability to induce QT interval prolongation has already been reported in anaesthetized guinea-pigs and rabbits (Alvarez et al., 2005). In the present study it was showed that QT interval prolongation and decreased blood pressure occur after free form of TA administration, evidencing its cardiovascular toxicity. There are some possible mechanisms to explain the antimonial toxicity. Antimony compounds can be genotoxic (De Boeck et al., 2003), and can induce cytotoxicity and apoptosis (Lecureur et al., 2002; Mann et al., 2006), effects that are important for its use as anti-tumoral, but also could contribute to its cardiotoxicity. Considering that both trivalent antimony and arsenic compounds are metalloids belonging to group V of the periodic table, they share many chemical properties and are thought to interfere with biological processes in a similar manner; just like that it is possible to suggest a similar mechanism for TA toxicity as a rationale. The QT interval prolongation, among other electrocardiographic abnormalities, was also observed in patients treated with arsenic trioxide (As_2O_3) for acute promyelocytic leukemia (Ohnishi et al., 2000). In addition to prolongation of the action potential duration, some studies have shown cellular Ca^{2+} overload, lipid peroxidation caused by reactive oxygen species generation and decreased intracellular ATP concentration (Yamazaki et al., 2006). It was reported by Drolet et al. (2004) that As_2O_3 is a potent blocker of both I_{Kr} and I_{Ks} in hERG and KCNQ1 + KCNE1 transfected CHO cells. Otherwise, it was reported that As_2O_3 (Ficker et al., 2004) and antimonial compounds (Kuryshev et al., 2006) reduce hERG/I_{Kr} (a specific cardiac ion channel that carries the rapidly activating delayed rectifier potassium current, I_{Kr}) currents, not by direct block, but by inhibition of hERG/I_{Kr} trafficking to the cell surface (Dennis et al., 2007). It should be pointed out that these experiments were carried out with hERG (human ERG) transfected cells, and there are significant differences in ERG protein expression between the species. In rats, rERG (rat homologue of hERG) protein and functional I_{Kr} expression are higher in atria than ventricles, whereas in mouse and human, ERG (mERG and hERG) expression is higher in the ventricles (Pond et al., 2000). Also, it was previously shown that antimony potassium can induce a lethal oxidative stress in cardiac myocytes (Tirmenstein et al., 1995), which may arise due to a deficiency of antioxidant defenses rather than direct reduction of molecular oxygen (Tirmenstein et al., 1997). The intracellular calcium increase by antimony can also induced cardiac myocyte death (Wey et al., 1997; Ohnishi et al., 2000). All these effects together could explain the malfunction of the heart muscle caused by myocyte injury, and could influence the arterial pressure control, beyond other undetermined mechanisms.

In fact, the main objective of our work was the study of the toxic effects of TA encapsulated in liposomes on the cardiovascular system, assuming that the mechanism of action of TA has not been changed. The specific investigation of ECG and blood pressure changes caused by TA in the rat model is first reported here. It was known that non-lethal doses of the TA reduce the ability of cardiac myocytes to mobilize calcium during excitation–contraction phase of cardiac cycle (Toraason et al., 1997), and that in neonatal rat cultured cardiac myocytes, the non-lethal concentration of antimony potassium tartrate was able to increase glutathione levels and the synthesis of stress proteins, which may be responsible for protection against the toxicity caused by high doses used in treatments (Snawder et al., 1999). In this way, the TA in liposomes is probably less able to induce cardiotoxicity because the control release of the high dose of TA used in this study.

Another important result reported here is the reduced effect on blood pressure of TA in liposomes, preventing hypotension, which is a common adverse reaction induced by the free form of TA. However, this effect only represents a major advantage of liposomes over the free form, when high doses are necessary. To treat schistosomiasis is necessary to use multiple IV doses of TA every day, for a month or more (Cioli et al., 1995), and it was reported some options of doses, from 26.0 to 28.5 mg Sb/kg IV, until a total of 2 g/day of TA, for 18–30 days (Honey, 1960). A previous study, showed that intraperitoneal or subcutaneous administration of 11 and 27 mg Sb/kg TA in pegylated liposome reduced significantly the worm burden of mice with Shistosoma mansoni infection and they all survived, even when treated with the higher dose, in contrast with free TA that was 100% lethal with the dose of 27 mg/kg (De-Melo et al., 2003). Additionally, the time-course of antimony release from liposomes used in the present work has already been determined by De-Melo et al. (2003) in mice serum and the results showed that about 7% of encapsulated antimony was found to be released within 24 h and 9 days was the time estimated to release 50% of Sb from liposomes. The distribution profile of free TA was also determined before by Ness et al. (1947) and it was shown that in 24 h only about 10% of Sb remained in blood. In the present work, 17 mg Sb/kg of TA in free or liposomal form was used, keeping the animal alive during the experiments. A higher dose of TA free form (18 mg/kg) in rats induced 100% death. To show the ability of liposomes to avoid the cardiovascular toxicity of TA, a near to lethal dose was used to demonstrate the advantage of TA encapsulation in liposomes.

The polydispersity index and the average size of this suspension of liposomes were very similar to those previously prepared liposomal TA (De-Melo et al., 2003), demonstrating the reproducibility of the preparation methodology.

The present work shows a reduction of TA cardiotoxicity resulting from the properties of liposomes, which probably induced to a lower concentration of TA available for association with the cardiac tissue as compared with the administration of the free drug. It is noteworthy that such a reduction of cardiotoxicity has been previously described, in the case of doxorubicin following its encapsulation in long-circulating liposomes (Papahadjopoulos et al., 1991), but this observation not necessarily indicates that this ability of liposomes could be observed for all cardiotoxic drugs. The present work is the first to demonstrate the prevention of cardiotoxicity of an antimonial drug encapsulated in liposomes.

5. Conclusion

The most important result emerging from this work is the ability of a liposomal system to ensure a reduced cardiotoxic profile for TA when compared to the free drug at the same high dose, showing that drug distribution was markedly modified by this nanocarrier. It can be speculated that lower doses of TA in liposomes could be used by IV route, to obtain the same effect of higher doses of TA solution in humans. Therefore, the nanocarrier, strategically used in this study, was able to reduce cardiac side effects of TA, and represents a potential interest in the future production of safer IV treatments.

Conflict of interest statement

Authors declare there are no conflict of interest.

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