Photodiagnosis and Photodynamic Therapy 28 (2019) 210–215

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

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

# Topical photodynamic therapy with chloroaluminum phthalocyanine liposomes is as effective as systemic pentavalent antimony in the treatment of experimental cutaneous leishmaniasis

Sávia Caldeira Lopes[a,1], Raphaela Ariany Silva[a,1], Marcus Vinícius Novais[a], Larissa Dutra Coelho[a], Lucas Antônio Ferreira[a], Paulo Eduardo Souza[b], Antônio Tedesco[c], Ricardo Bentes Azevedo[d], Marta Gontijo Aguiar[a,*], Mônica Cristina Oliveira[a,*]

[a] Department of Pharmaceutical Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Laboratory of Software and Instrumentation in Applied Physics and Laboratory of Electron Paramagnetic Resonance, Institute of Physics, University of Brasilia, Brasília, Brazil
[c] Department of Chemistry, Center of Nanotechnology and Tissue Engineering - Photobiology and Photomedicine Research Group, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
[d] Nanobiotechnology Laboratory, Institute of Biological Sciences, University of Brasilia, Brasília, Brazil

ARTICLE INFO

*Keywords:*
Chloroaluminum phthalocyanine
Liposomes
Topical treatment
Cutaneous leishmaniasis
Photodynamic therapy

ABSTRACT

*Background:* In the Americas, one of the main causative species of cutaneous leishmaniasis is *Leishmania (Leishmania) amazonensis*. The systemic antimonials remain the most largely used option for disease control. However, this drug has significant toxicity. The development of new alternative therapies, including the identification of effective drugs for topical treatment of cutaneous leishmaniasis, is of utmost interest. In this sense, photodynamic therapy emerges as a new strategy. The aim of this study was to develop the chloroaluminum phthalocyanine-loaded liposome, characterize it, and evaluate its stability and efficacy in the topical treatment of cutaneous leishmaniasis caused by *L. (L.) amazonensis*.

*Methods:* Liposomes composed of egg phosphatidylcholine were prepared by Bangham's method. Storage stability of phthalocyanine-loaded liposomes was evaluated at 30 and 60 days after preparation. For the *in vivo* evaluation, the animals were infected with *L. (L.) amazonensis* and divided into groups: chloroaluminium phthalocyanine-loaded liposome, blank liposome, meglumine antimoniate (200 mg$Sb^{+5}$/Kg/day), and control. The lesion size was determined weekly after the beginning of the treatment. Upon completion, parasites were recovered from the skin lesion and spleen and evaluated by limiting dilution assay.

*Results:* Chloroaluminum phthalocyanine-loaded liposomes were stable and showed adequate characteristics for topical administration. The topical chloroaluminum phthalocyanine-loaded liposome was as effective as systemic pentavalent antimony in reducing the parasitic load in the lesion and spleen in infected animals.

*Conclusions:* The present study showed that photodynamic therapy with chloroaluminum phthalocyanine-loaded liposomes is a promising strategy for the treatment of American cutaneous leishmaniasis caused by *L. (L.) amazonensis*.

## 1. Introduction

Leishmaniasis is a zoonotic infectious disease found worldwide and is considered one of the neglected tropical diseases. About 700,000 to 1.0 million new cases and 20,000 to 30,000 deaths occur annually. Cutaneous leishmaniasis, the most common form, is characterized by skin lesions on exposed areas of the body such as the face, arms, and legs [1]. In the Americas, one of the main causative species of cutaneous leishmaniasis is *Leishmania (Leishmania) amazonensis*.

Systemic antimonials remain the most largely used option for

* Corresponding authors.
*E-mail addresses:* saviacal@gmail.com (S.C. Lopes), raphaelaassilva@gmail.com (R.A. Silva), m_vinicius29@hotmail.com (M.V. Novais), larissadc16@gmail.com (L.D. Coelho), lucaufmg@gmail.com (L.A. Ferreira), psouza@unb.br (P.E. Souza), atedesco@usp.br (A. Tedesco), razevedo@unb.br (R.B. Azevedo), martagontijo@ufmg.br (M.G. Aguiar), itabra2001@yahoo.com.br (M.C. Oliveira).
[1] These authors contributed equally to this manuscript.

https://doi.org/10.1016/j.pdpdt.2019.08.020
Received 9 May 2019; Received in revised form 9 August 2019; Accepted 16 August 2019
Available online 24 August 2019

disease control, followed by amphotericin B, paromomycin, and miltefosine. These drugs present significant toxicity, high cost, and variable effectiveness [2]. The development of alternative therapies, including the identification of effective drugs for topical treatment of cutaneous leishmaniasis is a highly attractive proposition which would decrease the toxicity, reduce the side effects, lower the cost of treatment, and improve the quality of life of the patients [3].

The photodynamic therapy, which is an emerging new strategy that involves the combination of visible light, photosensitizer, and oxygen, is used for treating a large variety of diseases such as cancer and infectious diseases including cutaneous leishmaniasis [4]. In this therapy, a photosensitizer, in presence of molecular oxygen, is excited with visible light inducing the formation of reactive oxygen species, which are highly toxic to the target cells [5]. Chloraluminum phthalocyanine is a new generation of photosensitizer. Recently, *in vitro* studies have shown its antileishmanial activity against visceral and cutaneous species [5–8]. The limitation of topical treatments arises from the fact that depth penetration and selectivity depend on the drug's physicochemical properties [4]. The performance of a photosensitizer as an antileishmanial drug for topical application can be improved if it is loaded in a suitable carrier. Liposomes are colloidal vesicles used as an antileishmanial drug delivery system such as amphotericin B. This system is the most widely investigated delivery system for phagocyte-targeted therapies. Due to their composition variability and structural properties, liposomes are extremely versatile, leading to a large number of pharmaceutical applications [5,9]. In addition, liposomes are well known as nanocarriers capable of increasing skin penetration of encapsulated drugs [10]. Therefore, in the present study, liposomes loaded with chloroaluminium phthalocyanine were developed and the physicochemical properties were evaluated. The *in vivo* efficacy of these formulations was evaluated, as a new therapy, for the topical treatment of cutaneous leishmaniasis caused by *L. (L.) amazonensis*.

## 2. Materials and methods

Egg phosphatidylcholine was supplied by Lipoid GmbH (Ludwigshafen, Germany). Chloroaluminum phthalocyanine and TRIS buffer were obtained from Sigma Chemical Company (Saint Louis, USA). All other chemicals used in this study were of analytical grade.

### 2.1. Preparation of liposomes containing chloroaluminum phthalocyanine

Liposomes composed of egg phosphatidylcholine were prepared by Bangham's method [10]. Briefly, ethanolic solution of chloroaluminum phthalocyanine (2.9 or 5.7 $\mu g.mL^{-1}$) was added to the ethanolic solution containing egg phosphatidylcholine (30 mM), followed by homogenization. A lipid film was obtained by evaporating the ethyl alcohol under reduced pressure. Next, the lipid film was hydrated with 60 mM TRIS buffer, pH 7.4, up to a final concentration of 20 mM of egg phosphatidylcholine. After preparation, the vesicles were submitted to extrusion using polycarbonate membranes (0.4 μm, 10 times) and purified by ultracentrifugation at 150,000 x g at 10 °C for 90 min (Optima® L-80XP; Beckman Coulter, Indianapolis, USA).

### 2.2. Liposome characterization

#### 2.2.1. Determination of the diameter, polydispersity index, and zeta potential

The diameter of the vesicles and the polydispersity index were determined by photon correlation spectroscopy at 25°C and at an angle of 90°. The zeta potential of the vesicles was determined by dynamic light scattering associated with electrophoretic mobility at an angle of 90°. The measurements were performed using the Zetasizer Nano ZS90 equipment (Malvern Instruments Ltd, Worcestershire, UK). The samples were diluted with sodium chloride 0.9% (w/v) solution.

#### 2.2.2. Determination of the content of chloroaluminum phthalocyanine

The amount of encapsulated chloroaluminum phthalocyanine (ClAlPc) in liposomes was measured using UV-VIS spectrophotometry (UV mini-1240, Shimadzu, Kyoto, Japan). The analyses were performed evaluating the absorbance at wavelength equal to 674 nm. Initially, the vesicles were opened with ethyl alcohol at volume ratio of 1:2.3, and then the preparations were diluted in ethyl alcohol. The chloroaluminum phthalocyanine encapsulation percentage (EP) was calculated according to the following equation:

$$EP = \frac{[\text{amount of ClAlPC in purified liposomes}]}{[\text{amount of ClAlPC in the non-purified liposomes}]} \times 100$$

### 2.3. Stability study

The storage stability of liposomes containing chloroaluminum phthalocyanine was evaluated at 30 and 60 days after their preparation. These formulations were maintained at 4 °C (n = 3). After each interval time, the samples were evaluated for the % content of encapsulated chloroaluminum phthalocyanine, diameter, polydispersity index, and zeta potential. For quantification of chloroaluminum phthalocyanine, samples were submitted to ultracentrifugation at 150,000 × g at 20 °C for 90 min prior to UV-VIS spectrophotometry analysis.

### 2.4. Parasites and infection of animals

*L (L.) amazonensis* (strain IFLA/BR/1967/PH8) amastigotes were isolated from dorsal nodules of golden hamsters. The nodules were homogenized with an Ultra-Turrax (Ika, Germany) in Schneider's modified medium supplemented with 20% bovine fetal serum and 1% of a 100 U/mL penicillin and 100 μg/mL streptomycin solution. The tissue was centrifuged at 50 × g for 2 min for sedimentation (Hitachi, Himac). The supernatant was separated, centrifuged at 1700 x g for 15 min (Hitachi, Himac) and resuspended in Schneider's at $5.0 \times 10^7$ amastigotes/mL.

BALB/c mice (females, 5–6 weeks old) were inoculated with approximately $1 \times 10^6$ amastigotes of *L (L.) amazonensis* through subcutaneous injections at the base of the tail, after trichotomy. The study was approved by the Ethics Committee for Animal Experimentation from Federal University of Minas Gerais (CEUA/UFMG: 349/2015).

### 2.5. Treatment of infected animals

After development of ulcerated lesions (mean diameter, 8–11 mm; 45 days after inoculation), BALB/c mice were divided into four groups (n = 7), according to lesion size, to ensure similar mean size. For the treatment with chloroaluminum phthalocyanine-containing liposomes and blank liposomes, the animals were pre-anesthetized with a mixture of ketamine (100 mg/kg)/xilazine (10 mg/kg) by intraperitoneal *via.* The lesion was covered with 50 μL of formulation and protected from light using an aluminum foil to prevent light penetration. After 15 min, the aluminum foil was removed and the lesion containing the formulation was exposed to visible light irradiation at a wavelength of 660 nm, releasing 0–95 $J/cm^2$ at an intensity of 81 $mW/cm^2$ for 20 min. During the application of the laser, gauze soaked in 0.9% (w/v) sterile saline solution was used to prevent the animals' eyes from drying out. The distance between the light source and the lesion was 5 cm. After each session, the residues of the formulation were removed using 0.9% (w/v) sterile saline. The other treatment groups were: infected animals treated with meglumine antimoniate (Glucantime® at 200 mg$Sb^{+5}$/Kg/day), administered by intraperitoneal *via,* and infected untreated animals. The animals were treated on alternate days for 20 days (10 doses received). For this study, the treatment efficacy was evaluated through parasite determination at the site of infection and in the spleen. The lesion size was also monitored.

### 2.6. Parasite load

The parasite load in lesions and in the spleen was evaluated by the limiting-dilution assay, at three days after the end of treatment. The animals were euthanized in a $CO_2$ chamber and the cutaneous lesion and spleen were removed. The tissues were weighed and homogenized with an Ultra-Turrax homogenizer (IKA, Germany) in Schneider's medium containing 100 U/mL penicillin and 100 µg/mL streptomycin solution. Next, the tissues were centrifuged at 1620 × g (lesion) or 45 × g (spleen) for two minutes for sedimentation. The supernatants were separated and centrifuged at 1700 x g for 15 min (lesion) or 1620 × g for 2 min (spleen). The pellets were resuspended in 1 mL of Schneider's medium containing 20% bovine fetal serum, penicillin (100 U/mL) and streptomycin (100 µg/mL). The homogenate was submitted to serial dilutions in triplicate (1:10) in successive sterile 96-well culture plates and incubated at 23 °C. Each well was examined in an inverted microscope for the presence of parasites, and the parasite load was determined by the highest dilution at which parasites could grow over a 7-day period [12]. The results were expressed as the mean obtained from the triplicate.

### 2.7. Lesion size measurements and weight of the animals

During and after treatment, lesion size was followed up weekly using a digital caliper to measure its diameter (Mitutoyo, Brazil). The lesion size was determined by obtaining the average value between the longest line that could be traced from one border of the lesion to another and the line that bisected this distance at a 90° angle [11]. Further evaluations, through careful observation of paws and tails, included the appearance of relapses, nodules, and metastasis in other locations on the animals' skin. The determination of the weight of the animals was evaluated at the beginning (day 0) and on days 7 and 14 of treatment and day 21 after starting treatment. Other signs such as piloerection were used as indicators of toxicity.

### 2.8. Statistical analysis

The data were processed using GraphPad Prism 4 software. Normality and homogeneity of variance were assessed using the Kolmogorov–Smirnov and Bartlett's tests, respectively. The comparison of parasite loads, weights of animals, and lesion sizes among the groups was performed using one-way analysis of variance followed by Tukey's test. The difference was considered significant when the P value was $< 0.05$. The parasite load data were transformed into $\log_{10} + 1$.

## 3. Results

### 3.1. Liposome characterization

The physicochemical parameters of the liposomal formulations, such as the vesicle diameter, polydispersity index, and zeta potential are summarized in Table 1. The liposomes were prepared with two concentrations of chloroaluminum phthalocyanine: 2.9 (LIP 1) or 5.7 µg.mL$^{-1}$ (LIP 2). LIP-1 and LIP-2 presented an encapsulation percentage of chloroaluminum phthalocyanine and mean diameter of 76.7 ± 2.3% and 269 nm ± 45.0, and 67.4 ± 8.6% and 240 ± 50.1 nm, respectively. Both formulations were monodisperse (polydispersity index < 0.2). The presence of chloroaluminum phthalocyanine did not lead to an increase in the size of vesicles when chloroaluminum phthalocyanine-loaded liposomes were compared with blank liposome. In addition, no remarkable increase in vesicle diameter and polydispersity index was observed when increasing chloroaluminum phthalocyanine concentrations were associated with the system. The results showed that the two formulations LIP-1 and LIP-2 presented adequate characteristics for topical administration. Considering that the concentration of chloroaluminum phthalocyanine encapsulated in the LIP-2 formulation (3.6 ± 0.9 µg.mL$^{-1}$) was slightly higher than in LIP-1(2.2 ± 0.1 µg.mL$^{-1}$), LIP-2 was selected for the following studies.

### 3.2. Stability study

The stability study of liposome containing chloroaluminum phthalocyanine stored at 4 °C over a 60-day period was evaluated by accompanying the alterations of the vesicle diameter, polydispersity index, and the retention of chloroaluminum phthalocyanine. The diameter and polydispersity index of LIP-2 were not altered during 60 days of storage, indicating a good physicochemical stability of liposomes (Fig. 1). The fusion or aggregation of vesicles can lead to the release of encapsulated content, so that the maintenance of the diameter of the vesicles it is important to ensure the therapeutic efficacy of the liposomal preparation. The presence of negative charge on the surface of liposomes, as demonstrated by the potential zeta values (−14.9 ± 2.8 mV, immediately after preparation of formulation; −15.4 ± 0.8 mV, 30 days; −17.0 ± 0.4 mV, 60 days), may have prevented vesicle aggregation through electrical repulsion, and certainly contributed to avoiding changes in the diameter of vesicles. Besides, it is important to emphasize that the liposomal formulations remained monodisperse, which also contributes to an adequate therapeutic response (Fig. 1A). Concerning chemical stability, the retention of chloroaluminum phthalocyanine in LIP-2 over sixty days of storage, indicating that these liposomal preparations may be a viable pharmaceutical product (Fig. 1B).

### 3.3. In vivo efficacy of chloroaluminum phthalocyanine-loaded liposomes

The development of lesions in BALB/c mice infected with *L.* (*L.*) *amazonensis* normally evolves from nodules at the infection site to an ulcerated lesion. When the lesions presented a diameter of 9–11 mm, the treatments were started. The parasite load in the lesion and spleen was used to evaluate the efficacy of chloroaluminum phthalocyanine-containing liposomes in animals infected with *L.* (*L.*) *amazonensis*.

The data are presented in Fig. 2. The parasite load in the lesion decreased when the animals were topically treated with chloroaluminum phthalocyanine-containing liposomes. The number of parasites in the infected untreated group ($1.1 \times 10^6$) was significantly

**Table 1**
Physicochemical and chemical characteristics of liposomes containing or not chloroaluminum phthalocyanine.

| Samples[a] | Parameters evaluated | | | | |
|---|---|---|---|---|---|
| | Diameter ± SD (nm) | Polydispersity index ± SD | Zeta Potential ± SD (mV) | Concentration of ClAlPc ± SD (µg.mL$^{-1}$) | Encapsulation percentage ± SD |
| **LIP 1** | 269.0 ± 45.0 | 0.131 ± 0.04 | −18.4 ± 2.0 | 2.2 ± 0.1 | 76.7 ± 2.3 |
| **LIP 2** | 240.0 ± 50.1 | 0.126 ± 0.06 | −14.9 ± 2.8 | 3.6 ± 0.9 | 67.4 ± 8.6 |
| **B Lip** | 309.2 ± 26.2 | 0.143 ± 0.03 | −17.9 ± 1.4 | – | – |

[a] The liposomes were prepared in concentrations of 5 µM (LIP 1), 10 µM (LIP 2) of ClAlPc and without ClAlPc (B Lip – Blank liposome). Data expressed as mean ± standard deviation (SD) of three batches.

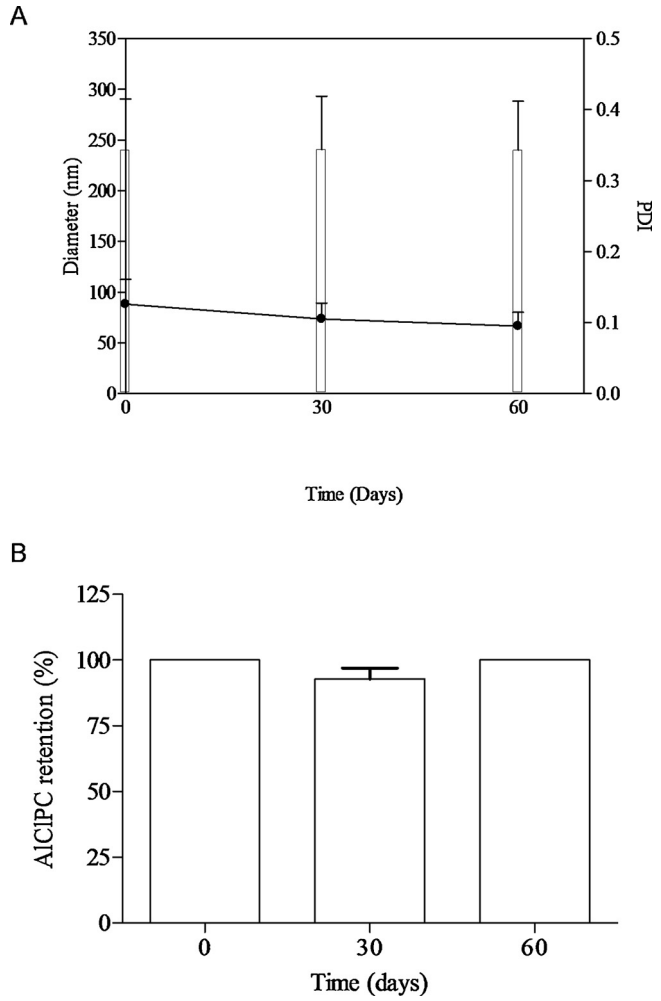


**Fig. 1.** Evaluation of the vesicle diameter, polydispersity index (PDI) (A), and chloroaluminum phthalocyanine retention (%) (B) during 60 days of storage at 4 °C. The 0-day column shows the vesicle diameter obtained immediately after preparation of the formulation. Data expressed as mean ± standard deviation (SD) of three batches.

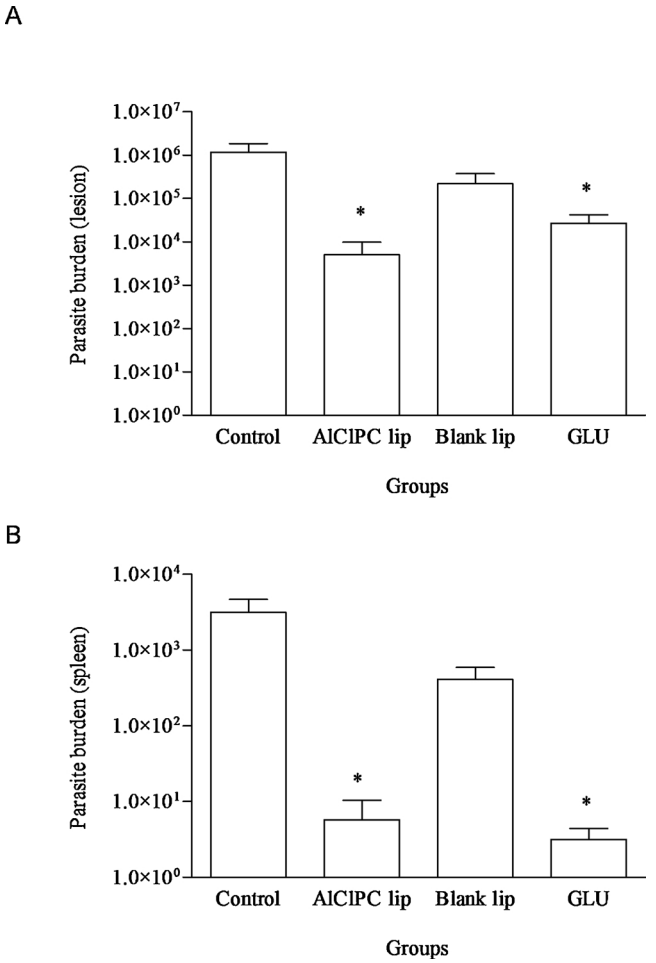


**Fig. 2.** *In vivo* efficacy of CLPH in *L (L.) amazonensis*-infected mice. Female BALB/c mice were infected with *L (L.) amazonensis* amastigotes in base of the tail. After the development of ulcerated lesions (8–11 mm), the animals were treated topically with chloroaluminum phthalocyanine-containing liposomes (AlClPC lip), without chloroaluminum phthalocyanine liposomes (Blank lip), or with meglumine antimoniate – Glucantime® administered by intraperitoneal route (dose of 200 mg$Sb^{+5}$/Kg/day) (GLU) for alternate days during 20 days (10 doses received). The control group was infected and did not receive any treatment (Control). Three days after interruption of treatment, parasite load recovered from lesion and spleen was evaluated by limiting dilution assay. The homogenate was submitted to serial and successive dilutions (1:10) and incubated at 23 °C. Each well was examined in an inverted microscope for the presence of parasites, and the parasite load was determined by the highest dilution at which parasites could grow. The results were expressed as the mean obtained from the triplicate measurements. (A) Parasite load in lesions (* $P < 0.05$ when compared to Control or Blank lip-treated groups). (B) Parasite load in the spleen (*$P < 0.05$ when compared to Control or Blank lip-treated groups). The bars represent the averages and standard deviations (n = 7).

higher than that observed in the group treated topically with ClAlPc -containing liposomes ($5.0 \times 10^3$) or meglumine antimoniate (Glucantime® at a dose of 200 mg $Sb^{+5}$/Kg/day; administration by intraperitoneal route) ($2.6 \times 10^4$). Statistical analysis showed a significant reduction in parasite load in the lesion of the animals treated with topical chloroaluminum phthalocyanine-containing liposomes as compared with the infected untreated or blank liposome treated groups ($p < 0.05$), presenting a reduction of 99% in the lesion parasite load (Fig. 2A).

A comparison of the activity of topical chloroaluminum phthalocyanine-containing liposomes with that of the standard injectable drug (meglumine antimoniate) was performed to determine the parasite load in the spleen (Fig. 2B). A significant decrease in the spleen parasite load was detected in mice treated with chloroaluminum phthalocyanine-containing liposomes ($5.5 \times 10^0$) and meglumine antimoniate ($3.1 \times 10^0$), as compared with the infected untreated ($3.1 \times 10^3$) or blank liposome-treated ($4.4 \times 10^2$) groups ($p < 0.05$). Therefore, topical treatment with chloroaluminum phthalocyanine-loaded liposome showed a comparable therapeutic efficacy to that of the conventional pentavalent antimony treatment administered systemically for the reduction of parasite load in both lesion and spleen.

Fig. 3 shows the evolution of the lesion size after the beginning of the treatment as a function of the time. At the beginning of the treatment, the animals treated presented lesions with an average diameter of 8.8 and 10.4 mm without significant differences among all the groups ($p > 0.05$). The lesion size of the animals treated with chloroaluminium phthalocyanine-loaded liposomes or meglumine antimoniate showed stabilization at the time interval evaluated since there was no significant difference in average diameter of lesions between the initial and final time of the treatment (12.8 and 8.9 mm, respectively) ($p > 0.05$) (Fig. 3A). In contrast, the control or blank liposomes-treated groups showed a gradual increase in the average lesion size with significant difference between the initial and final time intervals of treatment (13.3 and 13.1 mm, respectively) ($p < 0.05$) (Fig. 3B).

The animals did not present weight loss, the parameter used to evaluate the toxicity of the treatments. At the end of the treatments, the average body weight of the animals treated with topical

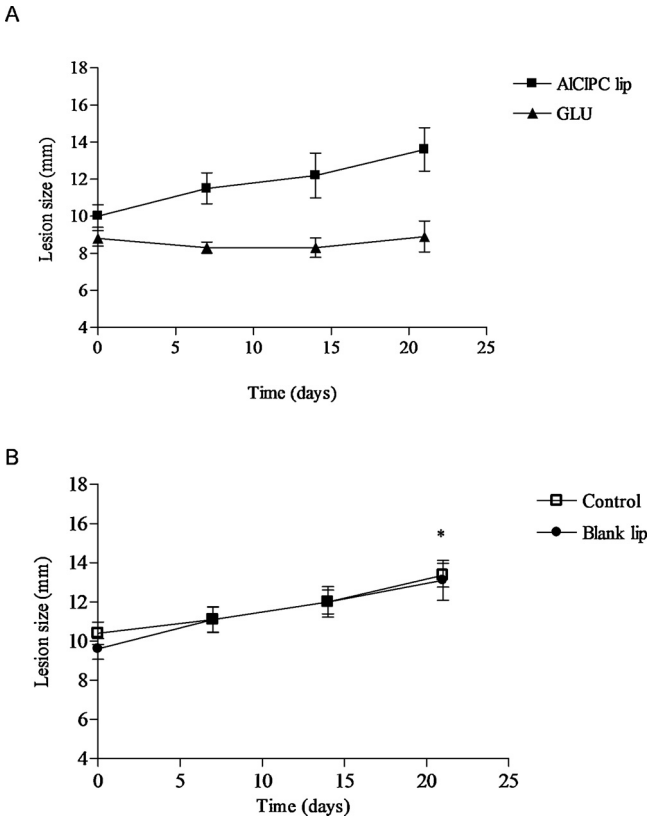


**Fig. 3.** Monitoring of the size of the lesion in response to different treatments. Female BALB/c mice were infected with *L (L.) amazonensis* amastigotes in base of the tail. The animals were treated topically with chloroaluminum phthalocyanine-containing liposomes (AlClPC lip), meglumine antimoniate – Glucantime® administered by intraperitoneal route (dose of 200 mg $Sb^{+5}$/Kg/day) (GLU) (A), without chloroaluminum phthalocyanine liposomes (Blank lip) for alternate days during 20 days (10 doses received). The control group was infected and did not receive any treatment (Control) (B). Lesion size is shown as average and standard deviation. Comparisons between average lesion size at initial time (time zero) and final time (21 days) of the treatment with AlClPC lip and GLU were not statistically significant ($P > 0.05$). The Control and Blank lip-treated group showed significant differences between the time zero and 21 days after starting the treatments (*$P < 0.05$) (n = 7).

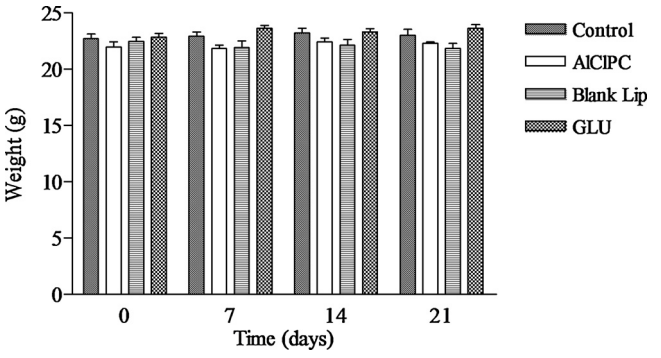


**Fig. 4.** Monitoring of the body weight of the animals treated topically with chloroaluminum phthalocyanine-containing liposomes (AlClPC-lip), without chloroaluminum phthalocyanine liposomes (Blank lip), intraperitoneally with meglumine antimoniate – Glucantime® (dose of 200 mg $Sb^{+5}$/Kg/day) (GLU) during alternate 20 days (10 doses received) or infected but did not receive any treatment (Control). Female BALB/c mice were infected with *L (L.) amazonensis* amastigotes in base of the tail. The animals were weighed at the beginning of the treatment (day 0) and days 7, 14, and 21 after starting the treatments. The bars represent the average and standard deviations. Comparisons between the weight of all groups were not statistically significant ($P > 0.05$) (n = 7).

chloroaluminum phthalocyanine-loaded liposomes (22.3 ± 0.3 g) was similar to the initial body weight (22.0 ± 1.2 g) ($p > 0.05$). Comparisons of the mean body weight of the animals of each group at days 0, 7, 14, and 21 were also not statistically different ($p > 0.05$) (Fig. 4). Other signs of toxicity assessed were not observed.

## 4. Discussion

Obtained in this study were chloroaluminum phthalocyanine-loaded liposomes that presented physicochemical characteristics suitable for topical administration. The size and polydispersity index (PDI < 0.3) of the vesicles were not significantly influenced by the concentration of chloroaluminum phthalocyanine used in the preparation of the liposomes. However, the amount of chloroaluminum phthalocyanine encapsulated in liposomes was greater at drug-to-lipid molar ratio equal to 0.0005 for LIP-2 in comparison with LIP-1 (0.00025).

The physicochemical properties of liposomes, such as surface charge, vesicle size, and membrane fluidity, are factors that influence their stability. The increase in particle size provoked by the aggregation or the fusion of unstable liposomes upon storage is a typical phenomenon of instability of the liposome formulation [13]. The stability of LIP-2 was observed in our study, since the vesicle diameter and chloroaluminum phthalocyanine concentration inside the liposomes were not altered over the time evaluated. This behavior may be due to the repulsion provoked by the presence of negative charge on its surface, which could prevent vesicle aggregation, ensuring the stability of the formulations. In addition, the high level of chloroaluminum phthalocyanine retention in LIP-2 may be explained by the lipophilic character of chloroaluminum phthalocyanine, which could enable a stronger interaction with lipids of the liposome bilayer, in turn preventing drug release during storage [14].

Recently, *in vitro* studies have reported the activity of the photodynamic therapy with chloroaluminum phthalocyanine against different species of leishmania causing visceral and cutaneous leishmaniasis [8,15,16]. Hérnandez et al. [5] compared the activity of chloroaluminum phthalocyanine encapsulated in liposomes and in solution. Photosensitizer-loaded liposomes were almost 10 times more photoactive than free drug against promastigote and intracellular amastigote forms of *L. chagasi* and *L. panamenis*. In fact, although chloroaluminum phthalocyanine is a good photosensitizer, its solubility decreases with the increase of the solvent polarity, being not soluble in water and showing a high tendency to self-aggregate in this medium. This phenomenon reduces the singlet oxygen quantum yield [14,17]. Thus, a strategy to overcome this drawback and to enable its *in vivo* administration is the encapsulation of chloroaluminum phthalocyanine in drug delivery systems, such as liposomes.

The incorporation of the photoactive agent in biological membranes seems to be strongly improved when the lipid composition of the liposome carrier mimics the lipid composition of the targeted membrane, improving the drug selectivity and the photodynamic therapy efficiency. Solubilization of the sensitizer into the lipid bilayer also provides an alternative means of controlling the release of entrapped drugs from liposomes [18]. Our *in vivo* study showed that the novel stable formulation of chloroaluminum phthalocyanine-loaded liposomes was effective in reducing the parasitic load in the lesion and spleen. A plausible explanation for the reduction of the parasitic load in the spleen after topical application of chloroaluminum phthalocyanine-loaded liposomes might be the reduced dissemination of the parasite from the lesion site [19].

Ribeiro et al. [20] reported the low activity of the topical chloroaluminum phthalocyanine liposome gel in C57BL/6 mice infected with *L. (L.) amazonensis* in the paw. Skin conditions represent important considerations in the topical treatment of cutaneous leishmaniasis. The treatment can be applied either to thickened lesions, such as what happens in the paw infection, where there is an increase of the paw

without ulceration, with an additional barrier to absorption or to open lesions, such as what happens in the infection at the base of the tail, in which the barrier properties of the epidermis have been completely lost. When applied to the totally damaged skin (open lesions), liposomes showed the ability to increase the drug skin penetration [10], which may explain the greater activity presented in our study.

In addition, the group treated with LIP-2 showed stabilization in the average lesion size. It is important to note that the disease is more aggressive in animals infected with amastigotes than in those infected with promastigotes, and the outcome of the therapy might be markedly influenced by this [21]. Moreover, BALB/c mice have been considered a rigorous non-cure model in which only the most active drugs are effective and thus any improvement can be attributed to the effects of chemotherapy [22]. Thus, the present study showed that photodynamic therapy is a promising strategy for the treatment of American cutaneous leishmaniasis caused by *L. (L.) amazonensis*.

## References


[1] WHO. Leishmaniasis Avaliable in: http://www.who.int/mediacentre/factsheets/fs375/en/ (Accessed on May 07, 2019).

[2] Tatiana S. Tiuman, Adriana O. Santos, Tânia Ueda-Nakamura, Benedito P. Dias Filho, Celso V. Nakamura, Recent advances in leishmaniasis treatment, Int. J. Infect. Dis. 15 (2011) e525–e532, https://doi.org/10.1016/j.ijid.2011.03.021.

[3] T.T. Pham, P.M. Loiseau, G.G. Barrat, Strategies for the design of orally bioavailable antileishmanial treatments, Int. J. Pharm. 1 (2013) 539–552, https://doi.org/10.1016/j.ijpharm.2013.07.035.

[4] J. Montanari, A.P. Perez, F. Di Salvo, V. Diz, R. Barnadas, L. Dicelio, F. Doctorovich, M.J. Morilla, E.L. Romero, Photodynamic ultradeformable liposomes: design and characterization, Int. J. Pharm. 330 (2007) 183–194, https://doi.org/10.1016/j.ijpharm.2006.11.015.

[5] I.P. Hernández, J. Montanari, W. Valdivieso, M.J. Morilla, E.L. Romero, P. Escobar, *In vitro* phototoxicity of ultradeformable liposomes containing chloroaluminum phthalocyanine against New World Leishmania species, J. Photochem. Photobiol. B 117 (2012) 157–163, https://doi.org/10.1016/j.jphotobiol.

[6] P. Escobar, I.P. Hernández, C.M. Rueda, F. Martínez, E. Páez, Photodynamic activity of aluminium (III) and zinc (II) phthalocyanines in Leishmania promastigotes, Biomedica 26 (Suppl. 1) (2006) 49–56.

[7] L.B. de Oliveira de Siqueira, V. da Silva Cardoso, I.A. Rodrigues, A.L. Vazquez-Villa, E.P. Dos Santos, B. da Costa Leal Ribeiro Guimarães, C. Dos Santos Cerqueira Coutinho, A.B. Vermelho, E.R. Junior, Development and evaluation of zinc phthalocyanine nanoemulsions for use in photodynamic therapy for *Leishmania spp*, Nanotechnology 28 (2017) 065101, , https://doi.org/10.1088/1361-6528/28/6/065101.

[8] V. Nesi-Reis, T.R. Navasconi, D.S.S.L. Lera-Nosone, E.L. Oliveira, P.M. Barbosa, W. Caetano, T.G.V. Silveira, S.M.A. Aristides, N. Hioka, M.V.C. Lonardoni, Phototoxic effect of aluminium-chlorine and aluminium-hydroxide phthalocyanines on Leishmania (L.) amazonensis, Photodiagn. Photodyn. Ther. 21 (2018) 239–245, https://doi.org/10.1016/j.pdpdt.2017.12.008.

[9] A. Laouini, C. Jaafar-Maalej, I. Limayem-Blouza, S. Sfar, C. Charcosset, H. Fessi, Preparation, characterization and applications of liposomes: state of the art, J. Colloid Sci. Biotechnol. 1 (2012) 147–168.

[10] G. Carneiro, M.G. Aguiar, A.P. Fernandes, L.A. Ferreira, Drug delivery systems for the topical treatment of cutaneous leishmaniasis, Expert Opin. Drug Deliv. 9 (2012) 1083–1097, https://doi.org/10.1517/17425247.2012.701204.

[11] A.D. Bangham, M.M. Standish, J.C. Watkins, Diffusion of univalent ions across the lamellae of swollen phospholipids, J. Mol. Biol. 13 (1965) 238–252.

[12] M.G. Aguiar, A.M. Pereira, A.P. Fernandes, L.A. Ferreira, Reductions in skin and systemic parasite burdens as a combined effect of topical paromomycin and oral miltefosine treatment of mice experimentally infected with Leishmania (Leishmania) amazonensis, Antimicrob. Agents Chemother. 54 (2010) 4699–4704, https://doi.org/10.1128/AAC.00809-10.

[13] T. Yang, F. Cui, M. Choi, H. Lin, S. Chung, C. Shim, D. Kim, Liposome formulation of paclitaxel with enhanced solubility and stability, Drug Deliv. 14 (2007) 301–308, https://doi.org/10.1080/10717540601098799.

[14] S.M. Nunes, F.S. Sguilla, A.C. Tedesco, Photophysical studies of zinc phthalocyanine and chloroaluminum phthalocyanine incorporated into liposomes in the presence of additives, Braz. J. Med. Biol. Res. 37 (2004) 273–284.

[15] S. Dutta, D. Ray, B.K. Kolli, K.P. Chang, Photodynamic sensitization of *Leishmania amazonensis* in both extracellular and intracellular stages with aluminum, Antimicrob. Agents Chemother. 49 (2005) 4474–4484, https://doi.org/10.1128/AAC.49.11.4474-4484.2005.

[16] P. Escobar, I.P. Hernández, C.M. Rueda, F. Martínez, E. Páez, Photodynamic activity of aluminium (III) and zinc (II) phthalocyanines in Leishmania promastigotes, Biomedica 26 (Suppl. 1) (2006) 49–56.

[17] B.H. Vilsinski, A.P. Gerola, É.O. Lemos, P.M. Barbosa, K.S.S. Campanholi, G.B. César, A. Tessaro, N. Hioka, W. Caetano, Spectroscopic study of aluminum phahalocyanine chlride (AlPcCl) in homogeneous and micro-heterogeneous media consisting of P-123 and F-127 polymeric micelles, Quim. Nova 38 (2015) 631–639, https://doi.org/10.5935/0100-4042.20150056.

[18] M. Hoebeke, The importance of liposomes as models and tools in the understanding of photosensitization mechanisms, J. Photochem. Photobiol. B 28 (1995) 189–196.

[19] R. Schmidt-Ott, T. Klenner, P. Overath, T. Aebischer, Topical treatment with hexadecylphosphocholine (Miltex) efficiently reduces parasite burden in experimental cutaneous leishmaniasis, Trans. R. Soc. Trop. Med. Hyg. 93 (1999) 85–90.

[20] J.B.P. Ribeiro, A.L. Miranda-Vilela, D. Graziani, M.R.A. Gomes, A.A.S. Amorim, R.D. Garcia, J. de Souza Filho, A.C. Tedesco, F.L. Primo, J.R. Moreira, A.V. Lima, R.N.R. Sampaio, Evaluation of the efficacy of systemic miltefosine associated withphotodynamic therapy with liposomal chloroaluminiumphthalocyanine in the treatment of cutaneous leishmaniasis causedby *Leishmania (L.) amazonensis* in C57BL/6 mice, Photodiagn. Photodyn. Ther. 13 (2016) 282–290, https://doi.org/10.1016/j.pdpdt.2015.08.006.

[21] T. Garnier, A. Mäntylä, T. Järvinen, J. Lawrence, M. Brown, S. Croft, *In vivo* studies on the antileishmanial activity of buparvaquone and its prodrugs, J. Antimicrob. Chemother. 60 (2007) 802–810, https://doi.org/10.1093/jac/dkm303.

[22] S.L. Croft, S. Sundar, A.H. Fairlamb, Drug resistance in leishmaniasis, Clin. Microbiol. Rev. 19 (2006) 111–126, https://doi.org/10.1128/CMR.19.1.111-126.2006.