Parasitology Research (2019) 118:3077–3084
https://doi.org/10.1007/s00436-019-06419-2

TREATMENT AND PROPHYLAXIS - ORIGINAL PAPER

Combination oral therapy against *Leishmania amazonensis* infection in BALB/c mice using nanoassemblies made from amphiphilic antimony(V) complex incorporating miltefosine

Virgínia M. Carregal[1] · Juliane S. Lanza[1] · Daniel M. Souza[2] · Arshad Islam[1] · Cynthia Demicheli[3] · Ricardo T. Fujiwara[4] · Luis Rivas[5] · Frédéric Frézard[1]

Received: 25 January 2019 / Accepted: 2 August 2019 / Published online: 10 August 2019

Abstract

Clinically available drugs for mucocutaneous and cutaneous leishmaniases (CL) include mainly pentavalent antimony (Sb(V)) complexes, liposomal amphotericin B, and miltefosine (HePC). However, they present at least one of the following limitations: long-term parenteral administration through repeated doses, severe side effects, drug resistance, and high cost. HePC is the only oral drug available, but the appearance of resistance has resulted in changes of its use from monotherapy to combination therapy. Amphiphilic Sb(V) complexes, such as SbL8 obtained from reaction of Sb(V) with *N*-octanoyl-*N*-methylglucamide, were recently found to be orally active against experimental CL. The property of SbL8 to self-assemble in aqueous solution, forming nanostructures, led us to investigate the incorporation of HePC into SbL8 nanoassemblies and the therapeutic efficacy of SbL8/HePC nanoformulation by oral route in a murine model of CL. HePC incorporation into the SbL8 nanosystem was evidenced by using a fluorescent analog of HePC. The antileishmanial activity of SbL8/HePC nanoassemblies was evaluated after daily oral administration for 30 days in *Leishmania amazonensis*-infected BALB/c mice, in comparison with monotherapies (SbL8 or HePC) and saline control. All the treatments resulted in significant reduction in the lesion size growth, when compared with control. Strikingly, only SbL8/HePC nanoassemblies promoted a significant decrease of the parasite burden in the lesion. This work establishes the therapeutic benefit of SbL8/HePC association by oral route in a CL model and constitutes an important step towards the development of new orally active drug combination.

Keywords Miltefosine · Antimony · Nanoparticles · Leishmaniasis · Oral route · Drug combination

Section Editor: Sarah Hendrickx

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00436-019-06419-2) contains supplementary material, which is available to authorized users.

✉ Frédéric Frézard
frezardf@gmail.com

Virgínia M. Carregal
mendesvc@yahoo.com.br

Juliane S. Lanza
julianelanza@gmail.com

Daniel M. Souza
daniel.ufop@gmail.com

Arshad Islam
arshad.cgl@gmail.com

Cynthia Demicheli
demichel@netuno.lcc.ufmg.br

Ricardo T. Fujiwara
fujiwara@icb.ufmg.br

Luis Rivas
luis.rivas@cib.csic.es

Extended author information available on the last page of the article

Introduction

Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania* and according to the World Health Organization (WHO) is among the six diseases of greatest impact in developing countries (WHO 2010). There are three main forms of leishmaniasis: the visceral leishmaniasis (VL), where some vital organs such as the liver and spleen are affected; the cutaneous leishmaniasis (CL), the most common form that can be divided into localized, disseminated, and diffused forms; and the mucocutaneous form (ML) (WHO 2019). Clinical manifestation depends on the host cell-mediated immune response and the specific protozoa and vector species.

Chemotherapy is essential to control leishmaniasis, as there is no vaccine approved for human use (Duarte et al. 2016; Thomaz-Soccol et al. 2018). Pentavalent antimony (Sb(V)) complexes, despite their use for more than 60 years, still continue as first-choice drugs in several developing countries for treatment of all forms of leishmaniasis. These include sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®). However, the use of antimonials is limited by the need for parenteral administration through repeated doses and long period of time, their side effects, and drug resistance (Frézard et al. 2009). Liposomal amphotericin B (AmBisome®) has been introduced more recently with good clinical results (Wortmann et al. 2010). But this drug also presents drawbacks such as toxicities, parenteral administration, high cost, and low stability at room temperature. In this context, much effort has been devoted to the discovery of new antileishmanial agents or therapeutic strategies, preferentially oral treatment to improve patient compliance in developing countries.

Miltefosine (HePC) is the only oral drug available for the treatment of VL and ML. However, the appearance of resistance led to change its use from monotherapy to combination therapy (Sundar et al. 2011; Freitas-Junior et al. 2012; Srivastava et al. 2017; Hendrickx et al. 2017). Potential advantages of combination therapy include the reduction of dose, treatment duration, and side effects. The combination of HePC with antimonial drugs was poorly explored despite the report of in vitro additive to mildly synergistic actions of both drugs against a standard *Leishmania* strain (Seifert and Croft 2006). The combined use of HePC and antimonials is also supported by the observation that *Leishmania* strains selected for resistance to Sb did not show cross-resistance to HePC (Sereno et al. 2001; Hadighi et al. 2007; do Monte-Neto et al. 2011).

Amphiphilic Sb(V) complexes were recently identified by our group as an effective oral drug delivery strategy for pentavalent antimonials (Fernandes et al. 2013). One of these complexes, named SbL8, was obtained through reaction of non-ionic surfactant *N*-octanoyl-*N*-methylglucamide with $KSb(OH)_6$ at a molar ratio of 3:1. A pharmacokinetic study of SbL8 by oral route in mice showed greater and more sustained levels of Sb in serum and liver, when compared with Glucantime® at the same dose of Sb. SbL8 further demonstrated antileishmanial activity by the oral route in murine models of VL and CL (Fernandes et al. 2013; Lanza et al. 2016). SbL8 self-assembles in aqueous solution, forming micelle-like nanostructures (Lanza et al. 2016). A unique property of these nanosystems is their kinetic stability after dilution in water (Fernandes et al. 2013), evidencing their potential as carriers of other lipophilic antileishmanial agents such as HePC.

With the aim of associating the benefits of nanotechnology and combination therapy, we investigated here the incorporation of HePC in SbL8 nanoassemblies and the therapeutic efficacy of SbL8/HePC nanoformulation by oral route in murine CL caused by *Leishmania amazonensis*.

Material and methods

Materials and drugs

N-Octanoyl-*N*-methylglucamide (L8, 98%), potassium hexahydroxoantimonate ($KSb(OH)_6$) and HePC were obtained from Sigma-Aldrich Co. (St Louis, MO, USA). Double-distilled deionized water was used throughout all the experiments. The amphiphilic complex SbL8 was synthesized, as previously described (Fernandes et al. 2013) by the reaction of $KSb(OH)_6$ with L8 in water at 1:3 M ratio, that consists in heating at 70 °C for 3 h under stirring until solvent evaporation. The resulting film was rehydrated at room temperature with water and then freeze-dried. The fluorescent analog of HePC (MT-11-BDP) (11-(4′,4′-difluoro-1′,3′,5′,7′-tetramethyl-4′-bora-3'a,4'a-diaza-s-indacen-2′-yl)undecylphosphocholine) used in the work was synthesized as described previously (Hornillos et al. 2006, 2008).

Animals and parasites

BALB/c mice (female, 4–6 weeks old, 18–22 g) were obtained from Centro de Bioterismo (CEBIO) of the Institute of Biological Sciences (ICB), Federal University of Minas Gerais (UFMG, Belo Horizonte, Brazil). Free access to a standard diet was allowed, and tap water was supplied ad libitum. The study involving animals was approved by the Ethical Committee for Animal Experimentation of the UFMG with protocol number 318/2013.

The *Leishmania* strain used in the CL model was *Leishmania* (*L.*) *amazonensis* MHOM/BR/1989/BA199, obtained from the cryopreservation bank of the *Leishmania* Biology Laboratory at ICB, UFMG. The cells were maintained in vitro as promastigotes at 24 ± 1 °C, pH 7.0, in alpha minimal essential medium (αMEM; Gibco®; Thermo Fisher

Scientific, Waltham, MA, USA) supplemented with 10% heat-inactivated bovine fetal serum (FBS; Cultilab, Brazil), 100 μg/mL kanamycin (Sigma-Aldrich Co.), and 50 μg/mL ampicillin (Sigma-Aldrich Co.), in BOD greenhouse (model: 2005; Johns-VWR Scientific, Toronto, ON, Canada). The promastigotes were grown in cell culture flasks of 25-mL volume (Corning Incorporated, Corning, NY, USA) with an initial inoculum of 1×10^6 cells/mL and transferred to a new medium after reaching the stationary growth phase, twice a week.

Preparation and characterization of SbL8/HePC nanoassemblies

SbL8 was dispersed in 0.15 M NaCl solution at final L8 concentration of 524 mM. HePC was added to the mixture as powder, at a final concentration of 1.33 mM. After vortexing, the mixture was incubated at 25 °C for 1 h, prior to administration. The HePC/Sb ratio was chosen based on the therapeutic doses of HePC and SbL8 reported in the literature in murine models (Godinho et al. 2012; Fernandes et al. 2013).

The formulations were characterized for particle size distribution (mean hydrodynamic diameter and polydispersity index) and zeta potential, using the Zetasizer equipment (Nano ZS90; Malvern Instruments, UK). Dispersion Technology Software, version 6.12, was used to collect and analyze the data obtained. SbL8 dispersions were diluted in 0.15 M NaCl solution at final concentration of 30 mM of Sb, kept at 25 °C during the entire experiment, and analyzed at a fixed angle of 90°.

To evaluate the incorporation of HePC into SbL8 nanosystem, the fluorescent analog, MT-11-BDP, was used as HePC model. An aliquot of 10 mM ethanolic solution of the MT-11-BDP was added to the bottom of a tube and the solvent was evaporated. The dispersion of SbL8 or L8 in saline was then added to reach an L8/HePC ratio of 394:1, followed by incubation for 1 h at 25 °C. A saline solution of MT-11-BDP without surfactant was used as control. For fluorescence measurement, the solutions were diluted in PBS at an L8 concentration of 1 or 13 mM. Fluorescence measurements were carried out using the Cary Eclipse™ spectrofluorometer (Varian Inc., Australia) and a 1-cm cuvette compartment with temperature control and magnetic stirring. Fluorescence emission spectra were recorded at 25 °C, with excitation wavelength (λ) set at 500 nm.

Antileishmanial activity in murine model of CL

Evaluation of antileishmanial activity of SbL8/HePC in the CL mouse model was performed as described previously (Lanza et al. 2016). BALB/c mice were first infected with 1×10^6 stationary phase promastigotes of *L. amazonensis* intradermally at the tail base. Chemotherapy was initiated 35 days post-infection, corresponding to the first ulceration sign of the infection papule, with daily doses for 30 days. Animals were divided into the following treatment groups ($n = 9$–10): 1—animals treated with SbL8 in saline at 200 mg Sb/kg/day by oral route; 2—animals treated with HePC in saline at 5 mg Sb/kg/day by oral route; 3—animals treated with SbL8/HePC formulation in saline at 200 mg Sb/kg/day and 5 mg HePC/kg/day by oral route; 4—animals treated with 200 μL of saline by oral route (negative control). Three animals in the group treated with SbL8 and two animals in the group treated with SbL8/HePC died in the course of the treatment. The size of the lesion was determined by means of an analog universal caliper, 150 mm, Digimess® (Brazil). The estimated lesion size was obtained by measuring horizontal and vertical lengths of the lesion, respectively perpendicular and parallel to the vertebral column of the animal. In this model, the horizontal and vertical lengths presented close values and the lesions were considered to be approximately circular. The size of the lesion of each animal was measured every 3 days from the beginning to the end of treatment. After chemotherapy (65 days post-infection), the animals were sacrificed and the lesion was removed for evaluation of the parasitic load by qPCR (Nicolas et al. 2002). Briefly, the lesion samples were macerated and an aliquot was added in a microtube containing lysis buffer and Proteinase K, vortexed and incubated at 56 °C overnight. After incubation, DNA was extracted with NucleoSpin® Tissue Kit (MN, Macherey-Nagel GmbH & Co. KG, Dürin, Germany), according to the manufacturer's instructions. The DNA concentrations were measured by spectrophotometry (Abs at 280/260 nm) and adjusted to 20 ng/μL. One microliter of each sample was used to a final volume of 20 μL per reaction that included ultrapure water, 5.0 μL of SYBR® Green PCR Master Mix (Warrington, UK), 10 pmol of each oligonucleotide, as sense (forward, 5′-CCTATTTTACACCAACCCCCAGT-3′) and antisense primers (reverse, 5′-GGGTAGGGGCGTTC TGCGAAA-3′) constructed for amplification of the mini-circle region present in the kinetoplast DNA (kDNA) of approximately 120 bp. The standard curve was constructed with serial dilutions of the known concentrations of DNA of *Leishmania amazonensis*, extracted from promastigote culture. Ultrapure water was used as negative control. The amplification protocol included an annealing temperature and extension of 60 °C, with melting curve construction, on the Applied Biosystems™ 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) and the analysis was made using the 7500 System Software. Data are presented as nanogram (ng) amount of *Leishmania* DNA per 20 ng of total DNA.

Toxicity evaluations

The animals under treatment were followed daily regarding mortality and occurrence of diarrhea. Each animal was also weighed just before and during the treatment to uncover possible toxic effect.

Sera were collected just after animal euthanasia and stored at − 80 °C. To evaluate hepatocellular lesion, enzymatic activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined using kinetic tests. The serum level of lactate dehydrogenase (LDH) was also determined as another important marker of tissue injury. The plasma levels of ALT, AST, and LDH were assessed in units/liter using commercial kits (Bioclin Quibasa, Belo Horizonte, Brazil).

Statistical analyses

One-way ANOVA with Tukey post-test and Kruskal-Wallis non-parametric test with Dunns post-test were used for statistical analyses of parasite load data and serum levels of enzymes, respectively, with significance level $P < 0.05$. Two-Way ANOVA (repeated measures) was used to compare the variation in lesion size between the experimental groups, followed by Dunnett's post-test, with a significance level of $P < 0.05$. The graphics and statistical analyses were performed using GraphPad Prism® (version 7) software.

Results

Characterization of SbL8/HePC nanoassemblies

SbL8/HePC nanoassemblies were prepared through simple addition of HePC to a concentrated SbL8 dispersion in saline. The particle size distribution and zeta potential of SbL8/HePC and SbL8 were evaluated following dilution in saline at 30 mM of Sb. Polydisperse particles with mean diameter in the range of 100 nm and negative zeta-potential were observed (Table 1), in agreement with previous report for SbL8 (Lanza et al. 2016). The data indicates a slight increase of the mean diameter and marked increase of the polydispersity index upon incorporation of HePC.

Indirect evidence of the incorporation of HePC into the SbL8 nanoassemblies was obtained using the fluorescent analog of HePC (MT-11-BDP) and exploiting its fluorescent properties. This analog (Fig. 1) was found to present very similar physicochemical and biological properties compared with HePC (Hornillos et al. 2008; De La Torre et al. 2014). Following incorporation of the HePC analog into SbL8 or L8 dispersion, the resulting solution was visually green fluorescent, contrary to the brown-orange solution of HePC analog in saline. The fluorescence differences of the HePC analog between its saline solution and in SbL8 dispersion was confirmed by their fluorescence emission spectra of these solutions after dilution in PBS at 32 and 2.5 μM (Fig. 2). It is noteworthy that the highest dilution corresponds to L8 concentration of 1 mM, which is well below the critical micellar concentration (CMC) of SbL8 (10 mM) and L8 (60 mM) (Fernandes et al. 2013). Even at concentration below the CMC value, MT-11-BDP still presents higher fluorescence intensity in SbL8 solution than in saline, as well as a shift of the maximum fluorescence emission wavelength. The fluorescence quenching of the MT-11-BDP may be attributed to the stacking of chromophoric moieties upon micelle formation. On the other hand, after incorporation into SbL8 and L8 micelles, intermolecular stacking is prevented allowing fluorescence. This data clearly establishes the incorporation of the fluorescent analog in SbL8 nanoassemblies and strongly suggests a similar behavior for HePC.

Efficacy of SbL8/HePC nanoassemblies in CL murine model

The antileishmanial activity of SbL8/HePC nanoassemblies was evaluated after oral administration in *L. amazonensis*-infected BALB/c mice, through measurement of lesion size growth and determination of parasite load in the lesion by qPCR.

Figure 3 displays the results of lesion size change and parasite load, following daily treatment with SbL8/HePC nanoassemblies by oral route for 30 days, in comparison with monotherapies (SbL8 or HePC) and saline control. All the treatments promoted significant reduction in the lesion size from day 9, when compared with control (Fig. 3a) ($P < 0.05$, two-way repeated measures ANOVA). On the other hand, only the group treated with SbL8/HePC nanoassemblies showed a significant reduction of parasite burden in the lesion, in comparison with the saline. Strikingly, the parasite load was also significantly lower in the SbL8/HePC group than those receiving SbL8 or HePC (Fig. 3b).

Table 1 Particle size distribution and potential zeta in SbL8, HePC, or SbL8/HePC dispersion in saline

Sample	Mean hydrodynamic diameter (nm)[a]	PDI[a]	Zeta potential (mV)[a]
HePC	226	0.585	− 2.3
SbL8	94	0.281	− 27.8
SbL8/HePC	134	0.597	− 29.4

[a] Representative values of the results obtained from 3 independent experiments

PDI polydispersity index

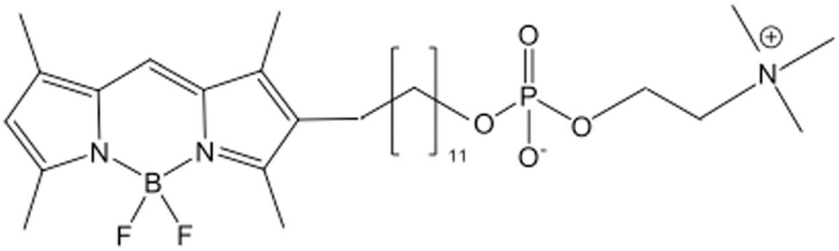

Fig. 1 Structure of the fluorescent HePC analog MT-11-BDP; modified from Hornillos et al. (2008)

Toxicity evaluations of SbL8 nanoassemblies

As a first attempt to evaluate possible side effects, the variation of body weight was registered individually during the treatment. The groups receiving SbL8 showed significant decrease of body weight, in comparison with the control group (data not shown). At the end of treatment of the mice infected with *L. amazonensis*, the serum levels of markers of the hepatic injury (AST and ALT) were assessed. LDH was also determined in serum as another important marker of tissue damages. However, no significant difference was observed in these markers between the experimental groups (Online Resource 1). Animal death was also found in some animal groups. Three out of 9 animals that received SbL8 died during the first and second weeks of treatment. A significant mortality (2 out of 9 animals) was also observed during the first week of treatment with SbL8/HePC nanoassemblies. In both groups, diarrhea was noticed, suggesting toxicity to the gastrointestinal system.

Discussion

Previous studies by our group have led to the discovery of amphiphilic Sb(V) complexes as orally active drug candidates for leishmaniasis. The antileishmanial activity of SbL8 by the oral route was established in murine models of both VL and CL (Fernandes et al. 2013; Lanza et al. 2016). SbL8 was found to self-assemble in aqueous solution, forming nanostructures with potential for incorporating and carrying lipophilic substances (Fernandes et al. 2013). As an attempt to associate the benefits of nanotechnology and combination therapy, we investigated here the incorporation of HePC into SbL8 nanoassemblies and the impact of this association on the therapeutic efficacy in vivo in experimental model of CL.

Previous attempts to characterize SbL8 nanoassemblies by SAXS, AFM, and NTA have indicated the formation of spherical core-shell nanoparticles with diameter in the range of 40–50 nm, that further aggregate to form larger (200–300 nm) nanostructures (Lanza et al. 2016). The present analysis of SbL8 by DLS is consistent with these findings. The increase in the mean particle diameter and polydispersity index upon addition of HePC suggests that HePC interferes in the aggregation of the SbL8 nanoparticles. The clear demonstration of the interaction of the HePC fluorescent analog with SbL8 strongly supports the incorporation of HePC into SbL8 nanoparticles. Indeed, HePC partitioning into SbL8 nanosystems is expected from HePC amphiphilic character, the existence of hydrophobic environment in SbL8 dispersion (Fernandes et al. 2013) and the high SbL8/HePC ratio used here. However, one may argue that the addition of the fluorophore modifies the physico-chemical properties of HePC and the resulting analog behaves differently from HePC. Although inclusion of BODIPY group in HePC slightly lowered its CMC (Barioni et al. 2015), the impact of the fluorophore on its biological properties is mild, with no change in uptake route in *Leishmania* and similar antileishmanial activity (Hornillos et al. 2008; De La Torre et al. 2014; Fernandez-Prada et al. 2016). An additional evidence for the ability of SbL8 nanoassemblies to promote the dispersion of an amphiphilic drug under the monomeric form was obtained with amphotericin B (Online Resource 2). The circular dichroism and UV/Visible absorption spectra of amphotericin B in SbL8 showed bands characteristics of the monomeric form, contrary

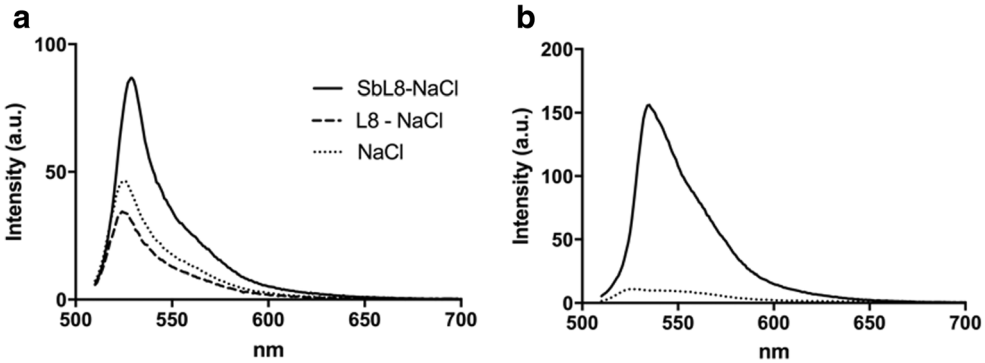

Fig. 2 Fluorescence emission spectra of HePC fluorescent analog (MT-11-BDP), following incorporation into SbL8 or L8 dispersion and subsequent dilution in PBS at final HePC concentration of 2.5 (**a**) or 32 μM (**b**). $\lambda_{excitation} = 500$ nm

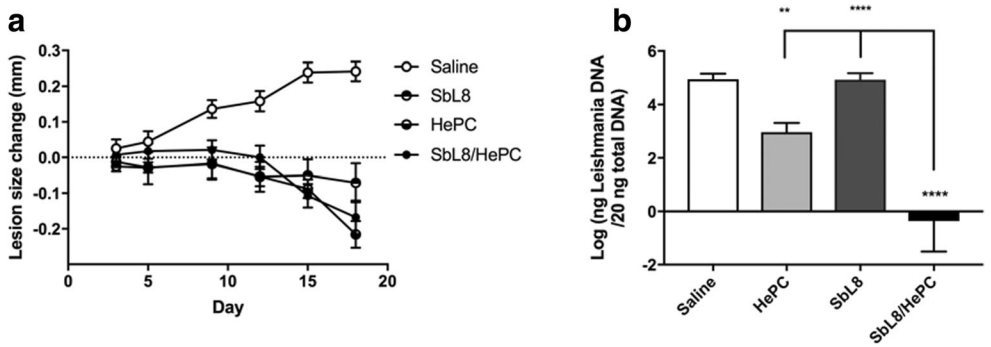

Fig. 3 Antileishmanial activity of SbL8/HePC nanoassemblies after oral administration in murine CL model. BALB/c mice ($n = 6–9$) were infected intradermally with *L. amazonensis* at the tail base. After 35 days of infection, animals were submitted to the following treatments for 30 days: SbL8/HePC nanoassemblies by oral route at 200 mg Sb/kg/day and 5 mg HePC/kg/day; SbL8 by oral route at 200 mg Sb/kg/day; HePC by oral route at 5 mg/kg/day; and saline by oral route. **a** Variation of the lesion size as a function of time, in relation to the initial treatment time ($n = 6–9$/group). All the treated groups showed significant reduction of the lesion size from day 9, in comparison with saline group ($P < 0.05$, two-way repeated measures ANOVA, followed by Tukey's multiple comparisons test). **b** Parasite load in the lesion determined by qPCR (4–6 per group). Data are shown as mean ± SEM. $^{**}P < 0.01$ and $^{****}P < 0.0001$ for comparison of each treated group with the control group, one-way ANOVA test, followed by Tukey's post-test for multiple comparisons

to the free drug in water that exhibited typical bands of the aggregated forms (Gaboriau et al. 1997). This data taken altogether supports the ability of SbL8 nanosystems to act as HePC carrier systems.

SbL8/HePC nanoassemblies given orally were as effective as HePC or SbL8 in reducing the lesion size of infected animals. On the other hand, only SbL8/HePC significantly reduced the parasite load in the lesion, in comparison with saline control. This formulation was also more effective in reducing the parasite load than HePC or SbL8 alone. In spite of the clear therapeutic benefit of SbL8/HePC nanoassemblies in the CL model, this effect was achieved using relatively high dose of Sb (200 mg Sb/kg) and prolonged treatment (30 days) and, in these conditions, toxicity may be an issue. Although the biochemical parameters of blood serum gave no evidence of hepatotoxicity, body weight loss and death were observed in animals that received SbL8. These effects are consistent with our previous report of animal death following oral administration of SbL8 (Lanza et al. 2016). We proposed previously that death was due to suffocation of animals after gavage, as a consequence of the formation of foam during administration of the formulation, its regurgitation, and transfer to the lungs. The high viscosity of the formulation also made the administration difficult and increased the risk of esophagus irritation and injury. The fact that death took place in the first weeks of treatment reinforces the idea that toxicity was related to administration rather than intrinsic drug side effects.

Regarding the therapeutic benefit of oral treatment with SbL8/HePC nanoassemblies, as evidenced in the present work, several possible contributing mechanisms can be proposed. As SbL8 and HePC are orally active and presumably act through different mechanisms, both compounds may exert their action independently, resulting in additive antileishmanial effects. It has been suggested that HePC may temporarily increase the tight junctions of intestinal epithelium, allowing its action as a permeation enhancer for other drugs (Ménez et al. 2006). Such an effect may enhance the permeation of SbL8 through the intestinal epithelium and increase its activity. HePC was reported to promote IFN-g-dominated antileishmanial immune response (Wadhone et al. 2009), evidencing an immunomodulatory action that may enhance the action of host immune-dependent drugs such as antimonials (Murray et al. 1988). As another possible contributing mechanism, SbL8 nanostructures may carry HePC through the intestinal epithelium and favor the uptake by the host-cells in which leishmania parasites reside. The precise mechanism involved should be the subject of future investigations.

Conclusions

The present study supports the incorporation of HePC in SbL8 nanoparticles, establishes the therapeutic benefit of oral treatment with SbL8/HePC nanoassemblies in a murine model of CL, and constitutes an important step towards the development of new orally active antileishmanial drug combination.

Acknowledgements The authors specially thank Nayara K. L. M. Moura, Larissa P. Carvalho, Flaviana R. Fernandes, and Pablo H. P. Matias for technical support.

Funding information This work was supported by the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant number: 425332/2018–7), Coordenação de Aperfeicoamento de Pessoal de Nível Superior (CAPES, grant number: PNPD20131163), and Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG, grant number: APQ-03129-16) for financial support. F.F. was recipient of fellowship from CNPq (grant number: 305659/2017-0) and Chaire Jean d'Alembert, Université Paris-Saclay, France (ANR-11-IDEX-0003-02). L.R was supported by Program of Redes Temáticas de Investigación Cooperativa RETICS-FEDER (grant number: RD16/0027/0010).

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

The study involving animals was approved by the Ethical Committee for Animal Experimentation of the UFMG with protocol number 318/2013.

Conflict of interest The authors declare that they have no conflict of interest.

References

Barioni MB, Ramos AP, Zaniquelli MED, Acuña AU, Ito AS (2015) Miltefosine and BODIPY-labeled alkylphosphocholine with leishmanicidal activity: aggregation properties and interaction with model membranes. Biophys Chem 196:92–99. https://doi.org/10.1016/j.bpc.2014.10.002

De La Torre BG, Hornillos V, Luque-Ortega JR, Abengózar MA, Amat-Guerri F, Ulises Acuña A, Rivas L, Andreu D (2014) A BODIPY-embedding miltefosine analog linked to cell-penetrating Tat(48-60) peptide favors intracellular delivery and visualization of the antiparasitic drug. Amino Acids 46:1047–1058. https://doi.org/10.1007/s00726-013-1661-3

Do Monte-Neto RL, Coelho AC, Raymond F, Légaré D, Corbeil J, Melo MN, Frézard F, Ouellette M (2011) Gene expression profiling and molecular characterization of antimony resistance in *Leishmania amazonensis*. PLoS Negl Trop Dis 5:1167–1176. https://doi.org/10.1371/journal.pntd.0001167

Duarte MC, Lage DP, Martins VT, Chávez-Fumagalli MA, Roatt BM, Menezes-Souza D, Goulart LR, Soto M, Tavares CAP, Coelho EAF (2016) Recent updates and perspectives on approaches for the development of vaccines against visceral leishmaniasis. Rev Soc Bras Med Trop 49:398–407. https://doi.org/10.1590/0037-8682-0120-2016

Fernandes FR, Ferreira WA, Campos MA, Ramos GS, Kato KC, Almeida GG, Corrêa JD, Melo MN, Demicheli C, Frézard F (2013) Amphiphilic antimony (V) complexes for oral treatment of visceral leishmaniasis. Antimicrob Agents Chemother 57:4229–4236. https://doi.org/10.1128/AAC.00639-13

Fernandez-Prada C, Vincent IM, Brotherton MC, Roberts M, Roy G, Rivas L, Leprohon P, Smith TK, Ouellette M (2016) Different mutations in a P-type ATPase transporter in *Leishmania* parasites are associated with cross-resistance to two leading drugs by distinct mechanisms. PLoS Negl Trop Dis 10(12):e0005171. https://doi.org/10.1371/journal.pntd.0005171

Freitas-Junior LH, Chatelain E, Kim HA, Siqueira-Neto JL (2012) Visceral leishmaniasis treatment: what do we have, what do we need and how to deliver it? Int J Parasitol Drugs Drug Resist 2:11–19. https://doi.org/10.1016/j.ijpddr.2012.01.003

Frézard F, Demicheli C, Ribeiro RR (2009) Pentavalent antimonials: new perspectives for old drugs. Molecules 14:2317–2336. https://doi.org/10.3390/molecules14072317

Gaboriau F, Cheron M, Leroy L, Bolard J (1997) Physico-chemical properties of the heat-induced 'superaggregates' of amphotericin B. Biophys Chem 66(1):1–12. https://doi.org/10.1016/S0301-4622(96)02241-7

Godinho JLP, Simas-Rodrigues C, Silva R, Ürmenyi TP, De Souza W, Rodrigues JCF (2012) Efficacy of miltefosine treatment in *Leishmania amazonensis*-infected BALB/c mice. Int J Antimicrob Agents 39:326–331. https://doi.org/10.1016/j.ijantimicag.2011.11.008

Hadighi R, Boucher P, Khamesipour A, Meamar AR, Roy G, Ouellette M, Mohebali M (2007) Glucantime-resistant *Leishmania tropica* isolated from Iranian patients with cutaneous leishmaniasis are sensitive to alternative antileishmania drugs. Parasitol Res 101:1319–1322. https://doi.org/10.1007/s00436-007-0638-0

Hendrickx S, Van den Kerkhof M, Mabille D, Cos P, Delputte P, Maes L, Caljon G (2017) Combined treatment of miltefosine and paromomycin delays the onset of experimental drug resistance in *Leishmania infantum*. PLoS Negl Trop Dis 11:1–10. https://doi.org/10.1371/journal.pntd.0005620

Hornillos V, Carrillo E, Rivas L, Amat-Guerri F, Acuña AU (2008) Synthesis of BODIPY-labeled alkylphosphocholines with leishmanicidal activity, as fluorescent analogues of miltefosine. Bioorg Med Chem Lett 18:6336–6339. https://doi.org/10.1016/j.bmcl.2008.10.089

Hornillos V, Saugar JM, De la Torre BG, Andreu D, Rivas L, Acuña AU, Amat-Guerri F (2006) Synthesis of 16-mercaptohexadecylphosphocholine, a miltefosine analog with leishmanicidal activity. Bioorg Med Chem Lett 16:5190–5193. https://doi.org/10.1016/j.bmcl.2006.07.004

Lanza JS, Fernandes FR, Corrêa-Júnior JD, Vilela JM, Magalhães-Paniago R, Ferreira LA, Andrade MS, Demicheli C, Melo MN, Frédéric F (2016) Polarity-sensitive nanocarrier for oral delivery of Sb(V) and treatment of cutaneous leishmaniasis. Int J Nanomedicine 11:2305–2318. https://doi.org/10.2147/IJN.S105952

Ménez C, Buyse M, Besnard M, Farinotti R, Loiseau PM, Barratt G (2006) Interaction between miltefosine and amphotericin B: consequences for their activities towards intestinal epithelial cells and *Leishmania donovani* promastigotes in vitro. Antimicrob Agents Chemother 50:3793–3800. https://doi.org/10.1128/AAC.00837-06

Murray HW, Berman JD, Wright SD (1988) Immunochemotherapy for intracellular *Leishmania donovani* infection: gamma interferon plus pentavalent antimony. J Infect Dis 157(5):973–978. https://doi.org/10.1093/infdis/157.5.973

Nicolas L, Prina E, Lang T (2002) Real-time PCR for detection and quantitation of *Leishmania* in mouse tissues. J Clin Microbiol 40:1666–1669. https://doi.org/10.1128/JCM.40.5.1666-1669.2002

Seifert K, Croft SL (2006) In vitro and in vivo interactions between miltefosine and other antileishmanial drugs. Antimicrob Agents Chemother 50:73–79. https://doi.org/10.1128/AAC.50.1.73-79.2006

Sereno D, Guilvard E, Maquaire S, Cavaleyra M, Holzmuller P, Ouaissi A, Lemesre JL (2001) Experimental studies on the evolution of antimony-resistant phenotype during the in vitro life cycle of *Leishmania infantum*: implications for the spread of chemoresistance in endemic areas. Acta Trop 80:195–205. https://doi.org/10.1016/S0001-706X(01)00154-1

Srivastava S, Mishra J, Gupta AK, Singh A, Shankar P, Singh S (2017) Laboratory confirmed miltefosine resistant cases of visceral leishmaniasis from India. Parasit Vectors 10:1–11. https://doi.org/10.1186/s13071-017-1969-z

Sundar S, Sinha PK, Rai M, Verma DK, Nawin K, Alam S, Chakravarty J, Vaillant M, Verma N, Pandey K, Kumari P, Lal CS, Arora R, Sharma B, Ellis S, Strub-Wourgaft N, Balasegaram M, Olliaro P, Das P, Modabber F (2011) Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. Lancet 377:477–486. https://doi.org/10.1016/S0140-6736(10)62050-8

Thomaz-Soccol V, Da Costa ESF, Karp SG, Letti LAJ, Soccol FT, Soccol CR (2018) Recent advances in vaccines against leishmania based on patent applications. Recent Pat Biotechnol 12:21–32. https://doi.org/10.2174/1872208311666170510121126

Wadhone P, Maiti M, Agarwal R, Kamat V, Martin S, Saha B (2009) Miltefosine promotes IFN-g-dominated anti-leishmanial immune response. J Immunol 182:7146–7154. https://doi.org/10.4049/jimmunol.0803859

World Health Organization (2010) Control of the leishmaniases. World Health Organ Tech Rep Ser 5:22–26. https://doi.org/10.1038/nrmicro1766

World Health Organization. (2019) It contains institutional information, technical news, publications, projects and services. Available in www.who.int/topics/leishmaniasis/en/. Accessed on January 15, 2019

Wortmann G, Zapor M, Ressner R, Fraser S, Hartzell J, Pierson J, Weintrob A, Magill A (2010) Lipsosomal amphotericin B for treatment of cutaneous leishmaniasis. Am J Trop Med Hyg 83(5):1028–1033. https://doi.org/10.4269/ajtmh.2010.10-0171

Affiliations

Virgínia M. Carregal[1] · Juliane S. Lanza[1] · Daniel M. Souza[2] · Arshad Islam[1] · Cynthia Demicheli[3] · Ricardo T. Fujiwara[4] · Luis Rivas[5] · Frédéric Frézard[1]

[1] Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, Minas Gerais 31270-901, Brazil

[2] Colégio Técnico da UFMG, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, Minas Gerais 31270-901, Brazil

[3] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, Minas Gerais 31270-901, Brazil

[4] Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, Minas Gerais 31270-901, Brazil

[5] Centro de Investigaciones Biologicas-CSIC, Ramiro de Maeztu, 9, Madrid E-28040, Spain