Pterocarpanquinone LQB-118 Induces Apoptosis in 
Leishmania (Viannia) braziliensis and Controls Lesions in Infected Hamsters

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Abstract

Previous results demonstrate that the hybrid synthetic pterocarpanquinone LQB-118 presents antileishmanial activity against Leishmania amazonensis in a mouse model. The aim of the present study was to use a hamster model to investigate whether LQB-118 presents antileishmanial activity against Leishmania (Viannia) braziliensis, which is the major Leishmania species related to American cutaneous leishmaniasis. The in vitro antileishmanial activity of LQB-118 on L. braziliensis was tested on the promastigote and intracellular amastigote forms. The cell death induced by LQB-118 in the L. braziliensis promastigotes was analyzed using an annexin V-FITC/PI kit, the oxidative stress was evaluated by 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) and the ATP content by luminescence. In situ labeling of DNA fragments by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was used to investigate apoptosis in the intracellular amastigotes. L. braziliensis-infected hamsters were treated from the seventh day of infection with LQB-118 administered intraleisonally (26 μg/kg/day, three times a week) or orally (4.3 mg/kg/day, five times a week) for eight weeks. LQB-118 was active against the L. braziliensis promastigotes and intracellular amastigotes, producing IC₅₀ (50% inhibitory concentration) values of 3.4±0.1 and 7.5±0.8 μM, respectively. LQB-118 induced promastigote phosphatidylserine externalization accompanied by increased reactive oxygen species production and ATP depletion. Intracellular amastigote DNA fragmentation was also observed, without affecting the viability of macrophages. The treatment of L. braziliensis-infected hamsters with LQB-118, either orally or intraleesionally, was effective in the control of lesion size, parasite load and increase intradermal reaction to parasite antigen. Taken together, these results show that the antileishmanial effect of LQB-118 extends to L. braziliensis in the hamster model, involves the induction of parasite apoptosis and shows promising therapeutic option by oral or local routes in leishmaniasis.

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Introduction

Leishmaniases are neglected diseases that occur in 98 countries and show an annual incidence of 1 to 1.5 million people worldwide [1–3]. American cutaneous leishmaniasis (ATL) presents with a spectrum of clinical forms, including mucosal and disseminated and diffuse cutaneous leishmaniasis. Leishmania (Viannia) braziliensis is the important species that causes ATL and is the major agent for the mucosal forms in Brazil [4,5]. In general, treatment failures and relapses are common for this form, leading to the mutilation or destruction of the affected nasopharyngeal area [5].

The treatment of leishmaniasis is restricted to a few extremely toxic drugs, quite costly and increasingly challenged by the development of parasite resistance to drugs [6,7]. Moreover, the treatment is complicated by intrinsic, species-specific differences, resulting in variable drug susceptibilities in determine geographical locations. Because of high cost or limited effectiveness, the supply of the new formulations of amphotericin B and oral miltefosine have been insufficient to meet the demand, especially in endemic regions [8–10]. The development of drugs that are less toxic, more effective, less costly and orally administrable is critical for the treatment of leishmaniasis in endemic countries [10].

Naphthopterocarpanquinone LQB-118 is a synthetic molecule produced through the hybridization of a naphthoquinone with a pterocarpan (isoflavonoid). Previous studies demonstrated that LQB-118 has antitumoral activity and induces apoptosis in cells.
derived from chronic myeloid leukemia patients [11]. Our group has recently demonstrated the antileishmanial activity of LQB-118 administered by the local or oral route in the treatment of BALB/c mice infected with L. amazonensis. This treatment was able to control the lesions and parasite load with the same efficacy as Glucantime, which was used as the reference drug [12].

Mice are naturally resistant to L. braziliensis infection and resolve the infection in approximately 5 weeks [13,14]. The infection of golden hamsters with L. braziliensis results in a localized lesion and the dissemination of the parasite, which resembles the infection profile in humans [15,16].

In the present study, we evaluated the activity of LQB-118 on L. braziliensis using golden hamster as the infection model. Our findings indicated that LQB-118 is therapeutically effective when administered orally or intrareionally and is active in vitro against L. braziliensis, selectively inducing DNA fragmentation in the intracellular amastigote.

Materials and Methods

LQB-118

LQB-118 was synthesized as previously described [17] in the Laboratory of Bioorganic Chemistry of the Federal University of Rio de Janeiro, Brazil. LQB-118 was dissolved in DMSO (dimethylsulfoxide) (Sigma Aldrich, St Louis, MO, USA).

Parasites

Leishmania braziliensis (MCAN/BR/98/R619) was routinely isolated from hamster lesions and maintained as promastigotes in Schneider’s insect medium (Sigma-Aldrich, St Louis, MO, USA) containing 20% heat-inactivated fetal bovine serum (Cultilab, Brazil) and 100 μg/mL gentamicin (Schering-Plough). The parasites were used after no more than five passages.

Animals

Female golden hamsters (Mesocricetus auratus) six to eight weeks old were obtained from Fundação Oswaldo Cruz. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Brazilian National Council of Animal Experimentation. The protocol was approved by the Ethics Committee on Animal Use (CEUA) of the Instituto de Biologia Roberto Alcantara Gomes of the Universidade do Estado do Rio de Janeiro-UERJ, by the number protocol 044/2009.

Antipromastigote growth

Stationary-phase, L. braziliensis promastigotes at 5 × 10^5 cells/mL were cultured in a 24-well culture plate (1 mL/well) at 28°C in Schneider’s medium plus 20% heat-inactivated fetal calf serum containing the indicated concentrations of LQB-118. The controls were treated with 0.2% DMSO, which was the highest concentration of DMSO present in the LQB-118 treatments (0–20 μM). To determine whether the antileishmanial effect of LQB-118 was reversible, the parasites were incubated with LQB-118 (0–20 μM) for 48 h at 26°C, and the number of parasites was then counted. The cells were centrifuged (1000 × g for 10 min), washed twice in PBS, their number adjusted and incubated again for another 48 h at 26°C with Schneider’s medium plus 20% heat inactivated fetal bovine serum, and then the promastigotes were counted in a Neubauer chamber.

Antiamastigote activity

Resident macrophages were obtained from the peritoneal cells of golden hamsters after the peritoneal injection of 10 mL of DMEM. The peritoneal cells (2 × 10^6/mL) were plated onto glass coverslips placed within the wells of a 24-well culture plate (0.5 mL/well) and incubated at 37°C in 5% CO₂ for 1 h. After removing the nonadherent cells, the monolayers were infected with 5 promastigotes for each macrophage for 4 h at 37°C in 5% CO₂. The infected macrophages were washed and incubated with several concentrations of LQB-118 for 48 h at 37°C in 5% CO₂. The monolayers were then stained with Giemsa, and at least 100 infected macrophages per sample were counted under optical microscopy. The supernatant was collected for nitric oxide analysis. The 50% inhibitory concentration (IC50%) was determined by logarithmic regression analysis using GraphPad Prism 5.

Determination of nitric oxide production

For the analysis of the production of nitric oxide (NO) by the infected macrophages, the culture supernatant was collected after 48 h of LQB-118 treatment, as described above. The production of NO was measured by assaying nitrite (NO₂⁻) using the Griess reaction, as described previously [18].

Measurement of ROS levels in L. braziliensis promastigotes

Intracellular ROS levels were measured in treated and untreated cells as described previously [19]. Briefly, promastigotes of L. braziliensis were cultured in Schneider’s insect medium supplemented with 10% HIFCS and incubated at 26°C in concentrations of LQB-118 ranging from 0 to 10.0 μM. After 48 h, the cultures were washed three times and the parasite concentration was adjusted to 1 × 10^7 cells/mL. Then, 20 μM of 2’,7’-dichlorodihydrofluorescein diacetate (H₂DCFDA) (Molecular Probes, Eugene, OR, USA) was added, and the samples were incubated for 30 min under dark conditions. The fluorescence was monitored using a Spectra Max GEMINI XPS spectrophuorometer (Molecular Devices, Silicon Valley, CA, USA) at excitation and emission wavelengths of 507 and 530 nm, respectively.

ATP content determination

Intracellular ATP concentration was measured in treated and untreated cells using a CellTiter-Glo luminescent assay (Promega), which generates a signal proportional to the ATP amount. Briefly, promastigotes of L. braziliensis were cultured in Schneider’s insect medium supplemented with 10% HIFCS and incubated at 26°C in the absence or presence of LQB-118 (2.5, 5, 10 and 20 μM). After 48 h, the cultures were washed three times and the parasite concentration was adjusted to 1 × 10^7 cells in 200 μL of PBS. A 50 μL aliquot of each sample was transferred to a 96-well plate, mixed with the same volume of CellTiter-Glo and incubated in the dark for 10 min, and the bioluminescence was measured using a GloMax-Multi Microplate Multimode Reader (Promega). ATP concentrations were calculated from the ATP standard curve. The respiratory chain inhibitor KCN 1 mM (inhibitor of the complex IV) was used as control.

Mitochondrial membrane potential (ΔΨm)

To determine the effect of LQB-118 on the ΔΨm in macrophages (2 × 10^6 cells/mL) were cultured in the presence of 3.125, 6.25, 12.5, 25, 50, 100 and 200 μM of LQB-118 at 37°C/5%CO₂. After 48 h, the cells were incubated for 10 min with JC-1 (Sigma-Aldrich), washing in Hanks balanced salt solution (HBSS) and fluorescence was measured spectrophluorometrically at both 530 and 590 nm using 485 nm as the excitation wavelength. The ratio between red and green fluorescence (i.e. 590/530 nm) determined the ΔΨm [20].
In situ detection of DNA fragmentation by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL)

DNA fragmentation into the cell was analyzed using an in situ cell death detection kit (Roche) according to the manufacturer’s instructions. Briefly, the hamster macrophages (4 x 10^5/well) were adhered in monolayers to Lab-Tek eight-chamber slides (Nunc, Roskild, Denmark), infected (5 promastigotes per macrophage) and treated with LQB-118 for 48 h at 37°C in 5% CO_2. The monolayers were washed twice in PBS and fixed in paraformaldehyde (4%) for 10 min. The cells were washed in PBS, incubated in a solution of 3% hydrogen peroxide and methanol for 10 min and then washed again with PBS. The monolayers were permeabilized with a solution of 0.1% Triton x-100 and 0.1% sodium citrate and then labeled with the TUNEL solution for 1 h at 37°C. Finally, the monolayers were washed with PBS and analyzed by fluorescence microscopy (Nikon Eclipse-80i).

Flow cytometric analyses of externalized phosphatidylserine

The phosphatidylserine externalization by the parasites was assessed using an annexin V-FITC staining kit (BD Biosciences, San Diego, USA). Briefly, promastigotes (2 x 10^6 cells/mL) were incubated in the absence or presence of LQB-118 (5, 10 or 20 μM) for 24 and 48 h at 28°C. The cells were washed twice and resuspended in 100 μl binding buffer (10 mM HEPES, 150 mM NaCl and 2.5 mM CaCl_2), containing 1 μl annexin V-FITC. After 20 min, the cells were washed twice and resuspended in 300 μL of binding buffer; then, at the time of data acquisition, 1.67 μg/mL of propidium iodide was added. The data were acquired using a BD Accuri C6 flow cytometer (BD Accuri, Ann Arbor, MI, USA) and analyzed with CFlow software.

In vivo activity

Golden hamsters (Mesocricetus auratus) were used for the infection model. The animals were held 5–6/group and infected in the hind footpad with 10^7 stationary-phase, L. braziliensis promastigotes. Beginning 7 days postinfection, each group was treated for eight weeks with LQB-118 (solubilized in DMSO) administered three times a week by the intralional route (26 μg/kg/day), LQB-118 (partially solubilized in PBS) given five times a week by the oral route (4,3 mg/kg/day) or meglumine antimoniate (Glucantime, Sanofi-Aventis) given by the intraperitoneal route (70 mg/kg/day). The groups of control animals received 10 μl DMSO (Sigma) by the intralional route three times a week or were left untreated. The lesion sizes were measured weekly with a dial caliper (Mitutoyo, Brazil). In the eight week of infection, the intradermal reaction of L. braziliensis antigen was evaluated. The left hind footpad was injected with 20 μl of total antigen (obtained by freezing and thawing) corresponding at 2 x 10^6 promastigotes. Twenty-four h later the footpad swelling was measured with dial calliper, and the intradermal reaction was expressed as the difference between thickness prior and to injection. The hamsters were euthanized at the end of the experiment using carbon dioxide chamber, and their infected paws were aseptically excised, skinned, weighted and ground in Schneider’s medium (Sigma) containing 20% fetal calf serum. The cell suspension was serially diluted, and the motile parasites were evaluated using limiting dilution analysis after 7 days.

Statistical Analysis

The statistical analyses of the in vitro drug effects on the promastigote and amastigote forms were performed using Student’s t-test. The statistical analyses of the in vivo experiments were performed using analysis of variance (ANOVA) and the Tukey post hoc test.

Results

In vitro antileishmanial activity against L. braziliensis

To evaluate whether the previously demonstrated antileishmanial activity of LQB-118 on L. amazonensis also occurred on L. braziliensis, promastigotes were initially cultivated in the presence of different concentrations of LQB-118 [0–20 μM] for 3 days. LQB-118 inhibited the growth of the parasites in a dose-
Macrophages. Nitric oxide production in the supernatants was measured and the infection index was determined by counting at least 100 amastigote, infected hamster macrophages were treated with different LQB-118 concentrations (0–20 \( \mu M \)) for 48 h. Figure 2A shows a dose-dependent increase in ROS production was achieved in cells treated with LQB-118 (Figure 2A). Interestingly, this increase in ROS production was paralleled to a severe depletion and DNA fragmentation. The promastigote forms treated with 3.5 \( \mu M \) (IC50) and 20 \( \mu M \) LQB-118 showed annexin labeling (AnnV+, PI–) at 48 h (Figure 3A). The treatment with 20 \( \mu M \) LQB-118 at 24 h and 48 h promoted the labeling of 16.9% and 30.3%, respectively (Figure 3B). As oxidative stress is other hallmark of apoptosis, we also analyzed the generation of reactive oxygen species (ROS) by LQB-118-treated promastigotes. A concentration-dependent increase in ROS production was achieved in cells treated with LQB-118 (Figure 3C). Interestingly, this increase in ROS production was paralleled to a severe depletion of ATP stocks (Figure 3D). To extrapolate these findings to the clinically relevant form of the parasite, hamster peritoneal macrophages were infected with L. braziliensis, treated with LQB-118 and DNA fragmentation was evaluated by TUNEL assay. Fluorescence analysis revealed that 7.5 \( \mu M \) LQB-118 was enough to induce DNA fragmentation in intracellular amastigotes (green-labelled structures in Figure 3E) and a concentration as high as 20 \( \mu M \) kept the selectivity against the parasite, as no label is seen in the host cell nucleus (Figure 3E).

### Induction of apoptosis in L. braziliensis promastigotes and amastigotes by LQB-118

To determine whether the irreversible effect of LQB-118 observed on L. braziliensis occurred because of the induction of apoptosis, cells treated with LQB-118 were investigated for labeling with annexin V-FITC/PI, ROS production, ATP depletion and DNA fragmentation. The promastigote forms treated with 3.5 \( \mu M \) (IC50) and 20 \( \mu M \) LQB-118 showed annexin labeling (AnnV+, PI–) at 48 h (Figure 3A). The treatment with 20 \( \mu M \) LQB-118 at 24 h and 48 h promoted the labeling of 16.9% and 30.3%, respectively (Figure 3B). As oxidative stress is other hallmark of apoptosis, we also analyzed the generation of reactive oxygen species (ROS) by LQB-118-treated promastigotes. A concentration-dependent increase in ROS production was achieved in cells treated with LQB-118 (Figure 3C). Interestingly, this increase in ROS production was paralleled to a severe depletion of ATP stocks (Figure 3D). To extrapolate these findings to the clinically relevant form of the parasite, hamster peritoneal macrophages were infected with L. braziliensis, treated with LQB-118 and DNA fragmentation was evaluated by TUNEL assay. Fluorescence analysis revealed that 7.5 \( \mu M \) LQB-118 was enough to induce DNA fragmentation in intracellular amastigotes (green-labelled structures in Figure 3E) and a concentration as high as 20 \( \mu M \) kept the selectivity against the parasite, as no label is seen in the host cell nucleus (Figure 3E).

### Effect of LQB-118 on the mitochondrial membrane potential (\( \Delta \Psi_m \)) of hamster macrophages

The therapeutic effect of LQB-118 was evaluated in L. braziliensis-infected hamsters. Seven days after footpad infection, the hamsters were treated with LQB-118 by the intralesional (three times a week) or oral (five times a week) route for eight weeks. The controls were hamsters treated with intralesional DMSO (three times a week) or Glucantime (five times a week) by the intraperitoneal route or left untreated. LQB-118 decreased the lesion size from the fourth week of the treatment (fifth week of the infection) (Figure 5A). Although not statistically significant, the lesion size was decreased from the fourth week of the treatment (fifth week of the infection) in the groups treated with LQB-118 by...
Figure 3. Evaluation of LQB118 inducing apoptosis on *L. braziliensis* – A) and B) Phosphatidylserine exposure on promastigotes. Promastigotes were incubated with 3.5 or 20 μM LQB118 to 24 or 48 h/28°C and then stained with Annexin V-FITC+propidium iodide and analyzed by flow cytometer. Controls were promastigotes incubated with 60 μM Miltefosine or the culture medium supplemented with 20% fetal bovine serum. In A only treatment at 48 h and in B quantitative evaluation of cells stained with annexin V-FITC at 24 and 48 h. (mean ± SD, n = 3). *P < 0.05 **P < 0.01. C) ROS generation and D) impairment of ATP production in LQB-118-treated promastigotes. Promastigotes of *L. braziliensis* were incubated for 48 h in the presence of LQB-118 in Schneider’s insect medium plus 10% HIFCS. A) ROS generation was quantified using H2DCFDA, B) Cellular ATP concentration was measured by bioluminescence assay. Results are presented as means ± standard error; n = 3. *P < 0.05; **P < 0.01. E) *In situ* DNA fragmentation of intracellular amastigote. Infected monolayers of hamsters peritoneal macrophages were treated with the indicated concentrations of LQB118 for 48 h. Monolayers were labeled using TUNEL and observed using fluorescence microscopy. Highlight the cells in 400× magnification. Legend Arrows indicate intracellular amastigotes, N – Macrophage nucleus.

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Figure 4. Changes in the ΔΨm of macrophage. Peritoneal hamster macrophages (2×10^6 cells/mL) were cultured in the presence of LQB-118 for 48 h at 37°C/5%CO2. The cells were incubated for 10 min with JC-1 and analysed fluorometrically. Results are presented as means ± standard error; n = 3. *P < 0.05; **P < 0.01.

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Figure 5. Activity of LQB-118 on golden hamsters infected with *L. braziliensis*. Golden hamsters (5/group) infected with *L. braziliensis* (10^7*) were treated on the seventh day of infection with LQB-118 intralesional (26 μg/kg/day) three times/week or orally (4.3 mg/kg/day) five times/week during eight weeks. Controls were untreated, treated with intralesional DMSO three times/week or Glucantime five times/week by intraperitoneal route. A) Lesion thickness was measured for nine weeks. The arrow indicates the start of treatment. Mean ± SD, # P<0.002 (in relation to untreated group). * p<0.001 (in relation to intralesional DMSO group); B) Intradermal reaction at *L. braziliensis* antigen was evaluated on the contralateral foot pad on eight week of infection. The swelling was measured 48 h later in the antigen-injected footpads. * p<0.04; ** p<0.01. Each point represents one animal and the horizontal bar indicates the mean. C) Parasite burden was assessment by limiting dilution at the end of treatment. *** p<0.001. ii, intralesional/subcutaneous; ip, intraperitoneal.

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DY showed the mitochondrial function of LQB-118-treated macrophages fragmentation without affecting macrophages DNA. Analysis of induces annexin V labeling and intracellular amastigote DNA K562-Lucena cell line [11]. In the present work, we demonstrated inducing cell death by apoptosis, as previously observed using macrophages. These effects suggest that LQB-118 may be of LQB-118 was independent of the production of nitric oxide by 

L. braziliensis fragmentation. These effects of LQB-118 on cell death by apoptosis, as demonstrated by PS exposure and DNA fragmentation (data not shown), ROS generation and ATP production impairment in promastigotes. 

Discussion

We previously demonstrated that the pterocarpanquinone LQB-118 presents antileishmanial activity against L. (Leishmania) amazonensis in vitro and in vivo using the mouse model [12]. In the present article, we used a hamster model to show that the antileishmanial effect of LQB-118 extends to another subgenus and includes L. (Viannia) braziliensis, the most important species causing ATL.

LQB-118 reduced the number of both the promastigote and amastigote intracellular forms of L. braziliensis in vitro, and this inhibitory effect on parasite growth was irreversible and occurred mainly with 10 and 20 μM LQB-118. The antimastigote action of LQB-118 was independent of the production of nitric oxide by macrophages. These effects suggest that LQB-118 may be inducing cell death by apoptosis, as previously observed using myeloid leukemia cells, such as those from the multidrug-resistant, K562-Lucena cell line [11]. In the present work, we demonstrated that the LQB-118 treatment of the promastigotes of L. braziliensis induces annexin V labeling and intracellular amastigote DNA fragmentation without affecting macrophages DNA. Analysis of the mitochondrial function of LQB-118-treated macrophages showed ΔΨm reduction occurred at doses much higher than the IC50 for intracellular amastigotes. Although annexin labeling is not exclusive to the externalized phosphatidylserine (PS) membrane phospholipids of Leishmania [21], we additionally observed promastigote DNA fragmentation (data not shown), ROS generation and ATP production impairment in promastigotes. The increase in ROS production and ATP stocks depletion suggest mitochondrial dysfunction, which could culminate with cell death by apoptosis, as demonstrated by PS exposure and DNA fragmentation. These effects of LQB-118 on L. braziliensis are consistent with our recent finding concerning the cell death mechanism of LQB-118 in L. amazonensis. LQB-118 kills the promastigote of L. amazonensis by apoptosis, which induces ROS production, oxidative stress, depolarization of mitochondrial membrane potential and reduction of ATP [22]. These results suggest LQB-118 induces mitochondrion-dependent apoptosis in Leishmania spp. On the other hand, LQB-118-induced apoptosis in tumor cells is believed to involve different routes, depending on the cell type: increasing ROS, intracellular calcium levels and the activity of caspase 9 or 12 [23].

The golden hamster is susceptible to L. braziliensis and reproduces the human infection, with progressive swelling and ulceration at the inoculation site that is followed by dissemination of the parasites [15,16]. The treatment of infected golden hamsters with LQB-118 was administered intralesionally and orally for eight weeks and significantly diminished the lesion size and parasite burden when administered by either means. The LQB-118 treatment was able promote increased of intradermal reaction to L. braziliensis antigen suggesting protective immune response. Intradermal reaction skin test (delayed type hypersensitivity) with Leishmania antigen is a good marker of cellular immune response in leishmaniasis e predictive of protection [24,25].

The lesion sizes were highly significantly (p<0.001) reduced by the intralesional treatment with LQB-118 in relation to the DMSO control group. DMSO was used to solubilize the LQB-118 doses administered by the intralesional route, but DMSO alone resulted in an increased paw size in relation to that of the untreated control group. Certain pharmacological activities are attributed to DMSO, and because of its anti-inflammatory activity, the use of DMSO has been suggested in several human diseases, including gastrointestinal and dermatologic disorders and brain edema [26]. In mice, intralesional DMSO treatment increased the paw edema induced by zimozan, whereas oral treatment had the opposite effect [27]. Because there was no significant parasitic load difference between the DMSO and untreated control groups, the DMSO most likely only induced paw swelling. Despite the increased paw swelling with the intralesional injection of DMSO, LQB-118 demonstrated a potent therapeutic action even when solubilized in this vehicle. Whether LQB-118 provides any additional anti-inflammatory effect is a question that requires further investigation. We observed no significant difference in effectiveness between the oral and intralesional routes of administration. Although the orally administered LQB-118 was partially solubilized in PBS, it was effective in the control of lesion size and parasite burden in relation to the untreated group (p<0.002 in the seventh week postinfection), showing that pterocarpanquinone is also active by this important route. Likewise, LQB-118 was orally active in controlling lesions in L. amazonensis-infected BALB/c mice [12]. Although oral miltefosine has been a breakthrough for leishmaniasis therapy, the drug resistance and reduced sensitivity of the parasite observed in New World species are factors limiting its use against ATL. [28]. The development of new antileishmanial molecules that are orally bioavailable is an important strategy in leishmaniasis control [10]. Together, the results of our study showed that the antileishmanial effect of LQB-118 extends to L. braziliensis in the hamster model and is related to the induction of apoptotic cell death in the parasites. These data are an important contribution to the preclinical studies of LQB-118, demonstrating the promising therapeutic action of this new molecule on leishmaniasis.

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Author Contributions

Conceived and designed the experiments: LC SAGS. Performed the experiments: LC ROP PMLD SAGS RFS EFCJ. Analyzed the data: LC ROP PMLD SAGS RFS EFCJ. Contributed reagents/materials/analysis tools: ECTS AJMS PRRC RFS SAGS. Wrote the paper: LC SAGS.
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