Effectiveness of the local or oral delivery of the novel naphthopterocarpanquinone LQB-118 against cutaneous leishmaniasis

Edézio Ferreira da Cunha-Júnior1†, Wallace Pacienza-Lima2†, Grazielle Alves Ribeiro1, Chaquip Daher Netto3,4, Marilene Marcuzzo do Canto-Cavalheiro1, Alcides José Monteiro da Silva3, Paulo Roberto Ribeiro Costa3, Bartira Rossi-Bergmann2 and Eduardo Caio Torres-Santos1*

1Laboratório de Bioquímica de Tripanosomatídeos, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil; 2Laboratório de Imunofarmacologia, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; 3Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; 4Laboratório de Química Orgânica, Instituto de Química, Universidade Federal do Rio de Janeiro, Macaé, Brazil

*Corresponding author. Tel: +55-21-3865-8131; Fax: +55-21-2290-0479; E-mail: ects@ioc.fiocruz.br
†These authors contributed equally to this work.

Received 17 February 2011; returned 10 March 2011; revised 22 March 2011; accepted 24 March 2011

Objectives: This paper describes the antileishmanial properties of LQB-118, a new compound designed by molecular hybridization, orally active in Leishmania amazonensis-infected BALB/c mice.

Methods: In vitro antileishmanial activity was determined in L. amazonensis-infected macrophages. For in vivo studies, LQB-118 was administered intralesionally (15 μg/kg/day, five times a week), intraperitoneally (4.5 mg/kg/day, five times a week) or orally (4.5 mg/kg/day, five times a week) to L. amazonensis-infected BALB/c mice throughout experiments lasting 85 or 105 days. At the end of the experiments, serum levels of alanine aminotransferase, aspartate aminotransferase and creatinine were measured as toxicological parameters.

Results: LQB-118 was active against intracellular amastigotes of L. amazonensis [50% inhibitory concentration (IC50) 1.4 μM] and significantly less so against macrophages (IC50 18.5 μM). LQB-118 administered intralesionally, intraperitoneally or orally was found to control both lesion and parasite growth in L. amazonensis-infected BALB/c mice, without altering serological markers of toxicity.

Conclusions: These results demonstrate that the molecular hybridization of a naphthoquinone core to pterocarpan yielded a novel antileishmanial compound that was locally and orally active in an experimental cutaneous leishmaniasis model.

Keywords: pterocarpan, antiparasitics, chemotherapy

Introduction

Antimony-containing drugs remained the first-line treatments for leishmaniasis in most of the world for about 70 years. In spite of the development of miltefosine and of novel formulations of conventional drugs, little impact on disease incidence has been observed in endemic areas. This trend may be explained partially by the high cost of the new alternatives, which hampers their use in developing countries. Furthermore, there are some concerns about the teratogenicity of, and the induction of resistance to, miltefosine. Thus, the development of new, safer, cheaper and orally available drug treatments for leishmaniasis is urgently needed. Quinones are important compounds with activity in biological systems and several studies have investigated the use of this class of natural products against Leishmania spp. and Trypanosoma cruzi. However, the further development of this group of compounds has been hampered by their high toxicity and low bioavailability. Atovaquone, a quinone currently used to treat Pneumocystis carinii infections, has shown in vitro activity against Leishmania infantum. However, its activity in L. infantum-infected mice was unsatisfactory. On the other hand, our group have recently demonstrated the antileukaemic activity of pterocarpan derivatives, a class of isoflavonoids. This article presents the results of the molecular hybridization of a naphthoquinone core with a pterocarpan moiety, which yields a hybrid compound that possesses selective antileishmanial activity and is orally bioavailable in mice.
Materials and methods

Chemicals

LQB-118 was synthesized in the Laboratory of Bioorganic Chemistry, Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Brazil. The compound was prepared in two steps from commercially available lawsone and ortho-iodophenol, as previously described. Sodium stibogluconate (Pentostam) was a gift from GlaxoSmithKline and meglumine antimoniate (Glucantime, Sanofi-Aventis) was acquired commercially.

Parasites

Leishmania amazonensis (MHOM/BR/77/LTB0016) was maintained as promastigotes at 26°C in Schneider’s insect medium (Sigma-Aldrich, St Louis, MO, USA) with 10% serum, 100 μg/mL streptomycin and 100 U/mL penicillin. Parasites were maintained until the 10th passage; subsequently, new cultures were obtained from infected animals.

In vivo activity

BALB/c mice (6/group) were infected in the footpad with 2×10⁶ L. amazonensis promastigotes. The method of treatment was similar to that described by Mendez et al., beginning 72 h following infection. Subcutaneous treatment consisted of injections of 10 μL of either LQB-118 (15 μg/kg/day) or sodium stibogluconate (100 μg/kg/day), five times a week, until the end of the experiment (day 105), when the animals were euthanized. Negative controls were similarly treated, but with sterile PBS. Additional animals were treated with LQB-118 at 4.5 mg/kg/day, either orally or intraperitoneally, five times a week until the end of the experiment (day 85), when the animals were euthanized. Control animals were either treated with meglumine antimoniate at 17 mg Sb⁷⁺/kg/day at the same frequency as LQB-118, or were left untreated. Lesion sizes were measured using a dial calliper every 3–4 days. At the end of each experiment the mice were euthanized and their infected paws were excised, skinned, weighed and minced in Schneider’s medium (Sigma) containing 5% fetal calf serum. The resulting cell suspensions were serially diluted and evaluated by limiting dilution analysis after 7 days. This study was approved by the Animal Ethics Committee of Oswaldo Cruz Foundation (license number LW07/2010). Statistical analysis was performed by analysis of variance with a Bonferroni post test.

Results

Selective antileishmanial activity of LQB118

Several naphthopterocarpanquinone derivatives were evaluated for antipromastigote activity against L. amazonensis and LQB-118 (Figure 1a) was selected because it had the lowest IC₅₀ (1.73 μM) and the easiest synthesis. In the next step we determined whether LQB-118 was able to act against intracellular amastigotes without being toxic to host cells. LQB-118
Figure 2. LQB-118 is effective in vivo, whether locally or systemically delivered, without altering serological markers of toxicity. BALB/c mice were subcutaneously infected with $2 \times 10^6$ L. amazonensis promastigotes in the rear paws. Animals were treated by intralesional (15 μg/kg/day five times a week) (a) or intraperitoneal or oral delivery (4.5 mg/kg/day, five times a week) (b) of LQB-118. Controls were treated with pentavalent antimonials [sodium stibogluconate in (a) or meglumine antimoniate in (b)], saline or left untreated, as indicated. Lesion development was measured with a dial caliper twice a week. At the end of the experiment the mice were euthanized and the parasite burden was estimated by dilution analysis (inserts). (c) Mice were infected and treated as described in (b). At the end of treatment, serum samples were collected for colorimetric determination of ALT (left panel), AST (middle panel) and creatinine (right panel) concentrations, as parameters of toxicity for the liver and the kidneys. As a positive control, animals were given a single dose of 1% carbon tetrachloride (CCl4) in peanut oil by an intraperitoneal route. Mean±SD; n = 6. *P < 0.05, **P < 0.01 and ***P < 0.001. sc, subcutaneous/intralesional; ip, intraperitoneal; NS, not significant among groups.
produced a concentration-dependent reduction in parasite load, with an IC_{50} of 1.45 μM (Figure 1b). Furthermore, the viability of uninfected macrophages was unaffected by concentrations of up to 10 μM LQB-118. The IC_{50} in macrophages was found to be 18.46 μM, which is 12-fold higher than the IC_{50} obtained against intracellular amastigotes.

**In vivo activity**

Considering the results of the in vitro experiments, two different protocols were developed to evaluate the activity of LQB-118 in a murine cutaneous leishmaniasis model. The two protocols were used to assess whether the test compound possessed in vivo antileishmanial activity when administered locally (subcutaneously at the site of infection) and systemically (intraperitoneally or orally), respectively. When administered subcutaneously, LQB-118 was as effective as pentavalent antimonial in preventing lesion development (Figure 2a). LQB-118 also promoted a significant reduction in the parasite burden compared with the PBS group (Figure 2a, insert). LQB-118 retained antileishmanial activity when delivered systemically, even when administered orally (Figure 2b). Orally delivered LQB-118 was as effective as intraperitoneally administered antimonial. Moreover, LQB-118 was slightly less effective when delivered intraperitoneally. Despite these differences in lesion development, both oral and intraperitoneal treatments effectively reduced the parasite burden, as shown in Figure 2b (insert). Furthermore, no significant differences in serum levels of ALT, AST or creatinine were measured in treated and untreated animals (Figure 2c). To verify the possible induction of resistance by LQB-118, we evaluated the susceptibility of parasites recovered from the systemically treated mice to LQB-118. Lesion amastigotes were allowed to differentiate into promastigotes by incubation in Schneider’s medium containing 10% fetal bovine serum at 26°C for 7 days. Next, the promastigote susceptibility assay was performed as before. Despite the long duration of treatment, no significant differences in IC_{50} were observed (untreated control, 1.48 ± 0.35 μM; intraperitoneal delivery, 1.21 ± 0.05 μM; and oral delivery, 1.23 ± 0.03 μM).

**Discussion**

Recently, new compounds have been designed and synthesized by our group by molecular hybridization of naphthoquinones with pterocarpanones (naturally occurring isoflavonoids). These hybrid pterocarpanquinones show in vitro antineoplastic and antiparasitic activity. In this article, a new pterocarpanquinone (LQB-118; Figure 1a) with potent activity against *L. amazonensis* (Figure 1b) is described. We demonstrate that LQB-118 is toxic to macrophages only at concentrations 12-fold higher than that required for activity against the intracellular forms of *L. amazonensis*. Having demonstrated selective in vitro activity of LQB-118, we evaluated its effectiveness in controlling lesion development in *L. amazonensis*-infected BALB/c mice. Early antileishmanial therapy has been adopted to maximize the effect of experimental drugs. Given that pharmacokinetic data for LQB-118 are not yet available, a long course of treatment, starting 72 h post-infection, was adopted in this work. BALB/c mice are extremely susceptible to *L. amazonensis* infection, with progressive swelling at the inoculation site followed by ulceration and metastasis. We found that LQB-118 significantly diminished the development of these skin lesions when delivered either locally or systemically (Figure 2a and b). Treatment resulted in both a reduction in swelling and ulceration and an important reduction in parasite burden. As previously reported, not even pentavalent antimonials can achieve a sterile cure. Significantly, the remaining parasites recovered from the LQB-118-treated mice did not demonstrate resistance following treatment. Additionally, the major serological markers of toxicity, such as aminotransferases and creatinine, were not significantly altered by the long course of systemic treatment (Figure 2c).

**Acknowledgements**

We are grateful to PDTIS/FIOCRUZ (Bioensaios V) for the use of its facilities and to GlaxoSmithKline for a sample of Pentostam.

**Funding**

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; fellowship to E. F. C.-J.), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; fellowship to G. A. R.), the Programa de Apoio a Núcleos de Excelência of the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), the Financiadora de Estudos e Projetos, the Program of Oncobiology—Universidade Federal do Rio de Janeiro, and the Programa Estratégico de Apoio à Pesquisa em Saúde (PAPES/FIOCRUZ; grant 403605/2008-3 to E. C. T.-S.).

**Transparency declarations**

None to declare.

**References**


