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Research paper

An effective *in vitro* and *in vivo* antileishmanial activity and mechanism of action of 8-hydroxyquinoline against *Leishmania* species causing visceral and tegumentary leishmaniasis

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ABSTRACT

The development of new therapeutic strategies to treat leishmaniasis has become a priority. In the present study, the antileishmanial activity of 8-hydroxyquinoline (8-HQN) was investigated against *in vitro* promastigotes and *in vivo* intra-macrophage amastigotes of three *Leishmania* species: *Leishmania amazonensis*, *Leishmania infantum* and *Leishmania braziliensis*. Studies were performed to establish the 50% *Leishmania* inhibitory concentration (IC_{50}) of 8-HQN, as well as its 50% cytotoxic concentration (CC_{50}) on murine macrophages and in human red blood cells. The inhibition of macrophages infection was also evaluated using parasites that were pre-treated with 8-HQN. The effects of this compound on nitric oxide (NO) production and in the mitochondrial membrane potential were also evaluated. Finally, the therapeutic efficacy of 8-HQN was assessed in a known murine model, *L. amazonensis*-chronically infected BALB/c mice. Our results showed that 8-HQN was effective against promastigote and amastigote stages of all tested *Leishmania* species, presenting a selectivity index of 328.0, 62.0 and 47.0 for *L. amazonensis*, *L. infantum* and *L. braziliensis*, respectively. It was effective in treating infected macrophages, as well as in preventing the infection of these cells using pre-treated parasites. In addition, 8-HQN caused an alteration in the mitochondrial membrane potential of the parasites. When administered at 10 mg/kg body weight/day by subcutaneous route, this product was effective in reducing the lesion diameter, as well as the parasite load in evaluated tissues and organs of infected animals. The results showed the *in vitro* and *in vivo* efficacy of 8-HQN against three different *Leishmania* species causing tegumentary and/or visceral leishmaniasis, and it could well be used for future therapeutic optimization studies to treat leishmaniasis.

1. Introduction

Leishmaniasis presents a broad spectrum of clinical manifestations and is caused by different species of protozoa belonging to the genus *Leishmania* (Desjeux, 2004). There are more than 20 parasite species responsible for the disease in humans, and it is

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endemic in 98 countries throughout Africa, Asia, Southern Europe and Latin America (Petersen, 2009; Ready, 2010). There are approximately 0.7–1.2 million cases of tegumentary leishmaniasis (TL), and 0.2–0.4 million cases of visceral leishmaniasis (VL) being registered annually. Because of its remarkable impact on global public health, the disease is considered one of the six major tropical diseases in the world (WHO, 2010).

Chemotherapy has been historically used to treat leishmaniasis, have being based on the parenteral administration of pentavalent antimonials compounds (Sundar and Chakravarty, 2010). Although these drugs present high efficacy, the occurrence of side effects such as myalgia, arthralgia, anorexia, fever and urticarial; associated with significant toxicity to the liver, kidneys and spleen of the patients have contributed to limit their use in clinical practice (Marsden et al., 1985; Barral-Netto et al., 1995; Croft and Coombs, 2003). Other drugs, such as amphotericin B (AmpB) have been employed with an increasing frequency. However, their use is limited because their high toxicity, which can cause nephrotoxicity, cardiac alterations, hemolysis and liver damage, as well as nausea and fever in the patients (Annaloro et al., 2009; Ribeiro et al., 2014). The World Health Organization has recommended the use of lipid-based formulations of AmpB based on its higher efficacy and safety (Bern et al., 2006), however, the main limiting factor has been the high cost (WHO, 2010). Pentamidine is unsuitable as a first-line treatment due to its toxicity. Clinical results using oral miltefosine have been encouraging in India, but it has been associated to teratogenicity, and should not be prescribed to pregnant women or to those of childbearing age (Bhattacharya et al., 2004). Therefore, the development of new and cost-effective therapeutic strategies to treat leishmaniasis has become a high-priority (Frézard et al., 2009).

An interest for medicinal chemists is the pharmacological observation that certain molecules that present biological activities, as well as their derivatives, might be also useful as antiparasitic agents (Lage et al., 2013; Awaad et al., 2014; Rabito et al., 2014; Yousefi et al., 2014; Annang et al., 2015; Dagnino et al., 2015). 8-hydroxyquinoline (8-HQN) is prevalent in a wide variety of biologically active compounds, and it present great interest in the field of inorganic and bioinorganic chemistry. It is a metal-binding compound (Ji and Zhang, 2005), and is known to its capacity in presents a variety of biological effects, such as potent protease inhibitor, candidate for the treatment of HIV-infected patients, cancer, neurodegenerative diseases such as Alzheimer's and Parkinson's disease, as well as parasitic and bacterial infections (Lentz et al., 1999; Collery et al., 2000; Prachayasittikul et al., 2013). A few studies have shown that 8-HQN is able to inhibit the growth of promastigote forms in some *Leishmania* spp. (Dardari et al., 2004; Paloque et al., 2012; Coa et al., 2015); however, its antileishmanial activity has not been still evaluated against *in vivo* amastigote forms of the parasites, as well as in *in vivo* models of disease.

In this context, the present study aimed to evaluate the antileishmanial activity of 8-HQN against *in vitro* stationary promastigotes and *in vivo* intra-macrophage amastigotes of three different *Leishmania* species, namely, *Leishmania amazonensis, Leishmania braziliensis* and *Leishmania infantum*. Studies were extended in order to determinate the 50% inhibitory concentration (IC_{50}) of 8-HQN about the three species, as well as its 50% cytotoxic concentration (CC_{50}) on murine macrophages and in human red blood cells (RBC_{50}), as well as by calculating the selectivity index (SI) of 8-HQN in these species. Also, its efficacy in treating infected macrophages and inhibiting the infection of these phagocytic cells when parasites were pre-treated with 8-HQN, were evaluated. Aiming to evaluate an *in vivo* treatment model, the efficacy of this compound in treating *L. amazonensis*-chronically infected BALB/c mice was also evaluated.

2. Material and methods

2.1. Chemicals and general details

SYTOX Green, Rhodamine 123 and ROS H2DCf-DA were purchased from Molecular Probes (Invitrogen, Brazil). Dimethyl sulfoxide (DMSO) was obtained from Merck (Brazil). M-199 medium, Hank's balanced salts, phosphate-buffered saline (PBS) and others were obtained from Sigma (Brazil).

2.2. Mice

Female BALB/c mice (8 weeks age) were obtained from the breeding facilities of the Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais (UFMG), Minas Gerais, Brazil. The animals were maintained under specific pathogen-free conditions. The study was approved by Committee on the Ethical Handling of Research Animals from UFMG, under the protocol number 0136/2012.

2.3. Parasites

L. braziliensis (MHOM/BR/1975/M2903), *L. amazonensis* (IFLA/BR/1967/PH-8) and *L. infantum* (MHOM/BR/1970/BH46) strains were used in this study. Parasites were grown at 24 °C in Schneider's medium (Sigma, St. Louis, MO, USA), which was supplemented with 20% heat-inactivated fetal bovine serum (FBS, Sigma) and 20 mM L-glutamine, pH 7.4. Stationary-phase promastigotes of the parasites were prepared as described (Coelho et al., 2003).

2.4. Antileishmanial activity

The 50% inhibitory concentration of *Leishmania* growth (IC_{50}) was assessed *in vitro* by cultivating stationary promastigotes (1×10^6 cells) in the presence of 8-HQN (10.0–0.1 $\mu g\,mL^{-1}$) in 96-well culture plates (Nunc, Nunclon®, Roskilde, Denmark), for 48 h at 24 °C. AmpB (10.0–0.1 $\mu g\,mL^{-1}$) was used as a control. Cell viability was assessed by measuring the mitochondrial oxidative activity with MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide]. Absorbances were measured by using a multi-well scanning spectrophotometer (Molecular Devices, Spectra Max Plus, Canada), at a wavelength of 570 nm. The products concentration needed to inhibit 50% of *Leishmania* viability was determined by applying the sigmoidal regression of concentration-response curves, using the different tested concentrations.

2.5. Cytotoxicity evaluation and selectivity index

Peritoneal macrophages were obtained from female BALB/c mice. For this, animals received an injection with 3% sodium tyoglicolate and four days after the peritoneal cavity was washed with 8 mL sterile RPMI 1640 medium. Then, the cell suspension was adjusted to 1×10^6 macrophages per mL. The 50% inhibitory concentration of the macrophages viability was evaluated by incubating these cells (5×10^5) with 8-HQN (10.0–0.1 $\mu g\,mL^{-1}$) in 96-well plates, for 48 h at 37 °C. AmpB (10.0 $\mu g\,mL^{-1}$) was used as a control. Cell viability was assessed by measuring the mitochondrial oxidative activity with MTT, and absorbances were measured by using a multi-well scanning spectrophotometer, at a wavelength of 570 nm. In addition, the selectivity index (SI) was calculated by the ratio between the CC_{50} and IC_{50} values, respectively.