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In vitro evaluation of (–)α-bisabolol as a promising agent against *Leishmania amazonensis*

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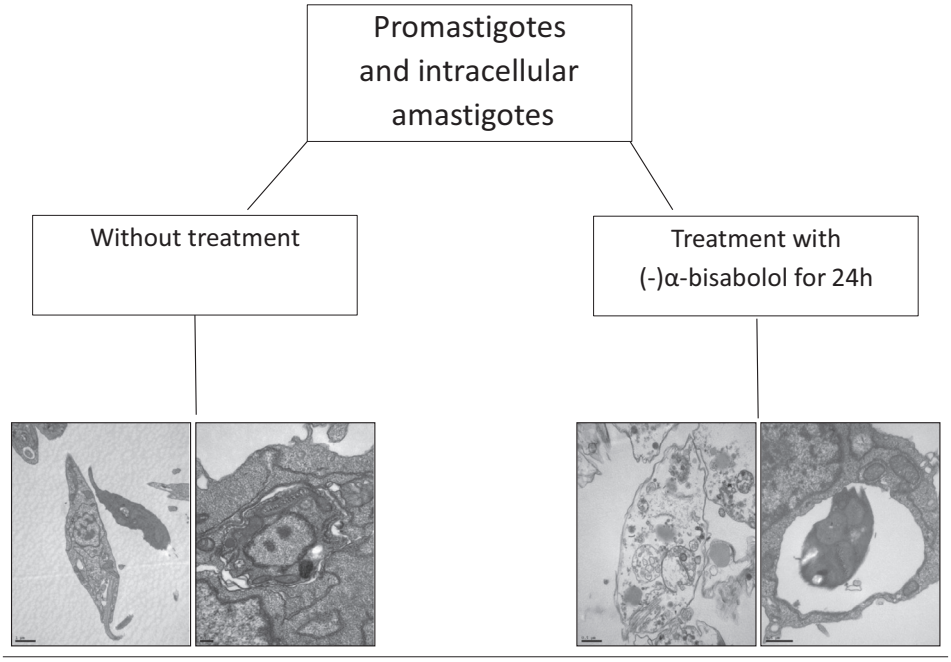
HIGHLIGHTS

- The (–)α-bisabolol is a sesquiterpene alcohol found in essential oils of plants.
- Antileishmanial activity of (–)α-bisabolol against *L. amazonensis* was evaluated.
- The (–)α-bisabolol showed cytotoxic effects *in vitro* against *L. amazonensis*.
- The (–)α-bisabolol at 8.07 μg/ml reduces in 50% the survival index of promastigotes.
- The (–)α-bisabolol at 4.29 μg/ml reduces in 50% the survival index of amastigotes.

GRAPHICAL ABSTRACT



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ABSTRACT

Current treatments for leishmaniasis present some difficulties due to their toxicity, the use of the intravenous route for administration and therapy duration, which may lead to treatment discontinuation. The aim of this study is to investigate new treatment alternatives to improve patients well being. Therefore, we evaluated the inhibitory effect of (–)α-bisabolol, a sesquiterpene alcohol found in various essential oils of different plant species, against the promastigotes and intracellular amastigotes forms of *Leishmania amazonensis*, as well as the cytotoxic, morphological and ultrastructural alterations of treated cells. Promastigotes forms of *L. amazonensis* were incubated with (–)α-bisabolol to determine the antileishmanial activity of this compound. The cytotoxicity effect was evaluated by testing against J774.G8 cells. After these tests, the infected and uninfected cells with *L. amazonensis* were used to determine if the (–)α-bisabolol was able to kill intracellular parasites and to cause some morphological changes in the cells. The (–)α-bisabolol compound showed significant antileishmanial activity against promastigotes with a 50% effective concentration of 8.07 μg/ml (24 h) and 4.26 μg/ml (48 h). Against intracellular amastigotes the IC_{50} (inhibitory concentration) of (–)α-bisabolol (24 h) was 4.15 μg/ml. The (–)α-bisabolol also showed a cytotoxic effect against the macrophage strain J774.G8. The value of 50% cytotoxic concentration was 14.82 μg/ml showing that (–)α-bisabolol is less toxic to macrophages than to the parasite. Ultrastructural studies of treated promastigotes and amastigotes showed several alterations, such as loss of cytoplasmic organelles, including the nucleus, and the presence of lipid inclusions. This study showed that (–)α-bisabolol has promising antileishmanial properties, as it can act against the promastigote forms and is able to penetrate the cell, and is also active against the amastigote forms. About 69% of the promastigotes forms suffered mitochondrial membrane damage after treatment with IC_{50} of (-)α-bisabolol, suggesting inhibition of the metabolic activity of parasites. These results open new prospects for research that can contribute to the development of products based on essential oils or isolated compounds from plants for the treatment of cutaneous leishmaniasis.

1. Introduction

Leishmaniasis is considered as an emerging or re-emerging disease. There has been an alarming increase in incidence, especially during the last two decades (Goto and Lindoso, 2010). It is caused by the obligate intracellular parasites belonging to the order Kinetoplastida (Honigberg, 1963 emend. Vickerman, 1976) and family Trypanosomatidae (Doflein, 1901, emend. Grobben, 1905), genus *Leishmania* (Ross, 1903) and is transmitted to vertebrate hosts by sand fly vectors.

The cutaneous form of leishmaniasis is the most common and represents 50–75% of new cases reported (World Health Organization, 1998). The other forms of the disease are mucosal and visceral leishmaniasis (Herwaldt, 1999).

The main drugs for the treatment of leishmaniasis are the pentavalent antimonials and the second-line drugs include pentamidine and amphotericin B (Gadelha et al., 1990; Monzote et al., 2007). Although treatment with pentavalent antimony is usually effective the disadvantages of this treatment, such as, parenteral administration, treatment duration, toxic effects, contraindications for heart and renal diseases, high costs and parasite resistance should be considered (Mayrink et al., 2006; Monzote et al., 2007). Side effects are also observed in pentamidine and amphotericin B treatment, so their administration must also be carefully monitored by specialized medical services.

Due to numerous side effects and difficulties in dealing with the main drugs, there is a growing interest in the search for new antileishmanial agents that have fewer side effects (Chan-Bacab and Peña-Rodriguez, 2001; Morales-Yuste et al., 2010). This has promoted research into natural products with activity against protozoa. Thus, plants and/or their compounds are being used to treat certain diseases, especially skin diseases like cutaneous leishmaniasis (Monzote et al., 2007).

The (–)α-bisabolol is a sesquiterpene alcohol found in various essential oils of different plants and because of its pleasant odor and pharmacological properties, it has been widely used in industry, in dermatology and cosmetic preparations (Gomes-Carneiro et al., 2005). Moreover, recent studies have demonstrated the leishmanicidal activity of (–)α-bisabolol against promastigote forms of *Leishmania infantum* (Morales-Yuste et al., 2010). Thus, this study aims to evaluate the antileishmanial activity of (–)α-bisabolol against promastigotes and the intracellular amastigote stage of *L. amazonensis*, which causes cutaneous leishmaniasis.

2. Materials and methods

2.1. Parasites

The parasite strain used was MHOM/BR/76/Ma-76 *Leishmania amazonensis* isolated from a patient with diffuse cutaneous leishmaniasis, maintained by serial passages in BALB/c mice and periodically reisolated in culture. The strain was characterized by the isoenzyme technique and lecithin (Schottelius and Gonçalves da Costa, 1982). All experiments with animals were conducted in accordance with the guidelines for experimental procedures of Oswaldo Cruz Foundation (Licence n° L.0001/07).

2.2. Sesquiterpene

The purity of (–)α-bisabolol (97%, Carl Roth, Karlsruhe, Germany) was determined by GC-FID and MS (conditions below). (–)α-bisabolol was diluted in dimethyl sulfoxide (DMSO) and medium for the assays, as described below. Gas chromatographic analysis was performed using an Agilent 6890 gas chromatograph (GC) (Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a DB-5 (5% phenyl/95% dimethylpolysiloxane) fused silica capillary column (25 m × 0.32 mm × 0.25 μm) and hydrogen was the carrier gas (1.0 ml min^{-1}). The injector temperature was kept at 250 °C and the oven temperature programmed from 70 to 280 °C at a rate of 10 °C min^{-1}. Detector (FID) was operated at 280 °C. The identity of the (–)α-bisabolol was also verified by GC-MS (5973 Agilent), using helium as the carrier gas and the same conditions as above. One microliter of a 1% solution of the (–)α-bisabolol in dichloromethane was injected in the splitless mode.