Experimental Parasitology 129 (2011) 145–151

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Leishmania amazonensis: Effects of oral treatment with copaiba oil in mice

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ARTICLE INFO

Article history:
Received 20 April 2011
Received in revised form 20 June 2011
Accepted 28 June 2011
Available online 13 July 2011

Keywords:
Leishmania amazonensis
Copaifera martii
Antileishmanial activity
Experimental treatment

ABSTRACT

Leishmaniasis is a severe public-health problem, with high rates of morbidity and mortality. Efforts to find new, effective and safe oral agents for the treatment of leishmaniasis have been ongoing for several decades, in order to avoid the problems with the currently used antimonials. In the present study, we found that a copaiba oil oral treatment (Group IV) caused a significant reduction in the average lesion size (1.1 ± 0.4 mm) against *Leishmania amazonensis* lesions compared with untreated mice (Group I) (4.4 ± 1.3 mm). To prove the safety of the oil, the toxicity and genotoxicity were also determined. Histopathological evaluation did not reveal changes in the copaiba oil-treated animals compared to the control animals. In the mutagenicity evaluation, (micronucleus test) the dose tested (2000 mg/kg) showed no genotoxic effects. Morphological and ultrastructural analyses demonstrated notable changes in parasite cells treated with this oleoresin. The main ultrastructural effect was mitochondrial swelling. We also demonstrated that in vitro copaiba oil treatment of *L. amazonensis* led to an increase in plasma membrane permeability, and depolarization in the mitochondrial membrane potential in parasite cells. Although the mechanism of action of the oleoresin is still unclear, these findings indicate that copaiba oil is a possible new drug, which would provide a safer, shorter, less-expensive, and more easily administered treatment for leishmaniasis.

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1. Introduction

Leishmania is the protozoan parasite responsible for several pathologies known collectively as leishmaniasis (McConville and Handman, 2007). The worldwide prevalence is 12 million cases, and the estimated population at risk is about 350 million in 88 countries on four continents. Currently, the estimated global annual incidence of new cases is 2 million (Alvar et al., 2006). However, it is clear that official data frequently grossly underestimate the reality, because leishmaniasis is not a notifiable disease in all the countries where it is endemic. Therefore, a substantial number of cases are never recorded (Bustamante et al., 2009).

The clinical manifestations of this infection depend on the species of *Leishmania* and the immunological status of the host. The disease can be classified as cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis, also known as Kala-Azar (Clem, 2010). *Leishmania amazonensis* is a species that causes cutaneous leishmaniasis (CL), which ranges from small cutaneous nodules to gross mucosal tissue destruction (Reithinger et al., 2007).

The pentavalent antimonial compounds have been used since 1940 to treat visceral and cutaneous leishmaniasis. Although new drugs or drug formulations such as liposomal amphotericin B (AmBisome), miltefosine, and paromomycin should be available for treatment of leishmaniasis, they all have limitations of cost, specific toxicities, or the need for parenteral administration (Lee and Hasbun, 2003; Croft, 2008; Dujardin et al., 2010). Consequently there is an urgent need to discover new drugs effective against leishmaniasis.

The current use of herbal therapy in *Leishmania*-endemic regions has renewed interest in evaluation of plant remedies used in traditional medicine as sources of potential antileishmanials (Iwu et al., 1994; Gachet et al., 2010; Tiuman et al., 2011). Interestingly, the use of copaiba oils to treat leishmaniasis has been cited in several ethnopharmacological studies with data obtained from western Amazonia, in Peru (Kvist et al., 2006), eastern Amazonia, in the state of Maranhão, Brazil (Moreira et al., 2002), and northern Amazonia, in French Guiana (Fleury, 1997; Grenand and Moretti, 1987). Recently, Santos et al. (2008) reported that copaiba oils from different species of *Copaifera* show activity against promastigote forms of *L. amazonensis*. These results led us to investigate the

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0014-4894/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2011.06.016

in vivo antileishmanial activity of copaiba oil from *Copaifera martii*, together with in vitro studies by electron microscopy, biochemical analysis, and flow cytometry to determine the targets of copaiba oil in *L. amazonensis*.

2. Materials and methods

2.1. Copaiba oil

The copaiba oil was collected from the trunk of *Copaifera martii* tree at Tapará, Pará (DC 349), and the sample was deposited in the Herbarium Chico Mendes (Maricá, Rio de Janeiro). The chemical characterization was performed by high-resolution gas chromatography (HRGC) analyses with a Hewlett–Packard (HP) model 5890 instrument equipped with a flame ionization detector as published in Santos et al. (2008).

2.2. Preparation of copaiba oil formulations

The available formulations were prepared as follows: The topical cream (TE) was prepared using glycerin monostearate 6% (w/w), stearic acid 2% (w/w), beeswax 1.5% (w/w), cetiol 11% (w/w), ethoxylated lanolin 1% (w/w), triethanolamine 1% (w/w), methyl paraben 0.18% (w/w), propyl paraben 0.2% (w/w) and distilled water 50% (w/w). Copaiba oil was added in tween 80 at the proportions of 1:1 and mixed until to uniformity. So, this mixture was added in the

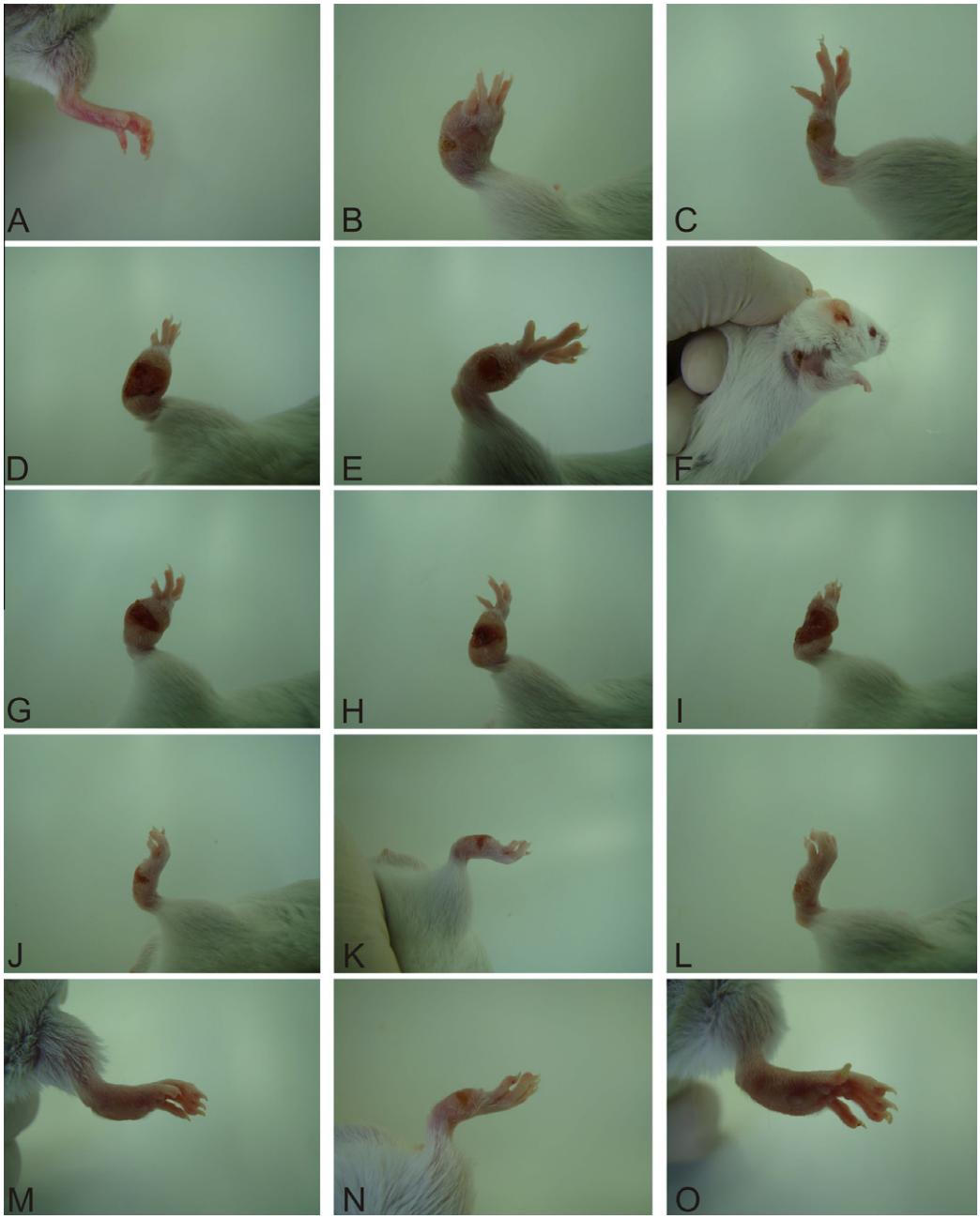

Fig. 1. Evaluation of cutaneous leishmaniasis development in mice treated with copaiba oil. (A) Group VII: Uninfected and untreated control; (B) Group I: infected and untreated control; (C) Group II: The reference drug Glucantime® (100 mg/kg/day) administered through intramuscular injection; (D–F) Group III: copaiba oil (100 mg/kg/day) by subcutaneous route; (G–I) Group V: Lesion treated topically with copaiba oil cream at a concentration of 4%, applied on the lesions in an amount of 1 mg/mm^2; (J–L) Group IV: copaiba oil emulsion at a dose of 100 mg/kg/day was administered orally by gavage. (M–O) Group VI: The animals received oral treatment by gavage (100 mg/kg/day) and also topical treatment with 4% copaiba oil cream applied on the lesions in an amount of 1 mg/mm^2.

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