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Activity of a paromomycin hydrophilic formulation for topical treatment of infections by *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania* (*Viannia*) *braziliensis*

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Abstract

Studies on in vitro skin permeation and in vivo anti-leishmanial activity in mice experimentally infected with *Leishmania* (*Leishmania*) *major* pointed out to the potential of a new paromomycin (PA) formulation (hydrophilic gel) for treatment of cutaneous leishmaniasis (CL). In this study, the activity of this formulation was evaluated in animals experimentally infected by *Leishmania* species that prevail in the New World. PA gel activity was compared to antimony treatment, since it is still the first choice treatment to the different clinical forms of leishmaniasis. The topical treatment activity with 10% PA gel in BALB/c mice infected by *Leishmania* (*Leishmania*) *amazonensis* was higher than that observed for parenteral antimony treatment, while the efficacy of these two regimes in hamsters infected by *Leishmania* (*Viannia*) *braziliensis* was similar. These results suggest that this formulation could be suitable for clinical studies and may represent an alternative novel formulation for topical treatment of CL.

Keywords: Cutaneous leishmaniasis; American tegumentary leishmaniasis; Topical treatment; Paromomycin; Hydrophilic formulation; Leishmaniasis

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1. Introduction

The pentavalent antimony (Sb) organic compounds, sodium stibogluconate (Pentostam™) and meglumine antimoniate (Glucantime™) developed in the 1940s, remains as the first choice treatment for both visceral and cutaneous leishmaniasis (CL) in most countries where the disease is prevalent. They have long been highly effective, presenting an efficacy around 90% (Grevelink and Lerner, 1996; Wortmann et al., 2002), but there is a growing evidence of variable efficacy, depending on species, geographic region, presence of resistant strains and therapeutic scheme (Romero et al., 1996, 2001a,b; Moreira et al., 1998; Sundar, 2001; Croft and Coombs, 2003). Moreover, several factors limit these drugs' usefulness, such as the need for parenteral administration for up to 30 days, the high frequency of side-effects with myalgia, arthralgia, anorexia, fever and urticaria, in addition to liver, kidney and spleen toxicity, revealed by abnormal laboratory tests (Marsden, 1985; Barral-Netto et al., 1995).

Cutaneous leishmaniasis (CL) in the America are caused mainly by *Leishmania* (*Viannia*) *braziliensis*, *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania* (*Viannia*) *guyanensis* and produces skin ulcers on the exposed parts of the body, such as the face, arms and legs. The lesions vary from mild to severe and may cause serious disability, leaving the patient permanently scarred. Additionally, cutaneous disease caused by *Viannia* subgenus may progress to mucosal involvement. Both cutaneous and mucocutaneous leishmaniasis require treatment.

In the last decades, much emphasis has been given to the development of alternative therapeutical approaches, including the identification of formulations for topical treatment of CL (Arana et al., 2001; Soto et al., 2002; Berman, 2003). This scheme may be interesting in those cases of non-disseminated disease, offering several advantages in comparison to conventional treatment: easy administration, lower adverse reaction incidence and an attractive cost–benefit ratio, mainly if one consider that CL occurs, many times, in areas of scarce medical assistance.

Topical formulations of paramomycin (an aminoglycoside antibiotic) at 15% associated to 12% methylbenzethonium chloride (MBCL), in a hydrophobic base (petrolatum ointment), have been evaluated presenting favorable results, especially against infections by *Leishmania* (*Leishmania*) *major* (El-On et al., 1988). Recently, this ointment showed a healing rate of 91.4% in a study performed in Guatemala in patients presumably infected by *L.* (*V.*) *braziliensis* and *Leishmania* (*Leishmania*) *mexicana* (Arana et al., 2001). Nevertheless, the efficacy of this formulation in infections by New World *Leishmania* species is not clear enough (El-On et al., 1984; Neal et al., 1995; Berman, 1997; Soto et al., 1998). Neal et al. (1995) reported that *Leishmania* species differ regarding to their susceptibility to PA and the New World species *L.* (*L.*) *amazonensis* and *L.* (*V.*) *braziliensis* are the least sensitive ones. Moreover, MBCL is toxic and irritant, causing ardency and burning sensations that restrict its use in many cases (Soto et al., 1998).

The activity of formulations containing only PA is usually low, promoting incomplete healing of cutaneous lesions in animals experimentally infected with *L.* (*L.*) *amazonensis* or *L.* (*L.*) *major* (Neal et al., 1995; El-On et al., 1984; Grogl et al., 1999). This limitation can be ascribed to the low cutaneous penetration of the drug. It was recently shown that PA cutaneous permeation, assayed in vitro on hairless mouse skin, in absence of stratum corneum, was about 20 times higher using hydrophilic formulations (cream and gel) in comparison to petrolatum ointment (Castro et al., 2003). In this study, stratum corneum was removed in order to simulate a typical lesion caused by *Leishmania* in that the lesion development leads to the loss of the main barrier to skin penetration of the drug. The anti-leishmanial activity of the formulation, evaluated in skin lesions in BALB/c mice experimentally infected by *L.* (*L.*) *major,* was similar to that of MBCL ointment (Fernandes et al., 2001; Ferreira et al., 2002).

The objective of the present study was to evaluate the activity of this new formulation in experimentally infected animals with strains, which are prevalent in the New World, *L.* (*V.*) *braziliensis* and *L.* (*L.*) *amazonensis*.

2. Material and methods

2.1. Preparation of formulations

The gel of PA was prepared by heating 1.5% hydroxiethylcellulose in water to 60–70 °C, under constant agitation, until a homogeneous and transparent gel was obtained. After cooling, PA was incorporated