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Amiodarone and itraconazole improve the activity of pentavalent antimonial in the treatment of experimental cutaneous leishmaniasis

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ABSTRACT

Leishmaniasis affect millions of people, causing morbidity and mortality, especially in developing tropical and subtropical countries. Unfortunately, the possibilities of treatment for these infections are still quite limited and most of the available drugs present serious side effects. The objective of this paper was to evaluate the therapeutic role of amiodarone and itraconazole in the treatment of cutaneous leishmaniasis caused by *Leishmania (Leishmania) amazonensis*. In order to perform this evaluation, hamsters were infected with 1×10^6 metaciclic promastigotes of the parasite in the hind footpad and, after the onset of the lesions, were treated with glucantime, amiodarone, itraconazole, glucantime and amiodarone, glucantime and itraconazole or amiodarone and itraconazole. The treatments' efficacy was evaluated per analysis of the size of the cutaneous lesions and by parasitic investigation of the infected foot (by histopathological examination and PCR) and possible side effects were analyzed taking into account the weight of the animals and some biochemical and metabolic parameters (glucose, urea, creatinine, AST, ALT and ALP). The results have shown that, in hamsters, amiodarone and itraconazole, either used isolated or in combination, are unable to stop the development of cutaneous lesions caused by *L. (L.) amazonensis*, but improve the activity of glucantime in the treatment of these lesions and seem to present no evident side effects. More studies are necessary in order to investigate the clinical potential of these combinations, so there can be the possibility of broadening the therapeutic options available, especially in resistant cases.

1. Introduction

Leishmaniasis are caused by many different protozoans' species from the Trypanosomatidae family and *Leishmania* genus, affecting men and different animals. This parasitosis is endemic in at least 98 countries in the entire world and more than two million new cases occur, each year, with high morbidity and mortality levels [1]. Among other factors, depending on the infecting species and the immune response of the host, the disease presents different clinical manifestations, such as: (a) the local cutaneous, characterized by the presence of lesions exclusively in the site of the vector insect bite; (b) the mucocutaneous, with destructive lesions in the upper respiratory tract; (c) the diffuse cutaneous, with multiple non ulcerated nodules, classically unresponsive to available treatments; (d) and the visceral, a systemic form affecting especially lymphatic nodes, the spleen, the liver and the bone marrow [2].

Leishmania (Leishmania) amazonensis is found and widely distributed in the Amazon rainforest region of Brazil, and has been reported as expanding to the Northeast, the Southeast and the Center West of the country, east of Paraguay and other Amazon areas of Bolivia, Peru Ecuador, Colombia and Venezuela [3,4]. Commonly, the

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species of *L. (L.) amazonensis* induce localized lesions, and nevertheless may also cause the diffuse cutaneous and even mucocutaneous and visceral forms of the disease [5].

Conventional therapy for all clinical forms of leishmaniasis include the pentavalent antimonials' (Sb^{+5}) use, such as antimonate of N-methylglucamine (Glucantime®) and sodium stibogluconate (Pentostam®) [6]. However, these drugs demand long term parenteral administration, present serious toxic side effects, especially on cardiac, renal and hepatic systems [7] and have shown gradually decreasing efficacy due to the appearance of resistance in many countries [8]. Amphotericin B, pentamidine and paromomycin are alternative drugs, also administered by parenteral via, that can present a variety of cure rates and many and frequent side effects [9,10]. Miltefosine, on the other hand, was introduced in India, in 2002, and represented a major advance in the treatment of visceral leishmaniasis, being the first drug possible to be orally administered, but the relatively high cost and the concerns related to teratogenicity and the potent development of resistance have limited its use [11]. Therefore, alternative treatments are intensely searched in order to achieve better results, with less side effects and higher patient adherence.

Amiodarone, an antiarrhythmic drug class III, commonly used to treat cardiopathies, including arrhythmias in the chronic phase of Chagas disease, have been subjected to recent studies investigating its use as antimycotic agent and anti trypanosomatid, since this drug has excellent pharmacokinetics properties and has relatively low cost [12,13]. It has been shown that amiodarone has direct activity against *Trypanosoma cruzi* and *Leishmania (Leishmania) mexicana*, affecting the viability of the parasitic forms in vitro experiments and reducing the parasitemia and the development of lesions, respectively, in mice infected and treated with this drug [14,15]. Amiodarone acts in the parasite in many different ways: (a) blocking the biosynthesis of membrane steroids; (b) promoting alterations in the potential of the mitochondrial membrane; (c) breaking the homeostasis of Ca^{2+} and inducing a rapid increase in the cytoplasmic calcium; (d) increasing the production of oxygen reactive intermediates. Even more than that, the efficacy of amiodarone in the infection caused by *Leishmania* can also be favored by its lipid nature (highly soluble), extense tissue distribution and important excretion through the skin, factors that assure distribution to infection sites [16].

Other drugs orally administered, especially azoles—such as ketoconazole, fluconazole and itraconazole—are also being applied experimentally in the treatment of leishmaniasis, especially cutaneous leishmaniasis. Taking into account that these antifungals inhibit the biosynthesis of ergosterol (the major sterol present in the plasmatic membrane of some microorganisms, including trypanosomatids) the use of these drugs in the treatment of infections caused by *Leishmania* is coherent by biochemical standards [17,18]. However, clinical essays with azoles are still limited and results obtained are ambiguous [19,20].

Currently, there are great expectations regarding the therapy with a combination of drugs designed to reduce dosages and length of the treatment, therefore improving tolerance and conformity of drugs already available [21]. Thus, the objective of this paper was to evaluate the therapeutic role of amiodarone and itraconazole in the treatment of experimental cutaneous leishmaniasis caused by *L. (L.) amazonensis*, in order to generate new perspective in the therapeutic approach of this important parasitic endemic disease.

2. Materials and methods

This study (Permit Number: 181/2011) was approved by the Ethics Committee for Animal Experimentation of the Universidade Federal do Triângulo Mineiro (Brazil). All animals received humane care in compliance with the 'Principles of laboratory animal care' formulated by the National Society for Medical Research and the 'Guide for the care and use of laboratory animals' prepared by the National Academy of Sciences (Washington, DC).

2.1. Parasites

The strain of *L. (L.) amazonensis* IFLA/BR/67/PH8 (isolated from phlebotomine *Lutzomyia flaviscutellata,* in Belém, Pará, Brazil, 1976) was kindly donated by Prof. Dr. Maria Norma Melo, of the Parasitology Department of the Instituto de Ciências Biológicas of the Universidade Federal de Minas Gerais (UFMG) and was kept in the laboratory for successive inoculations in hamsters and cultured at 26 °C in alfa-MEM medium (*Minimum Essential Medium*) (Gibco®), supplemented with 10% of fetal bovine serum (SFB) and 100 μg/ml gentamicin.

2.2. Drugs

Glucantime® (N-metilglucamine antimonate) was supplied by the Regional Health Management of Uberaba (Lot: 9E1030), amiodarone ($C_{25}H_{30}ClI_2NO_3$) was bought from Sigma-Aldrich and itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) was bought from Janssen-Cilag.

2.3. Experimental animal infection

A total of 105 Syrian hamsters (*Mesocricetus auratus*) were used, not isogenic, males aged between six and eight weeks, obtained from the biotery of the Laboratory of the Parasitology discipline of the Universidade Federal do Triângulo Mineiro (UFTM) and kept in the same place, at a controlled temperature of 23°C ± 1°C, light-dark cycle with food and water *ad libitum*.

Hamsters were inoculated by intradermal injection at the right hind footpad, with 100 μl phosphate-buffered saline (PBS) containing 1×10^6 *L. (L.) amazonensis* metacyclic promastigote from culture in alpha-MEM medium and quantified in Neubauer chamber (in triplicate).

2.4. Treatments

Hamsters were randomly divided in seven experimental groups (15 animals/group) and 20 days after inoculation, when infection was already well established and lesions were present, treatments were started. Each group was treated daily with the drugs (one dose per day and at the same time schedule) for 20 consecutive days (TRAT 1), as follows: GLUC Group - 100 mg Sb^{+5}/kg/day glucantime; AMIO Group - 50 mg/kg/day amiodarone; ITRA Group - 50 mg/kg/day itraconazole; GLUC + AMIO Group - 100 mg Sb^{+5}/kg/day glucantime and 50 mg/kg/day amiodarone; GLUC + ITRA Group - 100 mg Sb^{+5}/kg/day glucantime and 50 mg/kg/day itraconazole; AMIO + ITRA Group - 50 mg/kg/day amiodarone and 50 mg/kg/day itraconazole; CONTR Group (control group)—sodium chloride 0.9% (intraperitoneal via) and distilled water (oral via). After a 10 day interval, treatments were repeated in the same fashion for another 20 consecutive days (TRAT 2).

Glucantime was administered by intraperitoneal via and amiodarone (diluted in distilled water heated to 80 °C, in water bath) and itraconazole (diluted in distilled water and submitted to ultrasonic bath until the achievement of homogenous suspension) were orally administered by gavage.

The dosages of the drugs used were chosen according to published articles, involving animal experimentation [16,22].