Case report/Relato de Caso

American cutaneous leishmaniasis caused by *Leishmania (Viannia) braziliensis* resistant to meglumine antimoniate, but with good response to pentamidine: a case report

Leishmaniose cutânea americana causada pela *Leishmania (Viannia) braziliensis* resistente ao antimoniato de meglumina e com boa resposta terapêutica à pentamidina: relato de um caso

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RESUMO

Relatamos um caso de um militar brasileiro com leishmaniose cutânea, cuja lesão reativou após dois tratamentos sistêmicos com antimoniato de meglumina. Foi tratado com anfotericina B, mas precisou interromper por intolerância à medicação. Após isolamento de *Leishmania sp*, seis infiltrações intralesionais de antimoniato de meglumina foram realizadas, sem resposta. Promastigotas de *Leishmania sp.* foram novamente isoladas. Foi submetido a tratamento intramuscular com pentamidina (4mg/kg). Parasitas da primeira e segunda biópsias foram identificados como *Leishmania (Viannia) braziliensis*; os da primeira biópsia eram mais sensíveis ao antimoniato de meglumina *in vitro* do que os da segunda biópsia. A lesão não reativou.

Palavras-chaves: Leishmaniose cutânea. Terapêutica. Resistência medicamentosa.

ABSTRACT

This is a case report of a Brazilian soldier with cutaneous leishmaniasis. The lesion relapsed following two systemic treatments with meglumine antimoniate. The patient was treated with amphotericin B, which was interrupted due to poor tolerance. Following isolation of *Leishmania sp.*, six intralesional infiltrations of meglumine antimoniate resulted in no response. *Leishmania sp* promastigotes were again isolated. The patient was submitted to intramuscular 4mg/kg pentamidine. Parasites from the first and second biopsies were identified as *Leishmania (Viannia) braziliensis*; those isolated from the first biopsy were more sensitive to meglumine antimoniate in vitro than those isolated from the second biopsy. No relapse was observed.

Keywords: Cutaneous leishmaniasis. Therapeutics. Drug resistance.

INTRODUCTION

American tegumentary leishmaniasis (ATL) is a disease caused by protozoan parasites of the genus *Leishmania*. Several wild and domestic animals serve as reservoirs of the parasite and humans are the secondary host. Transmission occurs through the bite of different species of phlebotomine sand flies, which vary according to geographical region. The incubation period ranges from a few days to several months. The disease often manifests as a papule that evolves into a nodule and frequently ulcerates with a distinctive infiltrated border. A primary lesion is usually solitary, but more than one lesion might be observed[1].

Leishmania (Viannia) braziliensis is widely distributed in Brazil and is mainly responsible for cutaneous leishmaniasis (CL) and for occasional mucosal or mucocutaneous presentations. The last two forms are associated with significant morbidity[1].

Pentavalent antimonials ($Sb5^+$) are considered to be the first choice drug. In Brazil, a daily dose of 10 to 20mg/kg $Sb5^+$ administered for 20 days is recommended for CL. If no remission is observed, a second treatment is administered for 30 days. In the absence of a therapeutic response, the second drug of choice (amphotericin B) is used. The third drug of choice is pentamidine[1]. All of these medications are parenterally administered and may result in mild to severe side effects. Pentavalent antimonials can cause hyperamylasemia, ECG abnormalities, bone marrow suppression and hepatotoxicity, as well as constitutional symptoms, such as myalgia, arthralgia, headache, fever, nausea, vomiting, and pain at the site of drug application, when administered intramuscularly[1,2]. Amphotericin B may result in anemia, cardiac and nephrotoxic effects, hypokalemia, and constitutional side effects, such as nausea, vomiting, phlebitis, shivering and fever[1], which sometimes require the interruption of treatment. Pentamidine is known for its cardiac toxicity, nephrotoxicity, hypotension, hypoglycemia, but the major concern is the possibility of development of diabetes mellitus[1].

A case of CL caused by *Leishmania (Viannia) braziliensis* is reported in a Brazilian soldier, who probably acquired the disease in Ecuador during a military mission. The lesion was unresponsive to two complete courses of pentavalent antimonials and to six intralesional applications of the drug. The patient did not tolerate two different forms of amphotericin B (deoxycholate and liposomal), which caused constitutional symptoms. The lesion finally receded after treatment with 2.4g of pentamidine with minimal side effects.

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CASE REPORT

A 35 year-old white male military officer, weighing 77kg and living in Rio de Janeiro, Brazil, had been in Ecuador between May 2005 and June 2006 on a military mission. In March 2006, a furunculoid lesion developed on his right arm, which was treated with antibiotics. The lesion increased in diameter and ulcerated and a diagnosis of CL was made (imprint positive for the parasite). The patient was treated with pentavalent antimonial (meglumine antimoniate 10mL/day for 30 days, approximately 10mg/kg $Sb5^+$ per day), which resulted in apparent resolution of the lesion. Twenty days later, the lesion ulcerated again. Back in Brazil, the patient was submitted to a second treatment with meglumine antimoniate at the same dose for 30 days, with apparent healing of the lesion. Twenty days later, the lesion ulcerated again.

Next, the patient received 25mg of amphotericin B deoxycholate, but he did not tolerate the drug, due to high fever, malaise, headache and chills. New treatment with liposomal amphotericin B was initiated, but as the cumulative dose increased, the patient did not tolerate this drug either, again due to moderate fever, chills, myalgia, tachypnea, tachycardia, nausea and hypotension during infusion. In an attempt to minimize the adverse effects, the duration of infusion was increased to 4h and 100mg of hydrocortisone was administered during infusion and oral dipyrone was used every 6h; however, with the increasing cumulative dose, the adverse effects became more frequent and more severe, leading to suspension of the drug. A total dose of 0.775g of amphotericin B was administered over a 16-day period.

In August 2006, the patient still presented an ulcerated lesion on his right arm (**Figure 1**), but was otherwise in general good health. An immunoenzymatic assay for *Leishmania* was positive and the cutaneous Montenegro reaction was 12mm. A new skin biopsy was obtained and *Leishmania sp.* promastigotes were isolated by culture in NNN medium. Six attempts at intralesional injections of meglumine antimoniate at 2-week intervals were then performed, but the patient developed contact eczema throughout the lesion and the surrounding skin area following the final application. The lesion was still ulcerated and crusted (**Figure 2**). A new biopsy was obtained and *Leishmania sp.* promastigotes were again isolated. In March 2007, the patient was treated with intramuscular pentamidine isethionate at a daily dose of 4mg/kg on alternate days, receiving a cumulative dose of 2.4g over a 23-day treatment, with a brief interruption of one week due to pain and hemorrhaging at the site of injection.

Both isolates obtained during treatment with pentavalent antimonials were characterized as *Leishmania (V.) braziliensis* by isoenzyme electrophoresis[3]. In parallel, promastigote forms in the late log phase of growth were tested *in vitro* for sensitivity to meglumine antimoniate (Aventis-Pharma, São Paulo)[4]. The results showed a relevant difference between isolates, with the first isolate presenting an inhibitory concentration for 50% (IC_{50}) of 0.26 $\pm$ 0mg/mL and the second presenting an IC_{50} of 2.10 $\pm$ 0.16mg/mL.

No laboratory alterations were detected during and after treatment with pentamidine isethionate. The patient was negative for HIV 1 and 2 and HTLV-1. The lesion remained healed after a 34-month follow-up (**Figure 3**).

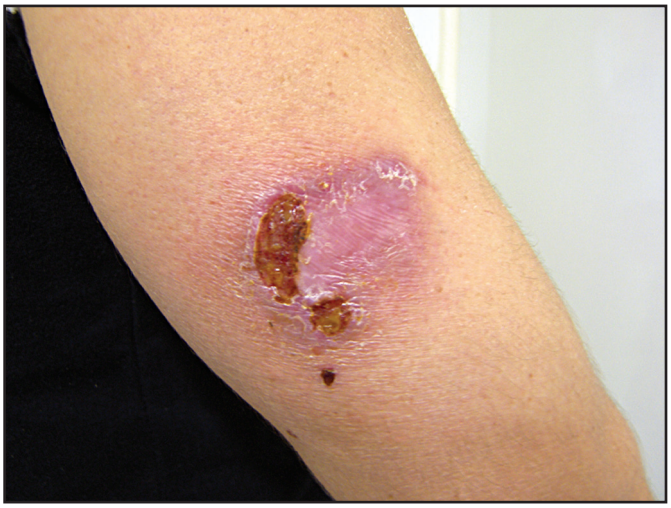

FIGURE 2 - Clinical aspect when the second biopsy was performed, 2007 February. Ulcerated lesion and purulent crusts in the periphery of the atrophic scar on the right arm.

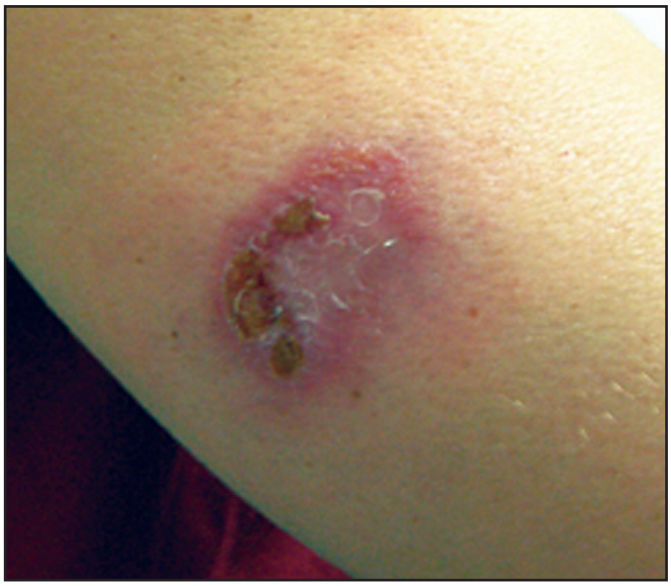

FIGURE 1 - Clinical aspect when the first biopsy was performed, 2006 August. Ulcerated lesion on the right arm, with purulent crusts.

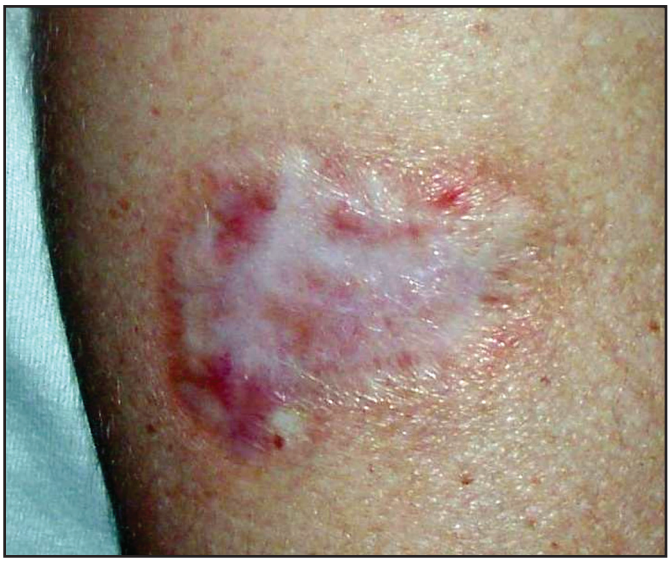

FIGURE 3 - Atrophic scar 34 months after treatment with pentamidine.

DISCUSSION

Cutaneous leishmaniasis is a common condition among soldiers involved in military campaigns in endemic areas. Military excursions to the Amazon region play a relevant role in the local incidence of the disease[5].

Success rates reported in the literature for the recommended doses of pentavalent antimonials vary widely. Many factors may influence the outcome of treatment, including drug subdoses and irregular treatment[6], and the immune status of the host, with more common failures in HIV-positive patients[7].

Several mechanisms have been suggested to be involved in the drug resistance of parasites. It is known that species causing leishmaniasis respond differently to treatment with pentavalent antimonials. Furthermore, some *Leishmania* populations may develop resistance to these drugs, probably as a result of natural clone selection[8]. *In vitro* sensitivity testing of the parasites isolated after the two intramuscular treatments and after the six intralesional injections of antimonials revealed differences between the isolates. According to Azeredo-Coutinho et al[9], *L. (V.) braziliensis* strains presented IC_{50} values varying from 0.8 to 9.5mg/mL and the strains isolated from patients poorly responsive to therapy showed significantly higher IC_{50} values than those isolated from patients who were cured after completion of the first antimonial treatment[9]. In the present study, the parasites isolated from the first biopsy were more sensitive to antimonials (0.26mg/mL) than those isolated after intralesional treatment (2.10mg/mL), a finding suggesting the development of resistance to the drug after successive cycles of treatment. This resistance was confirmed both *in vitro* and *in vivo*. These results suggest that even the appropriate therapeutic regimen can induce parasite resistance.

Amphotericin B has been recognized as an effective drug for the treatment of leishmaniasis, but is sometimes poorly tolerated. In Brazil, amphotericin B is the second drug of choice for the treatment of CL in the case of failure or contraindication to pentavalent antimonials[1]. Liposomal amphotericin B is associated with a lower frequency of side effects, but its high cost and the need for intravenous administration limit its use. The present patient did not tolerate either of the two forms of amphotericin B due to constitutional symptoms, although the total dose of the drug applied was 0.775g, approximately half the dose indicated for the treatment of CL[1].

Pentamidine has been gradually accepted in several Latin American countries as an excellent alternative to pentavalent antimonials and has been recommended as the drug of choice for the treatment of CL in some countries[10]. The drug is highly effective and side effects are generally well tolerated, with pain at the site of drug injection, nausea, fever and bitter taste being the most frequent symptoms[10]. Hypotension and hypoglycemia have been reported, as well as the induction of diabetes mellitus[1]. Therefore, monthly serum glucose monitoring for 6 months has been recommended following the administration of a pentamidine cumulative dose higher than 1.0g[1]. Several studies have compared the efficacy of pentavalent antimonials and pentamidine and some of them demonstrated a better performance of the latter[10].

The administration of pentamidine was well tolerated by the patient and the outcome was satisfactory. The patient presented no laboratory alterations during post-treatment follow-up and was lesion-free 34 months after the end of treatment.

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