SUCCESSFUL TREATMENT OF REFRACTORY CUTANEOUS LEISHMANIASIS WITH GM-CSF AND ANTIMONIALS

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Abstract. Therapeutic failure in the treatment of cutaneous leishmaniasis (CL) occurs in 5% of patients infected by Leishmania braziliensis. This study evaluates the use of topically applied granulocyte macrophage colony-stimulating factor (GM-CSF) combined with the standard dose of antimony to treat refractory cases of CL. Five patients who had received three courses or more of antimony were enrolled in an open-label clinical trial. One to 2 mL of the GM-CSF solution (10 μg/mL in 0.9% saline) was reapplied topically, and dressings were changed three times per week for 3 weeks, associated with standard parenteral antimony (20 mg kg⁻¹ day⁻¹ for 20 days). All the patients healed their CL ulcers; 3 healed within 50 days (21, 27, and 44 days) and 2 in 118 and 120 days after beginning therapy. There were no side effects. This study shows that combined topically applied GM-CSF and antimony can be effective and well tolerated in the treatment of relapsed CL.

INTRODUCTION

Cutaneous leishmaniasis (CL), caused by Leishmania spp., is a sandfly-transmitted protozoal disease endemic in many tropical countries of the Americas, Africa, and Asia. It is estimated that 400,000 new cases occur each year worldwide.¹ In areas of Leishmania braziliensis transmission, the typical initial clinical manifestation is a single skin ulceration, localized predominantly in the lower limbs, areas commonly exposed to phlebotomine bites.² Weeks to years after the onset of the cutaneous disease, mucosal lesion(s) involving the nasal mucosa, palate, pharynx, larynx, and/or vocal cords may develop.³ The pentavalent antimonials have been the treatment of choice for leishmaniasis for more than 50 years despite the need for daily parenteral injections for 20 to 30 days. There is a prolonged healing time of 3 to 4 months, and serious side effects include pancreatitis, liver enzyme abnormalities, and cardiac arrhythmia.²,³ Alternative drugs, such as amphotericin B and pentamidine, are of greater toxicity; however, amphotericin B is much less toxic and very effective if used in liposomes, although it is more expensive.⁴,⁵ Therapeutic failure is observed in about 5% of the patients with cutaneous ulcers due to L. braziliensis.⁷ The granulocyte macrophage colony-stimulating factor (GM-CSF) is a multipotential growth factor for marrow stem cells.⁸ Both Th1 and Th2 lymphocyte subsets respond to GM-CSF.⁹ In vitro, GM-CSF has been shown to activate macrophages to kill leishmania.¹⁰,¹¹ Besides its microbicidal activity, GM-CSF is known also to stimulate fibrosis and tissue wound healing.¹²,¹³ It has been shown that GM-CSF used in low doses has an effect in platelet function in the bone marrow.¹⁴ Moreover, GM-CSF locally applied in low doses improves the healing of chronic venous ulcer¹⁵ and also another cytokine, keratinocyte growth factor-2, applied locally accelerated wound healing in chronic venous ulcer.¹⁶ In double-blind randomized trials, we have shown that GM-CSF injected intraleisionally or applied topically¹⁷ as adjuvant therapy to antimony accelerates cutaneous leishmaniasis ulcer healing.¹⁶ The current study was designed to evaluate the effect of topically applied low doses of GM-CSF plus standard antimony in the healing of cutaneous leishmaniasis ulcers refractory to antimony.

MATERIALS AND METHODS

All patients lived in Corte de Pedra or surrounding communities, an endemic area of tegumentary leishmaniasis in the state of Bahia, Brazil. Each of the five patients had a confirmation of leishmaniasis by typical leishmania skin lesion and a positive skin test with Leishmania antigen or by parasite isolation in culture of lesion aspirates. Previous studies from our group have demonstrated L. braziliensis to be the etiologic agent in this region, and in the current study Leishmania was typed by using monoclonal antibody and zymodema analysis. All the patients had received at least three courses of intravenous pentavalent antimony in a dose of 20 mg kg⁻¹ day⁻¹ for 20 days and had at least three episodes of relapse of their cutaneous lesions. Additionally, the last treatment was done under observation in our clinic. After documentation of persistence of active lesions 90 days after the last therapy, the patients were considered refractory to antimony therapy and were enrolled in the study. This study was approved by the human ethical committee for research of the Hospital Universitário Prof. Edgard Santos, and informed consent was obtained from patients or guardians. The patients were treated topically with a commercially available GM-CSF (Leucomax; Novatis, São Paulo, Brazil) diluted to a final concentration of 10 μg/mL in saline.¹⁵,¹⁶ Ulcers were cleansed with 0.9% sodium chloride solution, and then 1 to 2 mL of the GM-CSF solution (10 μg/mL in 0.9% sodium chloride solution) was applied topically. A 1-mL portion was enough to cover a 10-cm² area. A nonadhesive wound compress (Adaptic; São Paulo, Brazil) was secured over the area with a cotton bandage and a short elastic compression bandage (Coban; 3M, São Paulo, Brazil) was applied. The GM-CSF solution was reapplied, and dressings were changed three times per week for 3 weeks.¹⁵,¹⁶ All patients received intravenous pentavalent antimonial (meglumin antimoniate) 20 mg per kg of body weight daily for 20 days. The patients were evaluated on Days 30, 60, 90, 120, 180, and 360 after treatment onset. On all occasions, two separate medical doc-
tors examined the patients. A cure was defined to be a patient whose previous ulcer (crater and border) had undergone complete re-epithelialization.

RESULTS

Complete healing was observed in all five patients included in the study. Table 1 shows the demographic data and time of healing of the 5 patients, 3 males and 2 females, with ages ranging from 14 to 25 years. The largest diameter size of the ulcer ranged from 14 to 120 mm. The majority of the lesions were below the waist and on the lower limbs (four patients). The other patient presented a lesion on the right arm. Four patients had positive Leishmania skin test, and one had negative test, but the parasite was cultivated from the lesion and was typed as L. braziliensis. The last course of antimony treatment had been given more than 90 days prior the inclusion in the study. Each of the five patients demonstrated complete healing by use of topical GM-CSF and antimony.

All patients cured with one new course of antimony combined to GM-CSF. Three patients had their ulcers healed before 50 days with a mean healing time of 66 ± 49 days (Days 21, 27, 44). The other two patients required 118 and 120 days for complete healing. Continuous follow-up of all cases reported in this study was performed over a period of at least 12 months after healing, and no relapse was observed. No systemic side-effects or contact allergic reactions from the therapy were reported by the patients or observed by the investigators.

DISCUSSION

This open clinical trial indicates that successful healing can be accomplished by the use of topically applied GM-CSF as adjuvant therapy to pentavalent antimony to treat cutaneous leishmaniasis patients refractory to antimony therapy. Our previous double-blind randomized data also showed that GM-CSF injected intralesionally or used topically as adjuvant therapy to antimony can be useful to reduce the healing time of cutaneous leishmaniasis ulcers. Previous studies have shown the ability of GM-CSF to improve wound healing. Robson and others demonstrated that topically applied GM-CSF, in a dose of 1 µg/cm² of wound areas, is as effective as a dose of 10 µg/cm² to reverse the inhibition of wound contraction caused by bacterial contamination in rats. Locally applied GM-CSF has also been useful in the treatment of chronic venous ulcers.

GM-CSF may decrease the healing time of cutaneous leishmaniasis ulcers by three potential mechanisms: increasing parasite killing by directly activating macrophages, enhancing scar formation, and modulating immunologic balance. Previous studies have shown that GM-CSF activates macrophages to kill Leishmania in vitro. GM-CSF has been described to improve healing and scarring of cutaneous lesions caused by agents other than Leishmania. Moreover, Doherty and others have shown that transfer of macrophages primed with GM-CSF and Leishmania antigens protected native BALB/c against challenge with Leishmania major. The proposed mechanism was the induction of a cell-mediated immune response to Leishmania antigen due to differentiation of CD4+ T cells to Th1. The healing of relapsing cutaneous leishmaniasis ulcers brought exceptional benefits to patients who had been through repeated courses of antimony. These patients are usually treated with other drugs with higher toxicity, such as amphotericin B, which needs to be administered during hospitalization; however, the drug is much less toxic and very effective if used in liposomes. Locally applied GM-CSF in conjunction with antimony should be evaluated in further studies in severe cases of leishmaniasis and in patients who do not respond to repeated courses of antimony.

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