

CONCISE COMMUNICATIONS

Randomized, Double-Blind Study of Stibogluconate Plus Human Granulocyte Macrophage Colony-Stimulating Factor versus Stibogluconate Alone in the Treatment of Cutaneous Leishmaniasis

Roque Almeida,¹ Argemiro D'Oliveira Jr.,¹
 Paulo Machado,¹ Olívia Bacellar,¹ Albert I. Ko,²
 Amelia R. de Jesus,¹ Niloufar Mobashery,²
 Jussamara Brito Santos,¹ and Edgar M. Carvalho^{1,a}

¹Serviço de Imunologia do Hospital Universitário Prof. Edgard Santos, Salvador, Bahia, Brazil; ²Division of International Health and Infectious Diseases, Department of Medicine, Cornell University Medical College–NY Hospital, New York, New York

The response to recombinant human granulocyte macrophage colony-stimulating factor (GM-CSF) in the treatment of cutaneous leishmaniasis was evaluated. Twenty patients with cutaneous leishmaniasis who had lesions for 60 days were enrolled in a double-blind placebo trial of GM-CSF with standard parenteral sodium stibogluconate (20 mg/kg⁻¹/day⁻¹) for 20 days. Ten patients were randomized to receive intralesionally injected GM-CSF (200 µg) at enrollment and 1 week after, and 10 patients received saline as placebo. GM-CSF- and antimony-treated patients healed faster than patients who received antimony alone (49 ± 32.8 vs. 110 ± 61.6 days, *P* < .05). Seven of 10 patients were healed of their lesions before 40 days after therapy in the GM-CSF group, compared with only 1 of 10 patients in the placebo group (relative risk, 7; 95% confidence interval, 1.04–47.00). Thus, GM-CSF plus antimony significantly increased the chance of lesion healing in 40 days.

Cutaneous leishmaniasis (CL) caused by *Leishmania* species is a sandfly-transmitted protozoal disease endemic in many tropical countries of the Americas, Africa, and Asia. Worldwide, ~400,000 new cases occur every year [1]. In infections with *Leishmania braziliensis*, an important etiologic agent in Brazil, the typical initial clinical manifestation is a single skin ulceration, localized predominantly in regions, such as the lower limbs, that are 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 >40 years despite the need for daily

intravenous injections for 20–30 days, prolonged healing time of 3–4 months, and serious side effects, which include pancreatitis, liver enzyme abnormalities, and cardiac arrhythmia [3, 4]. Alternative drugs, such as amphotericin B and pentamidine, are of greater toxicity [5, 6].

Granulocyte macrophage colony-stimulating factor (GM-CSF) is a multipotential growth factor for marrow stem cells [7]. Both T-helper type 1 (Th1) and type 2 (Th2) lymphocyte subsets respond to GM-CSF [8]. In vitro, GM-CSF has been shown to activate macrophages to kill leishmania [9–11]. In addition to its microbicidal activity, GM-CSF is known also to stimulate fibrosis and tissue wound healing [12]. The present study was designed to evaluate the effect of locally injected GM-CSF plus standard parenteral antimony in the healing time of CL ulcers.

Subjects and Methods

Twenty patients with CL were recruited for the study at an ambulatory clinic in an endemic region in Brazil. Previous studies have showed *L. braziliensis* to be the etiologic agent in this area [2]. Criteria for study enrollment were age between 10 and 50 years, presence of a single typical CL ulcer for <60 days duration, and confirmation of CL by compatible histology and either a positive serology or positive intradermal skin test for *Leishmania* antigen. The exclusion criteria were pregnancy, other associated acute or chronic illnesses, and history of allergy to GM-CSF and/or antimonial. Written consent was obtained from all patients older than 18 years and from the parents of younger patients, and the study

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Informed consent was obtained from patients or their parents or guardians, and human experimentation guidelines of the Hospital Univesitário Prof. Edgard Santos were followed in the conduct of this clinical research.

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^a E.M.C. is a senior investigator of CNPq (Brazilian National Research Council).

Reprints and correspondence: Dr. Edgar M. Carvalho, Hospital Univesitário Prof. Edgard Santos, Serviço de Imunologia, 3º andar, Rua João das Botas, s/n, Canela, 40110-160, Salvador, Bahia, Brazil (edgar@ufba.br).

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Table 1. Demographic data and mean lesion duration and size for patients with cutaneous leishmaniasis.

	GM-CSF + antimony	Placebo + antimony	<i>P</i>
Age (years)			
Mean (years)	31.80 ± 12.48	27.70 ± 12.94	NSS ^a
Range (years)	18–51	16–45	
Sex			
Male	8	5	NSS ^b
Female	2	5	
Lesion duration			
Mean (days)	36.10 ± 15.13	25.80 ± 12.43	NSS ^a
Range (days)	15–60	15–50	
Lesion size			
Mean (mm)	18.80 ± 5.75	17.90 ± 4.86	NSS ^a
Range (mm)	13–30	13–29	
Location of lesion			NSS ^b
Lower (%)	60	90	
Upper (%)	40	10	

NOTE. GM-CSF, granulocyte macrophage colony-stimulating factor; NSS, not statistically significant.

^a Mann-Whitney *U* test.

^b Fisher's exact test.

was approved by the ethics committee at Hospital Universitário Prof. Edgard Santos.

The selected patients were randomized into 2 study groups. The research team was blinded to the administered drug. The GM-CSF group of patients received 2 local injections of 200 µg of hr-GM-CSF (Sandoz, São Paulo, Brazil), at entry and 1 week later (designated as GM-CSF group). The control group received saline (designated as placebo group). All patients received intravenous pentavalent antimonial (sodium stibogluconate, Meheco, Beijing, China), at 20 mg per kg of body weight, daily for 20 days.

The patients were evaluated minimally on days 30, 60, 90, 120, and 180 after treatment onset. On all occasions, 2 separate medical doctors examined the patients. A cure was defined to be a patient whose previous ulcer (crater and border) had undergone complete reepithelialization. In the case of a disagreement between the 2 medical evaluations, the medical doctors reexamined the patient together. To maintain the double-blind nature of the experiment, questions on possible side effects of the treatment were deferred to a third medical doctor who was conversant with the known side reactions of GM-CSF, such as general malaise and muscle ache. All patients who had evidence of an active ulcer at 90 days after initiating the first treatment were defined as treatment failures and received an additional course of pentavalent antimonial treatment (20 mg/kg⁻¹/day⁻¹ IV for 20 days). The continuous variables were analyzed by Mann-Whitney *U* test and categorized data by Fisher's exact test. A *P* value <.05 was considered significant.

Results

As shown in table 1, patients in the GM-CSF and placebo groups were similar in age, sex, and ulcer size and duration (Mann-Whitney *U* test and Fisher's exact test). The majority of the lesions were below the waist and on the lower limbs in 15 (75%) of the patients. The number of female patients and the percentage of lesions on the lower limbs were greater in the placebo than in the GM-CSF group.

Patients who received GM-CSF plus antimony were healed of their ulcers faster than patients who received placebo plus antimony (49 ± 32.8 vs. 110 ± 61.6 days, *P* < .05, Mann-Whitney test). In the GM-CSF group, 7 of 10 patients were healed of their lesions within 40 days after starting therapy, whereas only 1 of 10 patients in the placebo group was healed of lesions in this time interval (*P* < .05; Fisher's exact test). The calculated relative risk for incomplete healing 40 days after initiation of therapy was 0.33 (95% confidence interval [CI], 0.13–0.88) for patients treated with antimony plus GM-CSF, compared with that for control patients treated with antimony alone.

The response to treatment for the GM-CSF and placebo groups is illustrated in figure 1 as the proportion of patients not cured. Most GM-CSF-treated patients (70%) were cured within 40 days after therapy onset, whereas only 10% of the placebo group was cured during this time. Eighty percent of the GM-CSF group was healed of lesions within 60 days, and all of them healed within 110 days after beginning therapy. In contrast, 50% of placebo patients healed within 120 days, and all of them healed by 270 days. Because of nonhealed ulcers at 90 days after therapy, a second course was given to 4 patients from the placebo group, and 2 of them had a third course of antimony. In contrast, only 2 patients in the GM-CSF group required a second course of treatment.

Discussion

This randomized, double-blind, placebo-controlled clinical trial indicates that the use of GM-CSF as adjuvant therapy

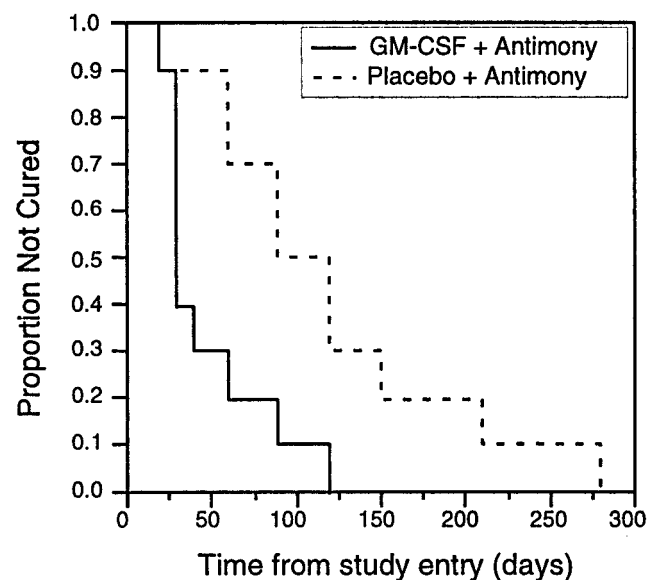


Figure 1. Kaplan-Meier curve showing proportion of patients not cured treated with granulocyte macrophage colony-stimulating factor (GM-CSF) + antimony (solid line) and placebo + antimony (dotted line) over 300 days of follow up.

with pentavalent antimonial significantly decreases the time to healing in CL. Differences in the characteristics of the randomized groups were not significant, although the GM-CSF group had a larger proportion of patients who were male and had longer lesion ulcer durations and more upper extremity lesions. Increased duration of lesions prior to initiation of therapy adversely affects treatment response and therefore would be expected to negatively influence the treatment benefit observed in the GM-CSF group. Sex of patient and site of the lesion have not been reported to be factors that influence the response during the treatment of *L. braziliensis* infections.

GM-CSF may decrease the healing time of CL ulcers by means of 3 potential mechanisms: increasing parasite killing by direct activating effect on macrophages [9–11], enhancing scar formation [13], and modulating the immunologic balance [14]. Previous study has shown that GM-CSF does activate macrophages to kill leishmania in vitro [9–11]. GM-CSF has been described to improve healing and scarring of cutaneous lesions resulting from causes other than *Leishmania* [13, 14]. Moreover, Doherty et al. [15] have shown that transfer of macrophages primed with GM-CSF and *Leishmania* antigens protected naive BALB/c mice against challenge with *Leishmania major*. The proposed mechanism was the induction of a cell-mediated immune response to *Leishmania* antigen, owing to differentiation of CD4 T cells to Th1. Our data suggest that GM-CSF may also influence human immune response to *Leishmania* by 1 or all the above mechanisms. A reduction in the healing time, from 110 to 50 days, and in the requirement for additional retreatment reduces the need for medical supervision, improves compliance to treatment, and reduces the economic burden resulting from a limited budget and lost work days.

The use of GM-CSF with sodium stibogluconate raises the possibility of reducing the duration of antimony therapy or dosage and thereby reducing drug toxicity. In addition, the combination of GM-CSF and antimony may be more effective in severe cases of leishmaniasis, and in patients with relative antimony resistance. This study did not address mechanisms to explain how GM-CSF decreases healing time of CL ulcers, but delineating these mechanisms may identify targets for development of other drugs as alternatives to antimony, which is the current treatment for CL in most countries.

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