SUCCESSFUL TREATMENT OF REFRACTORY MUCOSAL LEISHMANIASIS WITH PENTOXIFYLLINE PLUS ANTIMONY

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Abstract. Mucosal leishmaniasis is characterized by an intense inflammatory reaction and tissue damage with few parasites in the lesion. On the basis of previous observations that suggest a possible role of tumor necrosis factor alpha (TNF-α) in the pathology of this disease, an open-label study was performed to evaluate the efficacy of the treatment with an inhibitor of TNF-α (pentoxifylline) associated to antimony therapy in 10 patients with refractory mucosal leishmaniasis. Patients were treated with pentavalent antimony (20 mg per kilogram of body weight per day) plus orally administered pentoxifylline 400 mg 3 times daily for 30 days. Nine of 10 patients fulfilled the criteria for cure: they experienced complete reepithelization of the mucosal tissue 90 days after therapy and displayed no evidence of relapse at 1 year of follow-up. The TNF-α levels before therapy (776 ± 342 pg/mL) fell to 94 ± 57 pg/mL (P < 0.05) within 60 days after therapy. Our results indicate that pentoxifylline plus antimony therapy should be considered in all patients with mucosal leishmaniasis that is refractory to treatment.

INTRODUCTION

Mucosal leishmaniasis is a destructive disease that predominantly affects the nose. The disease is most common in areas of Leishmania braziliensis transmission and usually occurs months or years after cutaneous leishmaniasis. Complications of mucosal leishmaniasis include nasal septum perforation, hoarseness due to vocal cord involvement, and deformity of the nasal pyramid. Recommended therapy is pentavalent antimony in a dose of 20 mg per kilogram of body weight per day for 30 days. However, clinical failure—defined as presence of ulcer or granulomatous lesion—occurs in up to 42% of patients, and relapses have been documented in as many as 19% of patients after 3–10 years of follow-up.

The paucity of parasites in the mucosal destructive lesion highlights the current poor understanding of the pathogenesis of mucosal leishmaniasis. Mononuclear cells from patients with mucosal disease recognize Leishmania antigens by proliferation and secretion of Th1 cytokines. In addition, patients with mucosal leishmaniasis produce high levels of tumor necrosis factor alpha (TNF-α) in vivo and in vitro in response to Leishmania antigens. This information raises a question about whether hypersensitivity is part of the pathogenic mechanism. A role for TNF-α in the pathogenesis of mucosal leishmaniasis and other chronic inflammatory parasitic disease is suggested by the following: increased levels of TNF-α in serum and high expression in mucosal tissue; decrease of TNF-α levels after therapy of mucosal leishmaniasis; an association of TNF-α with cerebral malaria; and tissue damage in erythema nodosum leprosum; and clinical improvement in erythema nodosum leprosum by agents that inhibit TNF-α production.

Pentoxifylline is a xanthine derivative that suppresses TNF-α gene transcription and decreases leukocyte migration and adhesion. It has been approved for the U.S. Food and Drug Administration to be used as a vasodilator in patients with intermittent claudication due to chronic occlusive arterial disease of the limbs. Nausea, vomiting, dizziness, and headache are the most common side effects and occur in < 2.2% of patients. On the basis of observations suggesting that TNF-α participates in the pathogenesis of mucosal leishmaniasis, an open-label study was conducted that used pentoxifylline in combination with standard pentavalent antimony therapy in patients with mucosal leishmaniasis refractory to pentavalent antimony alone.

MATERIALS AND METHODS

Patients. All patients lived in Corte de Pedra or surrounding communities, an area endemic for tegumentary leishmaniasis in the state of Bahia, Brazil. During the period of the study (1994–1998), 53 patients with active mucosal involvement due to Leishmania were seen at the health post of Corte de Pedra, and 15 had not been cured after one or more courses of intravenously administered pentavalent antimony. One patient was excluded from the study because of severe diabetes, and another was excluded due to severe destruction of the nose and larynx, with intense secondary infection and wasting. All remaining 13 patients with typical lesions involving the nasal mucosa, a positive skin test with Leishmania antigen, and either parasite isolation or histopathological findings characteristics of mucosal leishmaniasis received an additional intravenous course of meglumine (Glucantime®; Roche, Rhoâne Poulenc, Paris) in a dose of 20 mg of pentavalent antimony per kilogram of body weight per day for 30 days. After documentation of persistence of active lesions at 90 days after therapy, 10 of 13 patients were enrolled in the study. Leishmania were cultured from 5 of 10 patients, including the 2 patients who denied having previous cutaneous leishmaniasis. The 5 parasites isolated were characterized by monoclonal antibodies and isoenzymes as L. (Viannia) braziliensis. Patients were treated with pentavalent antimony (20 mg/kg/day) plus orally administered pentoxifylline 400 mg 3 times daily for 30 days. The criteria for cure was complete reepithelization of the mucosal tissue 90 days after therapy and no evidence of a relapse during a 1-year follow-up period. This study was approved by the human ethical committee for research of the Hospital Universitário Prof. Edgard Santos, and informed consent was obtained from the patient or guardian.

Immunological response. Soluble crude Leishmania an-
tigen was prepared as previously described from an isolate previously characterized as *Leishmania amazonensis.* We determined the presence of TNF-α in supernatants of lymphocyte cultures of 7 patients before and 60 days after therapy. Mononuclear cells were obtained from heparinized venous blood by centrifugation with lymphocyte separation medium (Bionetics Laboratory Products, Kensington, MD). After washing with saline, cells were adjusted to a concentration of 3 × 10⁶/mL in RPMI 1640 culture medium (GIBCO, Grand Island, MA) supplemented with 10% heat-inactivated AB human serum. Cells were cultured at 37°C with 5% CO₂ in medium alone or with medium plus *Leishmania* antigen (10 μg). After 72 hr, the supernatants were harvested, the TNF-α levels determined by sandwich enzyme-linked immunosorbent assay, and the results expressed as picograms per milliliter.

**Nasal cytology.** As previously described, nasal lavages were performed with 5 mL of saline before and 60 days after therapy for quantitative analysis. The type of cells was determined in differential counts of swab smears after staining with DiffQuick® (American Scientific, San Diego, CA).

**Statistical analysis.** The Mann-Whitney test was used to compare the TNF-α and number of cells in the nasal quantitative cytology before and after therapy.

**RESULTS**

The clinical profile of the 10 study patients is shown in Table 1. The ages of the patients ranged 10–58 years (mean, 29 ± 17 years); the male:female sex ratio was 4:1. The duration of mucosal disease varied from 5 to 128 months (mean, 30 ± 47 months). All patients had nasal involvement. It was severe in 9 patients, with septal perforation documented in 5, including a patient with nasal pyramid deformity. Deep ulcerative lesions were found in 4 patients. Only one patient had a superficial ulcer characteristic of a moderate disease. The number of previous courses of antimony therapy ranged 2–8 courses (mean, 3 ± 1.9 courses). Patients were evaluated before and every 30 days after treatment with pentavalent antimony plus pentoxifylline by an otolaryngologist for at least 12 months. After this period, a follow-up examination was carried out every 90 days for a mean of 37 ± 16 months (range, 20–57 months). Clinical cure documented by complete reepithelization of the mucosa was observed in 9 of 10 patients. In 8 patients, complete healing was documented by Day 60 after therapy and in 1 patient at Day 90. One patient improved with therapy, but the lesion remained active. This patient had the longest duration of mucosal disease and had received the highest number (n = 8) of previous courses of antimony therapy.

The levels of TNF-α were evaluated before and after therapy in supernatants of lymphocyte cultures stimulated with *Leishmania* antigens. The mean concentration of this cytokine fell from 776 ± 342 pg/mL before therapy to 94 ± 57 pg/mL (P < 0.05), within 60 days after completing therapy. There was also a decrease in the total number of cells in the nasal lavage from 1,140 × 10⁶ to 410 × 10⁶ (P < 0.05). Additionally, while in the pretherapy stage, the predominant cell type was neutrophil; after therapy, epithelial cells were mainly observed. The percentage of neutrophils before therapy (62 ± 13%) was higher than that observed after therapy (15 ± 8%, P < 0.05).

**DISCUSSION**

Pentavalent antimony is the drug of choice for treatment of mucosal leishmaniasis, and amphotericin B is recommended when cure with antimony is not achieved. The high relapse rates observed with antimony therapy and the side effects caused by antimony and amphotericin B indicate the need for an alternative drug in the treatment of mucosal leishmaniasis. Mononuclear cells from these patients produce high levels of interferon gamma and TNF-α, cytokines that up-regulate nitric oxide synthesis. Although this system is important for *Leishmania* killing, an overproduction of these proinflammatory cytokines may lead to tissue damage. Thalidomide, an inhibitor of TNF-α synthesis, has been successfully used in the treatment of erythema nodosum leprosum, and a soluble TNF-α receptor is currently used for treatment of rheumatoid arthritis. Several lines of evidence suggest that host immune response may contribute to tissue damage in mucosal leishmaniasis. Levels of TNF-α are increased in serum and in supernatants of mononuclear cell cultures from patients with mucosal disease; an intense inflammatory reaction with rare or absent parasites is usually found in the mucosa.
Pentoxifylline associated with antimy was evaluated in 10 patients who failed to respond to at least 2 courses of standard SB5 pentavalent antimony therapy. The last course was administered by our team, and lack of response 90 days after therapy was observed. Therefore, it is unlikely that the therapeutic failure was due to inadequate treatment.

Pentoxifylline is a potent inhibitor of TNF-α production and neutrophil function.13,14 There is no evidence that pentoxifylline directly kills in vitro Leishmania or activates macrophages. It seems that the major contribution of pentoxifylline in the therapeutic response observed in this study was due to the decrease of the inflammatory reaction and consequent tissue damage. A possible vasodilatation effect of the drug allowing for access of defense mechanisms to inflamed tissue and consequent Leishmania killing cannot be ruled out. Quantitative nasal cytology and TNF-α levels previously used as markers of therapeutic response in mucosal leishmaniasis15,16,17 were assayed in these patients. All patients who were assessed by analysis of nasal lavage showed a decrease in the number of cells, and a significant decrease in TNF-α was observed 60 days after therapy. After at least 1 year of follow-up, none of the 9 treated patients with mucosal leishmaniasis had evidence of active nasal disease. The combined therapy with pentoxifylline and antimony was safe; only one patient reported nausea, but it did not require stopping pentoxifylline therapy.

These results indicate that pentoxifylline in conjunction with antimy therapy was able to induce cure quickly in patients with mucosal leishmaniasis refractory to antimy alone. Considering that antimy therapy failure rate is high and amphoterin B, the second choice drug, is associated with frequent side effects and needs hospitalization for administration, we recommend that all patients with mucosal leishmaniasis that fails to respond to antimy therapy be treated with the combination of antimy plus pentoxifylline before the use of other drugs is attempted. Currently, a randomized, double-blind study evaluating the efficacy of antimy plus pentoxifylline compared with antimy plus placebo in the control of mucosal leishmaniasis is being conducted in the endemic area of leishmaniasis of Corte de Pedra, Bahia, Brazil.

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