Clinical healing of antimony-resistant cutaneous or mucocutaneous leishmaniasis following the combined administration of interferon-γ and pentavalent antimonial compounds

E. Falcoff[1], N. J. Taranto[2], C. E. Remondegui[3], J. P. Dedet[4], L. M. Canini[2], C. M. Ripoll[3], L. Dimier-David[4], F. Vargas[4], L. A. Giménez[5], J. G. Bernabó[5] and O. A. Bottasso[6] [1]*Unité 196, INSERM, Institut Curie, 26 rue d'Ulm, 75231 Paris cedex 05, France;* [2]*Hospital San Vicente de Paul, Organ Salta, Argentina;* [3]*Hospital San Roque, San Salvador de Jujuy, Argentina;* [4]*Instituto Boliviano de Biologia de la Altura-Embajada de Francia, La Paz, Bolivia;* [5]*Roussel Argentina, Buenos Aires, Argentina;* [6]*División Inmunología, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Santa Fe 3100, Rosario (2000), Argentina*

Abstract

In an open trial, longer courses of pentavalent antimonials (Sb^v) at sub-optimal doses (10 mg/kg body weight), in association with recombinant human interferon-γ (IFN-γ) (100 μg/m² of body surface area) were administered, by daily intramuscular injections, to 13 patients with diagnoses of cutaneous or mucocutaneous leishmaniasis unresponsive to Sb^v. Four patients presented with large skin ulcers, and 9 had mucosal involvement as the main manifestation, the latter affecting the nose (3 cases), nose and septum (2 cases), nose and oral cavity (1 case), and nose, pharynx and larynx (3 cases). Except for one case with severe involvement of the upper respiratory tract, the lesions were fully resolved by the end of therapy (mean duration 40±12 [SD] d, range 30–60 d) in the 11 patients who completed therapy. The main side effects were headache and fever (7 cases), together with leucopenia and eosinophilia (4 cases). It is concluded that combined administration of low doses of Sb^v plus IFN-γ may provide a novel therapeutic approach for the treatment of antimony-resistant cutaneous or mucocutaneous leishmaniasis. The possible mechanisms by which IFN-γ contributes to resolution of the disease are discussed.

Introduction

Leishmania braziliensis braziliensis is the aetiological agent of mucocutaneous leishmaniasis (MCL), which involves the upper respiratory tract with significant necrosis of soft tissues.

There is a consensus that macrophages play a pivotal role in the protective response against intracellular parasites that replicate within phagolysosomes, such as *Leishmania*. Work with mice has demonstrated that efficient killing of parasites is mainly achieved by nitrogen oxidation products, generated from L-arginine, and to a lesser extent by reactive oxygen intermediates (MAÜEL *et al.*, 1991; NACY *et al.*, 1985, 1991). Secretion of these compounds is particularly enhanced in inflammatory and activated macrophages as a result of microbial endocytosis and/or stimulation with the cytokine interferon-γ (IFN-γ), which is secreted by T cells (HIBBS, 1991; NACY *et al.*, 1991). In this context, it could be expected that the ability of the host to cope with *Leishmania* would be largely dependent on the capacity of macrophages activated by IFN-γ to destroy the parasites, an assumption which has clearly been corroborated in a series of murine studies (MULLER *et al.*, 1989; SCOTT, 1991). Reinforcing this experimental evidence, BADARO *et al.* (1990) demonstrated that systemic doses of recombinant human IFN-γ (rHuIFN-γ) and compounds containing pentavalent antimony (Sb^v) are therapeutically effective in antimony-resistant visceral leishmanisis.

Since half the normal doses of Sb^v combined with rHuIFN-γ proved to be effective also in a patient with MCL refractory to antimony (BOTTASSO *et al.*, 1992), we decided to explore the clinical usefulness of such therapeutic approach in antimony-resistant patients with either large skin ulcers or mucocutaneous disease.

Methods

Patients

This open trial involved 3 study centres, 2 in Argentina and 1 in Bolivia. The selected sample consisted of 13 patients, one woman and 12 men (mean age±standard deviation [SD] was 42·6±13 years, range 17–61 years). Participants were incorporated on the basis of the following criteria: a prior history of leishmaniasis, and a present clinical picture of disease clearly refractory to antimony with parasitological confirmation or a positive Montenegro skin test. The latter was elicited by injecting, intradermally, 0·1 mL of leishmanin (30 μg of protein nitrogen/mL) into the volar aspect of the right forearm. The test was read 48 h later, and indurations >5 mm in diameter were considered positive. The parasitological diagnosis was made by the demonstration of amastigotes in Giemsa-stained smears of dermal scrapings, or culture of tissue aspirates in biphasic media. On some occasions aspirated material was inoculated into hamsters. Although many patients had a history of several unsuccessful courses of Sb^v, antimony resistance was considered present only when lesions remained unchanged one month after the end of a 28 d course of Sb^v, 20 mg/kg body weight/d. The minimum period between completion of prior antimony treatment and inclusion in the present study was 1 month.

Other criteria for eligibility included satisfactory renal, hepatic and cardiac functions and absence of circulating hepatitis B surface antigen or bacterial superinfection of the lesions. Patients with bacterial infection were admitted to the study, however, after appropriate antimicrobial treatment. Pregnant women, or women with insufficient evidence that pregnancy was unlikely to occur during the study period, were excluded.

The trial was conducted according to the rules of good clinical practice and the recommendations guiding biomedical research (Helsinki, 1964 and Venice, 1983). The protocol and consent form were approved by the Ethical Committee of the Facultad de Ciencias Médicas de Rosario in Argentina. Willingness of patients to participate in the study was recorded by signing the written informed consent.

Treatment

Patients received a daily intramuscular injection of rHuIFN-γ (RU 42369®, Roussel-Uclaf), 2 million iu/m² body surface, and Sb^v 10 mg/kg body weight (Glucantime®, Rhone-Poulenc) for 30 d. Treatment was terminated if lesions persisted unmodified after this period, but therapy was continued for 2 additional months if the ulcers were partially resolved. Treatment was discontinued and patients were withdrawn from the study if grade 3 adverse reactions occurred (MILLER *et al.*, 1981).

Clinical and laboratory assessment

Patients were evaluated daily for the presence of any complaint, such as fever, nausea, vomiting, diarrhoea, muscle, bone or abdominal pain, stiffness of the joints, headache, arthralgia, or palpitations. Laboratory investi-

gations included haematocrit, white blood cell and platelet counts, aminotransferases, creatinine, glucose and bilirubin serum levels, blood urea nitrogen level, and urinalysis. Tests were performed at inclusion, at 10 d intervals, and on completion of therapy. A 12-lead resting electrocardiogram was also performed, before starting, and at the end of, therapy.

Monitoring for efficacy

Efficacy was judged on the duration of clinical symptoms and macroscopic resolution of lesions, evaluated by direct observation and careful otorhinolaryngological examination. Changes in MCL lesions were recorded weekly during the period of treatment. The following definitions were adopted. Partial healing: clinical improvement but incomplete re-epithelization of the lesion by the end of treatment; complete healing: lesions fully resolved or flattened by the end of treatment; clinical healing: lesions completely resolved with no relapse during follow-up; failed treatment: persistence of ulcers, or occurrence of new lesions or relapses during follow-up. Patients were regularly examined at monthly intervals for a minimum period of 6 months.

Statistical analysis

Biochemistry variables were statistically analysed by the Wilcoxon matched-pairs signed-ranks test.

Results

Clinical data

Four patients presented with large single cutaneous ulcers located on the legs (patients no. 5 and 6), ankle (no. 13) and ear (no. 10). Nine cases had mucosal involvement as the main manifestation with lesions affecting the nose (patients no. 2, 9 and 12; nos 8 and 11 also had septal involvement), nose and oral cavity (no. 4), and nasal mucosa, oropharynx and larynx (nos 1, 3 and 7). With the exception of patient no. 11, who presented with an infiltrated lesion in the nasal septum, all mucosal lesions were ulcers. Cases no. 1, 2, 3 and 8 also had mucocutaneous junction ulcers, with lesions restricted to the corner of the mouth and the nasal opening. Chief symptoms were pain, nasal obstruction, and nasal discharge. Duration of illness varied from 1 to 25 years (mean 4·9 years).

Except for case no. 2 with a recent history of pulmonary tuberculosis, and case no. 5 with non-insulin-dependent type II diabetes, the remaining patients were free from concomitant pathological disorders. All patients except no. 3 had a positive skin test.

Leishmania amastigotes were seen in Giemsa-stained smears from 12 patients. The exception, patient no. 7, had been parasitologically diagnosed at prior admissions, and a biopsy showed histological infiltrates compatible with MCL. A positive culture was obtained from patient no. 3, and parasites were characterized by isoenzyme electrophoresis as *L. braziliensis braziliensis*. Inoculation of aspirate materials into hamsters produced no lesion.

Response to treatment

According to our criteria, 11 patients (nos 1, 2, and 5–13) attained complete healing by the end of therapy, which lasted 30–60 d (mean duration ±SD, 40±12 d), and 10 showed no sign of recurrence or development of new lesions during 6 months follow-up. Patient no. 11 did not complete the follow-up, but clinical examination at the fourth month revealed no relapse.

Patient no. 5, a diabetic with a large skin ulcer on the right leg, showed a marked rise in glucose serum level and pronounced inflammation of the subcutaneous tissue surrounding the nose after treatment for 8 d. Treatment was stopped, and after remission of the complications he was given a 4 weeks course of Sb^v, with no clinical response. One month later, he volunteered for reinclusion in the study, and achieved complete healing following 60 d of combined therapy.

Patient no. 4 removed himself from the study, due to abdominal pain, at day 21 of treatment, when his lesions were partially healed. Despite not receiving further treatment, a single examination after 4 months revealed no active lesion.

Only in patient no. 3, with severe involvement of the upper respiratory tract and genital mucosa, was treatment considered to have failed, with lesions remaining unchanged after therapy for 30 d.

Considering all those who completed treatment, the efficacy was 91·7% by the end of therapy (11/12), and 91% at the 6 months follow-up (10/11).

Tolerance

Adverse effects were recorded in 12 of the 13 treated patients (Table), and they were not associated with prolonged therapy. The most common of these events were fever, headache, leucopenia, and eosinophilia. With the exception of grade III hyperglycaemia in the diabetic patient, adverse reactions were not severe, and were reversible in all cases. Apart from a mild transient rise of serum glutamic oxalate aminotransferase in patient no. 11 on day 40, the laboratory tests were within normal values, and comparative analysis of data obtained before and after treatment showed no significant difference (data not shown). The same was true when comparisons of electrocardiographic tracings were made.

Table. Adverse events during treatment of 13 patients with antimony-resistant cutaneous or mucocutaneous leishmaniasis, using pentavalent antimony and recombinant human interferon-γ

Description	WHO grade I	II	III	Total
Headache	4	3	–	7 (53·8%)
Fever	4	3	–	7 (53·8%)
Leucopenia	2	2	–	4 (30·7%)
Eosinophilia	1	3	–	4 (30·7%)
Anaemia	2	–	–	2 (15·4%)
Weakness	1	–	–	1 (7·7%)
'Flu'-like symptoms	–	1	–	1 (7·7%)
Abdominal pain	–	1	–	1 (7·7%)
Bone pain	1	–	–	1 (7·7%)
Nausea	1	–	–	1 (7·7%)
Gastritis[a]	1	–	–	1 (7·7%)
SGOT[b] rise	1	–	–	1 (7·7%)
Hyperglycaemia[c]	–	–	1	1 (7·7%)

[a]Relationship with treatment doutbful.
[b]Serum glutamic oxalacetic transaminase.
[c]Not related to treatment.

A clear distinction between side effects due to Sb^v and those related to rHuIFN-γ administration was not easy to establish. Nevertheless, considering the well-known effects of Sb^v, one could infer that the leucopenia, eosinophilia, 'flu'-like symptoms and, to some extent, the headaches, were the result of therapy with rHuIFN-γ.

No adverse effect was recorded at the monthly visits during the follow-up period.

Discussion

Confirming and extending our earlier observation (BOTASSO *et al.*, 1992), the present results clearly demonstrated that most cases of antimony-resistant cutaneous or mucocutaneous leishmaniasis resolved completely after combined systemic administration of rHuIFN-γ and Sb^v. Antimony was given at a lower dose because our previous observation showed that the recommended dose of glucantime, 20 mg/kg, associated with rHuIFN-γ produced a diabetes insipidus-like syndrome (BOTASSO *et al.*, 1992), presumably related to the defect in the renal concentration capacity that antimonial drugs are known to produce (VEIGA *et al.*, 1983).

Since a long course of conventional Sb^v doses cured a relapsed patient (MARSDEN *et al.*, 1985), the healing of lesions in our series may have been due, to some extent, to this. Although such an assumption cannot be dis-

carded, we emphasize that our patients received a suboptimal antimony dose, and clinical improvement was clearly noticeable by the second week of treatment.

The mechanisms by which IFN-γ exerts a beneficial effect on MCL are presently unknown. Given its pleiotropic effects, one may assume that IFN-γ acts not only by increasing the macrophage microbicidal activity but also by enhancing the pharmacological action of Sb^v. Additionally, the cytokine may promote a shift in cell-mediated responses, whereby harmful reactions are replaced by protective immunity.

While some reports exist concerning the spontaneous resolution of mucosal and skin lesions (MARSDEN *et al.*, 1991), such an event is unlikely even partly to have accounted for our results, since most patients recruited in this study had a clear history of progressive and relapsing disease.

The question of whether IFN-γ is able by itself to promote healing of MCL is also unknown. Evidence from treatment of patients with antimony-sensitive infections indicates that peri-lesional treatment with low doses of IFN-γ has cleared some cutaneous lesions, but it was less effective than local injections of glucantime (HARMS *et al.*, 1989, 1991). Comparative controlled studies are required properly to assess the effectiveness of giving IFN-γ, alone or combined with Sb^v, in MCL and other forms of human leishmaniasis.

Acknowledgements

We are grateful to Drs L. Valda and H. Monasterios, of the Servicio de Dermatología, Hospital de Clínicas de La Paz, Bolivia, for their collaboration.

References

Badaro, R., Falcoff, E., Badaro, F. S., Carvalho, E. M., Pedral-Sampaio, D., Barral, A., Carvalho, J. S., Barral-Netto, M., Brandely, M., Silva, L., Bina, J. C., Teixeira, R., Falcoff, R., Rocha, H., Ho, J. L. & Johnson, W. D. (1990). Treatment of visceral leishmaniasis with pentavalent antimony and interferon gamma. *New England Journal of Medicine*, **322**, 16–21.

Bottasso, O. A., Cabrini, J. M., Falcoff, R. & Falcoff, E. (1992). Successful treatment of an antimony resistant American mucocutaneous leishmaniasis: a case report. *Archives of Dermatology*, **128**, 996–997.

Harms, G., Zwingenberger, K., Chehade, A. K., Talhari, S., Racz, P., Mouakeh, A., Douba, M., Nakel, L., Naiff, R. D., Kremsner, P. G., Feldmeier, H. & Bienzle, U. (1989). Effects of intradermal gamma-interferon in cutaneous leishmaniasis. *Lancet*, **i**, 1287–1292.

Harms, G., Chehade, A. K., Douba, M., Roepke, M., Mouakeh, A., Rosenkaimer, F. & Bienzle, U. (1991). A randomized trial comparing a pentavalent antimonial drug and recombinant gamma-interferon in the local treatment of cutaneous leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 214–216.

Hibbs, J. B. (1991). Synthesis of nitric oxide from L-arginine: a recently discovered pathway induced by cytokines with antitumour and antimicrobial activity. *Research in Immunology*, **142**, 565–569.

Marsden, P. D., Sampaio, R. N., Carvalho, E. M., Veiga, J. P., Costa, J. L. & Llanos-Cuentas, E. A. (1985). High continuous antimony therapy in two patients with unresponsive mucosal leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **34**, 710–713.

Marsden, P. D., Badaró, R., Netto, E. M. & Casler, J. D. (1991). Spontaneous clinical resolution without specific treatment in mucosal leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 221.

Maüel, J., Ransijn, A. & Buchmüller-Rouiller, Y. (1991). Killing of leishmania parasites in activated murine macrophages is based on an L-arginine-dependent process that produces nitrogen derivatives. *Journal of Leukocyte Biology*, **49**, 73–82.

Miller, A., Hoogstraten, B., Staquet, M. & Winkler, A. (1981). Reporting results of cancer treatment. *Cancer*, **47**, 207–214.

Muller, I., Pedrazzini, T., Farrel, J. P. & Louis, J. (1989). T-cell responses and immunity to experimental infection with *Leishmania major*. *Annual Review of Immunology*, **7**, 561–578.

Nacy, C. A., Fortier, A. H., Meltzer, M. S., Buchmeier, N. A. & Schreiber, R. D. (1985). Macrophage activation to kill *Leishmania major*: activation of macrophages for intracellular destruction of amastigotes can be by both recombinant interferon and non-interferon lymphokines. *Journal of Immunology*, **135**, 3501–3506.

Nacy, C. A., Nelson, B. J., Meltzer, M. S. & Green, S. J. (1991). Cytokines that regulate macrophage production of nitrogen oxides and expression of antileishmanial activities. *Research in Immunology*, **142**, 573–576.

Scott, P. (1991). IFN-γ modulates the early development of Th1 and Th2 responses in a murine model of cutaneous leishmaniasis. *Journal of Immunology*, **147**, 3149–3155.

Veiga, J. P. R., Wolff, E. R., Sampaio, R. N. & Marsden, P. D. (1983). Renal tubular dysfunction in patients with mucocutaneous leishmaniasis treated with pentavalent antimonials. *Lancet*, **ii**, 569.

Received 8 October 1992; revised 15 March 1993; accepted for publication 17 March 1993