MAJOR ARTICLE

Role of Imiquimod and Parenteral Meglumine Antimoniate in the Initial Treatment of Cutaneous Leishmaniasis

Iracema Arevalo,[1] Gianfranco Tulliano,[2] Ana Quispe,[2] Gerald Spaeth,[3] Greg Matlashewski,[4] Alejandro Llanos-Cuentas,[2] and Henry Pollack[1]

[1]Division of Pediatric Infectious Diseases, New York University School of Medicine, New York; [2]Instituto de Medicina Tropical Alexander von Humbolt, Universidad Peruana Cayetano Heredia, Lima, Peru; [3]Laboratory of Parasite Virulence, Department of Parasitology, Bâtiment Calmette, Pasteur Institute, Paris, France; and [4]Department of Microbiology and Immunology, Mcgill University, Montreal, Quebec, Canada

Background. Cutaneous leishmaniasis is a serious public health problem in the developing world. The main therapeutic agent—pentavalent antimony, developed >50 years ago—is expensive, often accompanied by severe adverse effects, and complicated by the emergence of drug resistance. Better therapies are urgently needed. In the present pilot study, we compared the use of imiquimod, an immunomodulatory molecule, to the use of meglumine antimoniate alone and in combination for the initial treatment of cutaneous leishmaniasis.

Materials and methods. Patients with newly diagnosed cutaneous leishmaniasis were enrolled from a single referral center in Lima, Peru, from August 2005 through October 2005. Patients were randomly assigned to 1 of 3 treatment groups and received either imiquimod 7.5% cream administered topically every other day for 20 days, intravenous meglumine antimoniate administered at a dosage of 20 mg/kg per day every day for 20 days, or combination therapy with both intravenous meglumine antimoniate and imiquimod 7.5% cream. Patients were evaluated weekly and at 1 and 3 months after treatment. Patients who had healed lesions at 3 months were considered to be clinically cured.

Results. Although several patients showed initial resolution of symptoms with imiquimod treatment alone, all of these patients experienced relapse after treatment discontinuation. Four (57%) of 7 patients treated with meglumine antimoniate alone and 7 (100%) of 7 patients treated with combination therapy were cured. Combination therapy was not only more effective than the other 2 treatments ($P < .05$) but also led to faster healing and better cosmetic results.

Conclusion. Combination therapy with imiquimod and meglumine antimoniate is a promising regimen for the initial treatment of cutaneous leishmaniasis that warrants additional larger studies.

Leishmania infection continues to be a major health problem, affecting >12 million people worldwide [1]. Leishmaniasis includes a wide spectrum of diseases, including visceral, cutaneous, and mucocutaneous clinical manifestations. Cutaneous leishmaniasis (CL), the most common form of leishmaniasis, is a disease that is prevalent throughout the world, with 1–1.5 million cases reported annually [2]. In South America, >14,000 cases of leishmaniasis are reported each year in the Andean regions of Colombia, Venezuela, Bolivia, and Peru, where the disease is endemic [1]. Most of the cases in the Andes region are CL due to *Leishmania (Viannia) braziliensis, Leishmania (Viannia) peruviana, Leishmania mexicana,* and *Leishmania amazonensis* [1].

Typically, lesions evolve from papules to nodules and then to ulcers with a central depression and raised, indurated borders. In South America, lesions are most commonly ulcerative and may persist for months or years. They often result in disfiguring scars, especially on the face, that can have deep psychological consequences and result in diminished employment opportunities [1, 2]. New treatments that would speed up the

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Reprints or correspondence: Dr. Henry Pollack, Div. of Pediatric Infectious Diseases, New York University, 550 First Ave., New York, NY 10016 (henry.pollack@med.nyu.edu).

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healing process and at the same time decrease scarring would be an important contribution to the management of this disease.

The mainstay of treatment for all types of leishmaniasis is pentavalent antimonial salts (meglumine antimoniate and pentostam [GlaxoSmithKline]). These are administered intravenously and can have serious adverse effects. The treatment success rate using antimonials is only 60%–80% [1]. In Peru, the treatment failure rate for the initial course of antimonial treatment is ~20%. Many of these cases can be cured with a second course of treatment or the use of other, much more expensive antileishmanial medications, such as amphotericin B. Because of these therapeutic limitations, there is an urgent need for new, more effective treatments that will improve both the efficacy and the tolerability of current therapeutic regimens.

To this end, we previously reported the use of imiquimod, a potent immune response modifier when associated with standard pentavalent antimonial treatment in patients who did not respond to a previous course of antimonials alone [3,10]. We were able to show that the addition of imiquimod cream to the treatment regimen significantly increased the rate of cure at the end of the second round of treatment in patients with presumed antimony-resistant CL, compared with persons treated with meglumine antimoniate alone [3, 10]. Furthermore, we demonstrated that imiquimod was safe and not only increased the rate of cure but also reduced the amount of scarring.

In the present randomized 3-arm pilot study, we examined responses to treatment with imiquimod 7.5% cream, meglumine antimoniate, and a combination of imiquimod plus meglumine antimoniate during the initial treatment of patients with new diagnoses of CL.

PATIENTS, MATERIALS, AND METHODS

Study subjects. This study was carried out at Cayetano Heredia Hospital in Lima, Peru, from August 2005 through October 2005. Adult patients (>18 years of age) with a confirmed diagnosis of CL and who had been newly referred to the outpatient *Leishmania* clinic were enrolled in the study after signing written informed consent forms. The patients were from cities in Peru where CL is endemic. The clinical diagnosis of CL had been confirmed in all patients by direct smear (using Giemsa staining), by culture (in Novy-Macneal Nicolle media), and/or by PCR prior to enrollment. All patients had been skin-tested for leishmaniasis (using the Montenegro skin test). Patients with mucosal involvement, other known diseases (e.g., AIDS, tuberculosis, bartonellosis, leprosy, or sporotrichosis), immunodeficiency, lesions >25 cm^2 in area, and those with a history of previous treatment for leishmaniasis were excluded, as were women who were breast-feeding or pregnant.

All patients had a complete clinical history recorded and physical examination performed at the time of enrollment. If bacterial superinfection of a lesion was observed, the patient was administered a regimen of daily cleansing and an oral antibiotic prior to the start of study medication. Lesions were measured and photographs were taken before, during, and at the conclusion of treatment.

This study was approved by the Institutional Review Board of New York University School of Medicine (New York, New York) and the Ethical Committee of the Universidad Peruana Cayetano Heredia (Lima, Peru).

Study design. Patients were recruited and assigned randomly to 1 of the following 3 treatment groups: (1) meglumine antimonate (glucantime; Aventis Pasteur), (2) imiquimod cream (Dutriec SRL), or (3) meglumine antimoniate and imiquimod cream. Meglumine antimoniate was administered daily at a dosage of 20 mg/kg by slow intravenous infusion over a 10-min period. Imiquimod was applied every other day as a 7.5% topical cream directly to the lesion(s). Imiquimod was provided in a syringe that contained a total of 10 doses. Each dose contained 125 mg of imiquimod. The amount of drug dispensed was based on the surface area of the lesion: if the lesion was ⩽3 cm in length, 1 dose of imiquimod was applied; if the lesion was >3 cm in length, 2 doses of imiquimod were applied. After application of the cream, each individual lesion was covered with an occlusive dressing (tegaderm patch [3M]) that was maintained for 6 h to ensure adequate exposure to the medication. Patients were instructed to remove the patch after 6 h, to wash the lesions with soap and water, and to record any adverse events daily in a log. The patients were examined and treatments were administered daily in the morning by the same study physician. Photographs of each lesion were obtained, and clinical outcome was recorded on days 0, 10, and 20 of active treatment and at each follow-up visit. For patients treated with meglumine antimoniate, serum liver enzyme levels,

Table 1. Demographic characteristics of study subjects with cutaneous leishmaniasis.

Variable	Patients (n = 20)
Age, years	
Mean ± SD	34.9 ± 15.9
Median (range)	32 (18–87)
Sex	
Male	11
Female	9
Occupation	
Andean farmer	15
Biologist	1
Household worker	2
Student	2

NOTE. Data are no. of patients, unless otherwise indicated.

Table 2. Characteristics of lesions in patients with cutaneous leishmaniasis, by treatment group.

Variable	Imiquimod group (n = 6)	Meglumine antimonate group (n = 7)	Combination imiquimod and meglumine antimonate group (n = 7)
Location of lesion, no. of lesions			
Face	3	4	3
Upper extremity	3	2	2
Lower extremity	1	4	4
Duration of disease, months			
Mean ± SD	4.2 ± 2.0	5.07 ± 4.6	6.2 ± 8.7
Median (range)	5.5 (1–6)	4 (1–12)	3 (1.5–26)
Lesion area, cm^2			
Mean ± SD	4.0 ± 4.1	7.1 ± 8.7	8.1 ± 10.4
Median (range)	2.7 (0.4–12.5)	1.5 (0.18–25.5)	5.0 (0.9–33)
Type of lesion			
Ulcerative	5	6	6
Nodular	1	1	1
Adenopathy			
Present	3	5	4
Absent	3	2	3
Bacterial superinfection			
Present	0	1	1
Absent	6	6	6

NOTE. Data are no. of patients, unless otherwise indicated.

creatinine levels, and electrolyte levels were measured on days 0, 10, and 20 of treatment. The duration of treatment was 20 days for all of the treatment regimens, and patients were then evaluated at 1 month and 3 months after treatment.

Clinical results were defined as follows: (1) clinical cure was defined as complete reepithelialization without signs of inflammation; (2) clinical improvement was defined as a reduction in lesion size and inflammation but without full reepithelialization; (3) treatment failure was defined as no improvement, with the lesion either unchanged or larger than at the start of treatment; and (4) relapse was defined as signs of activity, such as edema, erythema, or an open ulcer of a lesion that was considered to be clinically cured at the end of treatment. In patients with >1 lesion, the final response score was based on the lesion that showed the least improvement. At the termination of the treatment period or during follow-up visits, patients for whom therapy had failed were offered outpatient treatment with a second course of intravenous meglumine antimoniate, according to the established guidelines of the Peruvian Ministry of Health. The following scale for evaluating adverse effects was used: mild, defined as causing no significant interference with daily activities; moderate, defined as causing mild interference with daily activities but not requiring treatment; and severe, defined as moderate or severe interference with daily activities, requiring treatment or intervention.

Statistical analysis. Analysis of variance was used to compare the means of continuous variables (duration of disease and area of lesion) associated with 3 treatment groups. Clinical outcomes of 2 alternative treatments were compared with outcomes of the standard treatment using Fisher's exact test. The Cochran-Armitage exact trend test was used to compare all 3 groups together; tests of significance were performed with an $\alpha = 0.05$ and were 2-sided. All statistical analyses were performed using Sigma stat software, version 9.0 (SPSS).

RESULTS

A total of 20 patients were enrolled in the study from August through October 2005. Eighteen (90%) of 20 patients had positive direct smear and PCR results. Eleven (55%) of 20 patients had positive Montenegro skin test results. Nine (45%) of 20 patients had a culture positive for *Leishmania*. The demographic characteristics of the patients are presented in table 1. The mean age was 34.9 years. Eleven patients were male, and 9 were female. The majority of the patients were farmers. There were no differences in demographic characteristics among the 3 treatment groups. Twenty lesions were recorded among the 20 patients enrolled in the study. The characteristics of the lesions are outlined in table 2. All lesions were located on exposed areas: 10 lesions were located on the face, 7 lesions

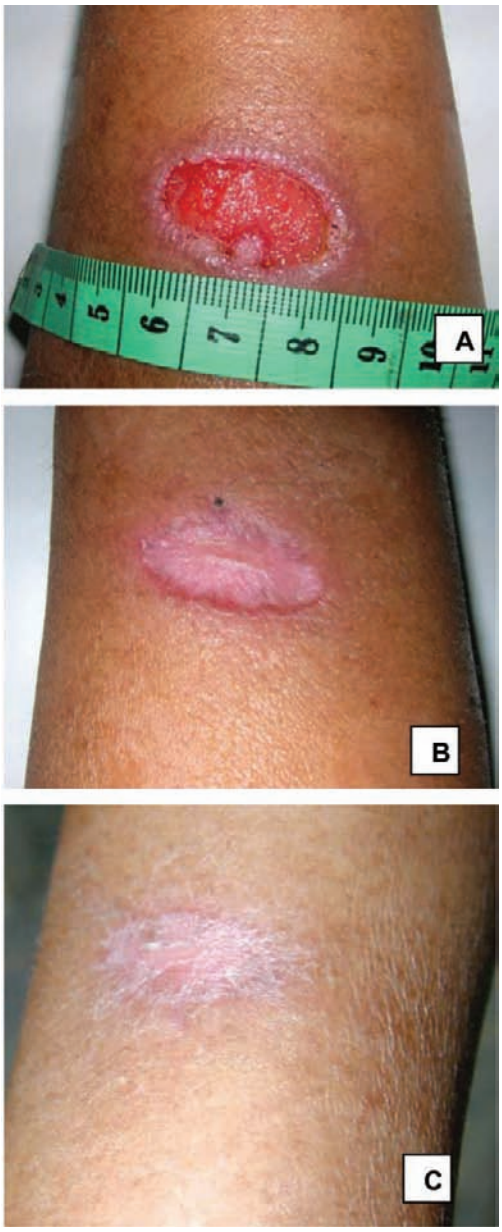

Figure 1. Photographs of a skin lesion on a 46-year-old patient with cutaneous leishmaniasis treated with imiquimod 7.5% cream and meglumine antimoniate, showing the lesion before treatment *(A)*, when the lesion measured 3 cm × 1.8 cm; on day 20 of treatment *(B)*, when the lesion had undergone complete reepithelialization; and 1 month after the end of treatment *(C)*.

were located on the upper extremities, and 9 lesions were located on the lower extremities. Twenty-three lesions were ulcerative, and 3 were nodular.

Six patients were treated with imiquimod, 7 patients were treated with meglumine antimoniate, and 7 patients were treated with meglumine antimoniate and imiquimod together. The mean durations of the time from the initial presentation of lesions to initiation of treatment were 4.2, 5.1, and 6.2 months for patients treated with imiquimod, meglumine antimonate, and imiquimod plus meglumine antimonate, respectively. There were no differences in the type, number, or distribution of lesions among the 3 treatment groups.

Response to treatment. All patients completed the 3 weeks of treatment. The clinical responses at day 10 and day 20 of treatment and at the 1-month and 3-month follow-up visits for each treatment group are presented in table 3.

Overall, combination therapy was associated with more-rapid resolution, a higher end-of-treatment response, and a higher sustained treatment response at the end of 3 months. Although no difference was found in lesion size at day 10 and day 20 in the group treated with imiquimod, compared with those treated with meglumine antimoniate alone, qualitative differences were observed in the scar tissue and pigmentation.

In the group treated with imiquimod alone, 4 (67%) of the 6 patients initially showed clinical improvement, often by the third dose of imiquimod, with a reduction in the size of the lesion and partial reepithelialization of almost all lesions by approximately day 10 of treatment. Two patients (33%) were considered to be clinically cured at the end of treatment. The 4 patients who showed initial improvement but subsequently experienced failure of therapy with imiquimod were treated with meglumine antimoniate. Of the 2 patients who were considered to be cured at the end of treatment, 1 patient had experienced relapse by 1 month and the other patient had experienced relapse by 3 months. Both patients were subsequently treated with meglumine antimoniate, and 1 patient, who completed the full course of treatment, was cured. The other patient voluntarily discontinued therapy after 2 weeks because of flu-like symptoms related to the use of meglumine antimoniate.

In the group treated with meglumine antimoniate alone, 4 (57%) of 7 patients were cured at the end of treatment and remained cured at 3 months after treatment. The other 3 patients (43%) required a second course of meglumine antimoniate, and all remained lesion-free at 3 months after the completion of the second treatment course.

In the group treated with imiquimod and meglumine antimoniate combined, 5 (72%) of the 7 patients were cured at the end of treatment. For the 2 remaining patients, clinical improvement continued after the end of treatment; 1 patient's lesions had healed by 1 week and the other patient's lesions had healed by 2 weeks after the completion of treatment. All 7 patients remained clinically cured 3 months after treatment. In addition, the rate of healing of lesions in the combined therapy group was more rapid than that seen in the group of patients who were treated with either imiquimod or meglumine antimoniate alone (figure 1). By day 10, combination therapy with imiquimod cream and meglumine antimoniate showed a statistically significant reduction in lesion size ($P < .05$) that

Table 3. Treatment results.

Treatment group	No. of patients	No. (%) of patients			
		With initial response at day 10[a]	With clinical cure at day 20[b]	With clinical cure at 1 month of follow-up	With clinical cure at 3 months of follow-up[c]
Imiquimod	6	4 (67)	2 (33)	1 (17)	0 (0)
Meglumine antimonate	7	5 (71)	4 (57)	4 (57)	4 (57)
Combination imiquimod and meglumine antimonate	7	6 (85)	5 (72)	7 (100)	7 (100)

[a] Initial response was defined as a reduction in the size of the lesion.
[b] End of treatment.
[c] $P < .05$ for cure at 3 months of follow-up.

persisted through the duration of treatment. Combination therapy was found to be more effective than meglumine antimonate and imiquimod alone at 3 months of follow-up in a stepwise fashion using the Cochran-Armitage exact trend test (2-sided $P = .001$).

Adverse events. Among patients treated with imiquimod cream, 10 (77%) of 13 reported mild adverse events including localized pruritus, erythema, and edema. Adverse effects observed in patients treated with meglumine antimoniate were generally more severe: 12 (86%) of 14 patients reported arthralgia, myalgia, and flu-like symptoms. Nine (64%) of the 14 patients treated with meglumine antimonate had elevated liver enzyme levels, none of which resulted in the discontinuation of therapy. However, 1 patient voluntarily discontinued treatment with meglumine antimoniate on day 15 of retreatment because of flu-like symptoms, arthralgia, and myalgia. Adverse events were first observed at the conclusion of the first week of treatment. No serious adverse events were observed in any study patients.

DISCUSSION

The present study provides important new information about the use of combination therapy in patients with CL and, in particular, provides information on the use of combination therapy in treatment-naive patients. Combination imiquimod–meglumine antimoniate therapy was associated with a higher percentage of clinical cure (defined as being lesion-free at 3 months) and a higher sustained treatment response than treatment with either meglumine antimoniate or imiquimod alone. The higher treatment response eliminated the need for a second course of meglumine antimoniate, with its many potentially serious adverse effects and higher costs, in those patients receiving combination treatment. Combination therapy also caused a more rapid reduction in lesion size. These results suggest that combination therapy is more effective than monotherapy for the treatment of newly diagnosed CL caused by *Leishmania* species that are endemic in Peru. By contrast, none of the patients treated with imiquimod alone and only 57% of patients treated with meglumine antimoniate alone remained clinically cured 3 months after treatment was stopped.

However, the initial response to imiquimod, measured by the reduction in the size of a lesion between day 0 and day 10, was as good as that observed with meglumine antimoniate. Healing was often observed by the end of the first week of treatment, but the effect was transient, the lesions never fully resolved, and all patients experienced relapse after the cessation of treatment. Similar results have been recently reported with imiquimod in the treatment of Old World CL [7].

Normally, scars caused by CL have a distinctive central depressed surface, covered by hyperpigmentated skin and rounded contours. These features make the scarring very pronounced, particularly on the face. In this trial, we confirmed a previous observation that imiquimod reduced lesion scarring formation, particularly by reducing hyperpigmentation [3]. Although rigorous criteria for scar formation were not used in the present study, the results are consistent with a recent report that showed that imiquimod treatment reduced keloid formation following surgery [6].

Imiquimod (1-[2-methylprophyl]-1H-imidazo[4,5c] quinoline-4-amine) is a synthetic, low–molecular weight imidazoquinoline compound that acts as an immune response modulator. It has been shown to induce Th1 responses and to activate dendritic cells and monocytes when applied to the skin. Previous studies have shown that imiquimod and its more potent analog, S28463, directly activate macrophages and mediate intracellular killing of *Leishmania* amastigotes in the absence of T cells [8, 9] by activating the NF-kB and AP1 signaling pathways [8] through binding to Toll-like receptor 7 [5]. This results in the secretion of proinflammatory cytokines, including IL-12 [4, 5], that play a pivotal role in the development of the Th1 immune response, which is central to the resolution of *Leishmania* infection [4]. The specific mechanism of action of imiquimod in CL, however, is unknown. Why treatment failed is not clear, and whether a longer course of treatment would have been more successful remains to be determined.

In summary, this study suggests that topical imiquimod may

be a useful and important addition to the current initial treatment regimen for CL. When used in combination with meglumine antimoniate, it not only led to a higher rate of cure, but also increased the speed of healing and improved the overall cosmetic effect. When used alone, without meglumine antimoniate, imiquimod brought about rapid initial healing of lesions in almost 50% of subjects but failed to achieve a response that was maintained after treatment was stopped. The results of this pilot study warrant larger studies to confirm the benefits of combination imiquimod-meglumine antimoniate therapy for the initial treatment of patients with CL. Additional studies are also necessary to assess whether a longer or more intensive course of treatment with single-agent imiquimod therapy could lead to more durable results and whether a shorter course of combination therapy, with its lower cost and fewer adverse effects, might be as effective as standard therapy in these patients.

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