RANDOMIZED, CONTROLLED, DOUBLE-BLIND TRIAL OF TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS WITH PAROMOMYCIN PLUS METHYLBENZETHIONIUM CHLORIDE OINTMENT IN GUATEMALA

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Abstract. A double-blind, randomized trial was undertaken in Guatemala to determine the therapeutic efficacy of an ointment for the treatment of cutaneous leishmaniasis that contained 15% paromomycin and 12% methylbenzethonium chloride and that was applied twice a day for 20 days. The treatment group included 35 patients, and the placebo group included 33 patients. The initial clinical response rate (13 weeks after completing the treatment) was 91.4% in the treatment group and 39.4% in the placebo group. The final clinical response rate at the 12-month follow-up examination was 85.7% (31 of 35) in the treatment group and 39.4% (13 of 33) in the placebo group (P ≤ 0.001). In general, the treatment was well tolerated and was never interrupted because of adverse effects. The number of adverse effects reported in the placebo group was lower than in the treatment group (16 events versus 30 events). All adverse effects reported by patients disappeared within 1 week of completing the treatment. Our findings show that the combination of paromomycin with methylbenzethonium chloride for 20 days is a good alternative for antimonial treatments of cutaneous leishmaniasis in Guatemala.

INTRODUCTION

More than half a century after the introduction of antimonials into the chemotherapy of leishmaniasis, pentavalent antimonials—either sodium stibogluconate or meglumine antimonate—remain the drugs of choice in the treatment of all forms of leishmaniasis. They are widely used despite their toxicity, difficulty of administration, high cost, and the promising good clinical response showed by some other chemotherapeutic agents.1–5 The development of new drugs for the treatment of leishmaniasis has been impeded by the lack of a simple and rapid drug evaluation system, applicable to the various Leishmania species infecting humans. In addition, leishmaniasis, like most of the protozoan diseases, is largely a problem of developing countries and therefore offers little commercial incentives for pharmaceutical companies to develop cheap and effective antileishmanial drugs.6

The development of a compound used for the topical treatment of cutaneous leishmaniasis (CL) is an attractive option for overcoming the problems of antimonials. One line of research has focused on paromomycin (PR) ointment in different combinations. Paromomycin is an aminocyclitol amionoglycoside antibiotic used for the treatment of leishmaniasis. Diverse preparations of topical PR have been tested on CL in animals and humans. Randomized, placebo-controlled trials that used 15% PR plus 10% urea topically applied during 2 weeks, for example, have been conducted in Tunisia,7 Iran,7 and Honduras.8

The topical application of PR (15%) plus methylbenzethonium chloride (MBCL; 12%) in a soft paraffin-based ointment was effective against Leishmania major in both experimental animals and in humans.9–11 In a study of 52 patients with CL in Ecuador, it was reported that after 20 applications of PR ointment over 10 or 20 days, 90% of lesion were cured at 100 days after treatment and 85% at the 1-year follow-up examination, but only 9% of the lesions of untreated patients healed spontaneously after 50 days.12 However, this controlled trial under field conditions did not include randomization or placebo treatment. In Colombia, the application of PR ointment in combination with systemic meglumine antimonate treatment did not show an additional benefit when compared with meglumine antimonate alone.13

In order to shed light on the question whether PR ointment is an efficacious alternative treatment for CL, a double-blind, randomized trial was undertaken in Guatemala that used an ointment containing 15% PR and 12% MBCL applied twice a day for 20 days.

MATERIAL AND METHODS

Patients. The study population was selected from patients attending the leishmaniasis clinic of the Universidad del Valle de Guatemala. Persons were eligible for the study if they met the following criteria: either male or female sex, aged 10–60 years, and parasitologically confirmed CL. Further, all patients had to provide written informed consent to participate in the study, and they had to be available for follow-up examinations for 12 months. People were excluded from the study for any of the following reasons: > 4 lesions or an active lesion measuring > 5 cm in diameter; previous use of antimony-containing drugs; serious concomitant medical problems; and evidence of mucosal involvement of leishmaniasis.

Topical treatment. The PR ointment, paromomycin sulfate 15% plus MBCL 12%, and the placebo ointment tubes, which contained only soft paraffin, were prepared and randomly numbered by Teva Pharmaceutical Industries, Petach Tikva, Israel, and provided to the investigators by Dr. F. Modabber (Tropical Diseases Research/World Health Organization [TDR/WHO]). Both active and placebo ointment were supplied in tubes of 14 g; they were identical in appearance and were marked only by a consecutive number. Neither the researchers nor the patients knew if the active substance or the placebo were in the tube. The codes identifying the contents of each tube were kept in Geneva by the TDR/WHO representative (F. M.) until the study was completed. The ointment was applied topically twice a day for 20 days across the lesions in 2 different directions at 90 degrees to each other. Patients were instructed to wash their
lesions with soap and water before applying the ointment. After the applications, the lesion was left uncovered. The amount of ointment used during each application varied depending on the lesion size; however, a 14-g tube per patient was enough to treat all the patients.

**Patient evaluation.** Confirmation of the CL infection was based on either observation of amastigotes in thin smears obtained from the edge of the ulcer and stained with Giemsa stain, or by culture of promastigotes from aspirates taken through the skin adjacent to the ulcer, following previously described methods. Only patients with positive cultures or clearly distinguishable amastigotes entered the study. As reported in previous studies, most of the patients (75%) we studied are infected with *L. braziliensis*, and the rest (25%) are infected with *L. mexicana*; therefore, no characterization of *Leishmania* parasites was performed. Surprisingly, despite the high incidence of *L. braziliensis* infections, very few cases of mucocutaneous leishmaniasis were found in the study area (Arapa B, unpublished data).

All patients were evaluated at the end of Weeks 1, 2, 3, 4, 6, 9, 13, 26, and 52 after therapy. The treatment response was classified as follows:

1. **Initial clinical response:** a patient whose lesions had completely reepithelialized and who had no evidence of inflammation or indurations by the 13-week follow-up examination.
2. **Final clinical response:** a patient who had an initial clinical response and had no disease reactivation during the follow-up period between 13 and 52 weeks.
3. **Reactivation:** the appearance of a lesion within or at the border of a previously healed lesion.
4. **Treatment failure:** an increase in lesion size of >100% compared with the size at the first day of treatment; lack of a clinical response by the 13-week follow-up examination; or reactivation of a lesion. Patients who experienced treatment failure were removed from the study and treated with meglumine antimonate (20 mg antimony per kilogram of body weight per day, administered intravenously, for 20 days).

**Statistical analysis.** Comparison of response rates over the first 13 weeks was performed by Cox’s log rank test. Comparison of the final response rates and rates of adverse effects were performed by the chi-square test or Fisher’s exact test (Epi Info version 6.04b, Centers for Disease Control and Prevention, Atlanta, GA). All values are 2-tailed, and *P* < 0.05 was considered statistically significant.

**Ethical considerations.** The Ethical Review Committee of the Universidad del Valle de Guatemala approved the study. Informed consent was obtained from all adult participants and from parents or legal guardians of minors.

**RESULTS**

**Patient characteristics.** A total of 76 patients were enrolled. Table 1 shows that randomization successfully allocated subjects with similar characteristics into the treatment and control groups. All 76 patients had their diagnoses confirmed by smear, culture, or both; 36 (47%) of 76 were positive for both culture and smear, 34 (45%) had only positive smears for *Leishmania* amastigotes, and 6 (8%) had only a positive *Leishmania* cultures. The 76 patients had a total of 91 lesions.

**Initial clinical response.** All patients but 4 received their treatment without interruption. The 4 patients who did not finish their treatment, and 4 additional patients who were lost after their 21-day clinical evaluation were not included in the final analysis. Out of the 68 patients who completed their evaluation at the 13-week examination, 35 belonged to the treatment group (PR-MBCL ointment) and 33 to the placebo group.

The initial clinical response rate was 91.4% (32 of 35) in the treatment group and 39.4% (13 of 33) in the placebo group. The cure rate in both treatment groups at the different control times is shown in Figure 1. Among the 3 patients who failed to respond to therapy in the treatment group, 2 (5.7%) showed a reduction in lesion size, and only one (2.9%) showed no clinical improvement at all. In the 20 failures to respond to therapy in the placebo group, 2 patients (6.1%) showed a reduction in lesion size, 1 (3.1%) showed no improvement in the lesions, and 15 (45.4%) showed increased lesion size, including 5 patients who had to be removed from the study before Week 13 because the area of their lesion had more than doubled.

**Final clinical response.** Between Weeks 13 and 52, only one patient (3.1%) of the 32 in the treatment group, and none of the 13 patients in the placebo group, with healed clinical lesions at the 13-week follow-up examination experienced reactivation of the lesion. The reactivation occurred 6 months after the beginning of the treatment. As a result, the final clinical response at the 12-month follow-up examination was 85.7% (31 of 35) in the treatment group and 39.4% (13 of 33) in the placebo group (*P* = 0.000025). All patients in both treatment groups who failed to respond to therapy were treated with meglumine antimonate at 20 mg/kg once a day parenterally for 10 days. All patients were cured with only one course of treatment.

**Adverse effects.** In general, the treatment was well tolerated and had never to be interrupted because of adverse effects. Of the 35 patients who received PR-MBCL ointment, 22 (62.8%) reported a total of 30 adverse effects. These effects included local pruritus (46.7%), sensation of
FIGURE 1. Percentage of patients who responded to treatment with paromomycin (PR) ointment or placebo by week of follow-up examination

- Burning (30%), local pain (20%), and local edema (3.3%). All adverse effects reported by patients disappeared within 1 week after finishing the treatment.
- No statistically significant difference was found between the number of patients reporting adverse effects in the treatment and placebo group (22 of 35 versus 14 of 33; \( P = 0.14 \)). However, the number of adverse effects reported by the placebo group was lower (16 events) than the number of adverse effects in the treatment group (30 events).

**DISCUSSION**

Topical treatment of CL is attractive compared with the systemic treatment with antimonials because of the easy application, particularly in remote areas (Behrend M, unpublished data) and the reduced cost. Likewise, the treatment with orally administered antileishmanial drugs, which have been tested successfully in Guatemala, are expensive and the treatment course prolonged, which make them, from a public health services perspective, not a good choice. The main question with topical treatments is related to its therapeutic efficacy and its potential adverse effects. The controlled trials on therapeutic efficacy of topical CL treatment with PR ointment are summarized in Table 2. They all used PR mixed with either urea (10%) or MBCL (in concentrations of 5 or 12%) together or not with meglumine antimonate. The therapeutic effect was measured either against a placebo group or a quas placebo group (i.e., without treatment) or against a group treated with meglumine antimonate. There was a wide variation in the study protocols used, in the determination of cure rates, and in the pathogenicity of the *Leishmania* parasites, so a comparison of the therapeutic efficacy is difficult. However, the following pattern seems to emerge: urea (10%) in addition to PR seems to have little or no therapeutic efficacy. The poor results with PR when combined with urea emphasize the antileishmanial properties of MBCL shown by El-On and Messer and the synergetic anti leishmanial effect of MBCL and PR. Additionally, PR as an antibiotic may reduce secondary infections and promote healing. Only in a recent controlled trial in Ecuador has the combination of PR with urea showed a similar cure rate of CL (70% in 30 patients) as PR-MBCL (78.2% in 23 patients) and meglumine antimonate (88.6% in 35 patients) after 1 year of observation; however, the treatment period was very long: twice a day for at least 12 weeks.

The addition of MBCL to PR apparently has a high therapeutic efficacy if applied long enough—that is, for > 15 days. This was reconfirmed by our study in Guatemala, which showed a therapeutic efficacy of 54% (cure rate of treatment group minus cure rate in placebo group divided by cure rate in treatment group). In other words, the probability of cure with the PR-MBCL in 20 days was 2.17 times higher than in the placebo group. This result is no different from the cure rates obtained in the same endemic area, where a 90% cure rate has been obtained when meglumine antimonate is used at doses of 20 mg/kg for 10–20 days (\( P = 0.95 \)). In terms of cost, however, if we could prepare the ointment locally, it would cost US$6.00, as compared with...
the standard treatment with meglumine antimonate, which costs US$280 in Guatemala.

Our study on the socioeconomic consequences of CL in Venezuela has shown that the direct and indirect costs for the patients are high and the negative social consequences severe. This is why an efficacious treatment of this generally self-limiting disease is important both for patients and health services. Operational research has shown that the topical treatment, with few well-defined exceptions, can be easily applied by basic health staff (Behrend M, unpublished data), so this option should be pursued further.

In addition, topical treatment has no systemic effects and cannot therefore prevent relapses, or in some areas the development of the mucocutaneous forms of the disease. However, the recurrence of cutaneous lesions and parasite persistence has been reported in patients treated for CL; there are also reports describing the detection of leishmanial DNA by means of polymerase chain reaction in patients with visceral leishmaniasis who received treatment with antimonials and were believed to be cured.23 26

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