Objective: To determine the efficacy and safety of imiquimod in combination with meglumine antimoniate in treating cutaneous leishmaniasis.

Design: Prospective, randomized, assessor-blind, parallel-design, placebo-controlled trial.

Setting: Two primary care health clinics.

Patients: One hundred nineteen patients (59 patients in the imiquimod group and 60 in the placebo group) were included in the study.

Interventions: Patients were randomly assigned to receive a combined 4-week course of imiquimod or placebo with meglumine antimoniate treatment (20 mg/kg of pentavalent antimony daily for 2 weeks) in an endemic area of *Leishmania tropica*.

Main Outcome Measures: The primary end point was clinical cure, defined as more than 75% reduction in the size of lesions compared with baseline at week 8.

**Results:** At the end of the 4-week treatment period, clinical cure was similar in both groups (11 patients [18.6%] in the imiquimod-treated group vs 18 patients [30.0%] in the placebo group) ($P = .15$). Four weeks after the end of treatment, 26 patients (44.1%) and 29 patients (48.3%) in the imiquimod-treated and placebo groups, respectively, were cured ($P = .64$). Pruritus and burning sensation were reported by 3 patients treated with imiquimod and by no patients treated with placebo.

Conclusion: This study showed no beneficial effect of combining a 4-week course of treatment with 5% imiquimod cream and a standard course of treatment with meglumine antimoniate in patients with cutaneous leishmaniasis in an endemic area of *L. tropica*.

**Trial Registration:** [isrctn.org Identifier: ISRCTN77659407](https://isrctn.org) and Cochrane Skin Group Identifier: CSG Trial No. 32

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Leishmaniasis is caused by 20 different species of the intracellular protozoan *Leishmania* and is transmitted by the bite of infected sandflies. Cutaneous leishmaniasis (CL) caused by *Leishmania tropica* (anthroponotic CL [ACL] in urban areas) or by *Leishmania major* (zoonotic CL in rural areas) is endemic in Iran. The initial sign of infection is the appearance of a papule at the feeding site of the sandfly. The papule develops into an ulcer with a violaceous border, healing spontaneously in several weeks to months, usually with scarring. This evolutionary course is much longer in ACL lesions than in zoonotic CL lesions, and the lesions may not ulcerate.

Systemic pentavalent antimonials (sodium stibogluconate and meglumine antimoniate [MA]) have been used as the standard treatment of CL since 1929. They are only parentally available, which is painful, toxic (especially for the heart and liver), and unaffordably costly in most endemic areas. In addition, resistance of *Leishmania* to antimonials has been reported, and several courses of treatment may be necessary.

Several other physical, topical, systemic, and immunological agents have been used in the treatment of CL, with controversial results. The variation in the efficacy of treatment modalities for CL is due to several factors, including poor design of the studies, differences in the causative species of *Leishmania*, consideration of the self-healing nature of CL, and differences in the criteria used to evaluate the efficacy of drugs.

In a 1999 study, imiquimod was shown to be an effective treatment of an experimental model of leishmaniasis. It is an immune response modifier that is unique in its activation of local immune functions.
Imiquimod activates Langerhans cells and enhances their migration to draining lymph nodes, indirectly stimulating production of a helper T-cell type 1 response that has been shown to be involved in resistance to Leishmania parasites. Imiquimod is approved by the US Food and Drug Administration as a 5% cream for the treatment of genital warts and has shown promising results in the treatment of other viral infections and skin cancers. Imiquimod is safe and the most frequently reported adverse reactions are local skin reactions, which are mild to moderate in most cases.

In an open study of 12 patients with CL resistant to MA alone, imiquimod in combination with MA cured 90% of the patients. Later, a randomized, double-blind clinical trial of patients with CL caused by Leishmania peruviana showed the efficacy of combination treatment with imiquimod and MA.

In this study, the efficacy of combination treatment with intramuscular injections of MA and topical imiquimod cream in ACL was evaluated in a randomized single-blind clinical trial. The results were analyzed according to the principles of good clinical practice.

**METHODS**

**PARTICIPANTS**

Inclusion criteria were (1) parasitologically proven cases of CL based on positive smear or culture, (2) otherwise healthy subjects, (3) age 12 to 60 years, and (4) willingness to participate in the study and sign the informed consent (by the patient or his or her parent or guardian in patients younger than 18 years).

Exclusion criteria were (1) pregnant or lactating women, (2) duration of lesions longer than 6 months, (3) number of lesions more than 5, (4) any lesions larger than 5 cm, (5) history of any standard course of treatment with antimonials, (6) history of allergy to antimonials, (7) serious systemic illnesses (as judged by the physician), and (8) participation in any drug trials in the last 60 days.

**STUDY SETTING AND LOCATION**

The study was carried out in the city of Mashad in the Khorasan Razavi province in northeast Iran, which is an endemic area for ACL caused by L. tropica. The eligible patients were referred to primary care health clinics established by the Undersecretary of Public Health at the Mashad University of Medical Sciences.

**INTERVENTIONS**

The patients who met the eligibility criteria of the trial were randomly divided into 2 groups according to a list made by a simple randomization block design. All patients were treated daily with 20 mg/kg of pentavalent antimony (Glucantime [equivalent to 60 mg/kg of MA]; Rhodia Laboratories, Rhone-Poulenc, France) given as intramuscular injections for 14 days. This treatment schedule was selected based on the national protocol of the treatment of CL issued by the Iran Ministry of Health.

The patients were also treated with 5% imiquimod cream (Al-dara; Laboratoires 3M Santé, Cergy Pontoise, France) or with placebo (petrolatum; Paveh Pharmaceuticals, Paveh, Iran) 3 times per week (Mondays, Wednesdays, and Fridays) for 28 days. Imiquimod was provided as 250-mg sachets; a box of 12 sachets was given to each patient. Because identical placebo sachets could not be prepared, plain petrolatum in 10-g tubes was used as a control. To keep the trial blinded, the physicians who were responsible for evaluation of patients were uninvolved in the process of allocation and drug dispensing and were unaware of the drug used by the patients. A written instruction sheet was given to patients, asking them to apply a thin layer of cream on lesions at bedtime, to massage it into the skin thoroughly, and to wash the lesions 6 to 10 hours after application with soap and water. The patients were visited 2, 4, 8, and 20 weeks after initiation of treatment, and the size of their lesions was measured and recorded at each visit. The ballpoint method was used to determine the greatest diameter of induration of each lesion, which was multiplied by the diameter perpendicular to it.

**END POINTS**

The primary end point of this study was the clinical cure of the patients, defined as more than 75% reduction in the size of lesions compared with baseline. The secondary end points included adverse effects and the rate of clinical improvement (defined as 50%-75% reduction in the size of lesions compared with baseline). These end points were assessed at the end of the treatment period (week 4) and 4 weeks later. The relapse rate (defined as a reappearance of lesions at the site or periphery of previously healed lesions or an increase in the size of lesions after initial improvement) was assessed 16 weeks after the end of treatment.

**ETHICS**

This study (including the protocol and consent form) was reviewed and approved by the Ethics Committee of the Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences, on September 1, 2003. The study was conducted in accord with the ethical principles provided by the Declaration of Helsinki and by ethical codes provided by the Undersecretary of Research at the Iran Ministry of Health. Informed consent was obtained from patients at patient allocation.

**RANDOMIZATION AND BLINDING**

The randomization sequence was generated by the use of a randomization table. A simple block randomization list with a block size of 4 was prepared by a team member (F.G.) who was not involved in the recruitment and follow-up of the patients. The randomization allocation concealment was performed by sending the randomization numbers in envelopes to a pharmacist who was responsible for giving the assigned treatment after each eligible patient was enrolled. The recruited patients were referred to the pharmacist to receive their assigned drugs. The pharmacist evaluated the compliance of the patients by counting the remaining sachets of imiquimod. This study was a single-blind trial in which the outcome assessors (A.F., A.K., and M.H.G.) were unaware of the drugs used by the patients.

**STATISTICAL ANALYSIS**

Forty-five patients per treatment group were needed to have 80% power to detect a significant difference in the expected cure rate of 50% in the placebo group and the desired cure rate of 80% in the imiquimod-treated group at week 8 with a type I error level of .05. To compensate for 20% estimated dropout, 54 patients would be required in each group. In this study, 119 patients were recruited (59 patients in the imiquimod-treated group and 60 patients in the placebo group).
A 2-sided $P<.05$ was considered significant. Data obtained and recorded were entered in SPSS 11.0 for Windows software (SPSS Inc, Chicago, Ill). Data were reported as mean±SD. Because of the abnormal distribution of the size of lesions, which was tested using the 1-sample Kolmogorov-Smirnov test, the median plus minimum and maximum (and interquartile range) were reported for this variable. An intent-to-treat analysis was performed at 2 time points (end of the treatment period [week 4] and 4 weeks later [week 8]). The rates of clinical cure, clinical improvement, and withdrawal were compared between the 2 groups using $\chi^2$ test. Fisher exact test was used to compare the rates of relapse and drug-related adverse events. Confidence intervals were measured for some proportions.

**RESULTS**

From August 1, 2004, through July 25, 2005, 508 patients with CL were screened (Figure). Among them, 119 eligible patients were recruited in this clinical trial; 59 patients with 128 lesions were treated with imiquimod, and 60 patients with 124 lesions were treated with placebo. Forty-five patients in the imiquimod-treated group and 52 patients in the placebo group completed the 4-week course, and 42 patients in the imiquimod-treated group and 47 patients in the placebo group completed the 8-week trial. The numbers of and the reasons for withdrawals were not significantly different between the 2 groups (28.8% [95% confidence interval, 18.8%-41.4%] in the imiquimod-treated group vs 21.7% [95% confidence interval, 13.1%-33.6%] in the placebo group) ($P=.37$).

There were no statistically significant differences between the 2 groups regarding demographics and disease characteristics before the initiation of treatment (Table 1). Clinical evaluation of the patients at the end of the treatment period and 4 weeks later showed no statistically significant differences between the 2 groups (Table 2).

The only adverse effects related to topical treatment were moderate pruritus and burning sensation in 3 patients treated with imiquimod. Although none of the patients treated with placebo complained of these adverse effects, the difference between the groups did not reach statistical significance ($P=.12$). Sixteen weeks after the end of treatment in the patients available for assessment, relapse was observed in 1 of 32 patients treated with imiquimod and in 3 of 37 patients treated with placebo ($P=.63$).

**COMMENT**

In this clinical trial, the efficacy of combining a 4-week course of treatment with 5% imiquimod cream and a course of treatment with 20 mg/kg of pentavalent antimony daily for 2 weeks was assessed in treating CL in an endemic area of *L. tropica*. According to the results of this study, the addition of 5% imiquimod cream for 4 weeks did not improve the response to the treatment with MA. This study was performed in an endemic area of CL caused by *L. tropica*, but it cannot be proven that the causative agent was *L. tropica* in all of the patients, as the identification of *Leishmania* species was infeasible in this setting. Because identical placebo vehicles could not be prepared, petrodatum was used in the control group, but the investigators (A.F., A.K., M.H.G., and M.N.-K.) responsible for recruitment, allocation, and assessment of patients were unaware of topical drugs used by the patients. The duration of treatment with imiquimod was 4 weeks in this study. This duration was selected based on published study findings11,12 about the use of imiquimod in CL. The duration of treatment with imiquimod cream in other indications is usually much longer (12-16 weeks).8-10

Cutaneous leishmaniasis is a major public health problem in many developing countries. Unfortunately, no ideal therapy for CL is available, and its treatment remains a challenge. More than 7 decades have passed since the first use of pentavalent antimonials in the treatment of leishmaniasis, but they remain the mainstay of treatment.1 Clinical trials for CL are usually confronted with several hurdles, including the diversity of *Leishmania* species, rapid self-healing nature of the disease, wide spectrum of clinical disease manifestations, and heterogeneity of the geographical regions where the disease most commonly occurs.4,13

Arevalo et al11 conducted an open-label prospective study in 12 patients with 21 active CL lesions caused by *L. peruviana* who had previously not responded to systemic MA. Each patient was treated with a combination of topical imiquimod every other day and with supervised intramuscular administration of 20 mg/kg of pentavalent antimony daily, delivered for 20 days. Twelve lesions in 6 patients were cured, and 9 lesions in 6 other patients improved. Local adverse effects attributed to imiquimod were mild to moderate erythema (40%), edema (30%), and sensations of burning (10%).

Miranda-Verástegui et al12 recruited 40 patients with CL for whom an initial course of antimony therapy had failed in a randomized, double-blind clinical trial. All patients received MA (20 mg/kg of pentavalent antimony daily, delivered intravenously or intramuscularly) and were randomized to receive topical 5% imiquimod cream...
or vehicle control every other day for 20 days. The cure rate in the imiquimod-treated group was 50% at 1 month (vs 15% in the placebo group), 61% at 2 months (vs 25%), and 72% at 3 months (vs 35%) (P < .05 at all time points).

In contrast to those study findings, no significant differences were found in the present study between patients treated with imiquimod vs placebo in combination with MA. This study was conducted in an endemic area for Old World CL caused by *L tropica*, but the other 2 studies were from parts of Peru that were endemic for the New World CL caused by *L peruviana*. Different causative *Leishmania* species in 2 areas may explain the observed contradictory results. All participants in the Peruvian studies had been treated with standard doses of MA before being recruited, while none of the patients in the present study were treated previously, which may also account for the different findings in these studies.

Seeberger et al. treated 12 patients with 22 lesions of Old World CL with topical imiquimod cream (3 times/wk), and 3 patients who received placebo served as the control group. Twenty of 22 lesions improved after 2 weeks of treatment with imiquimod, but at 6 weeks of treatment, 11 of 12 patients having 19 lesions showed progression. The responsible *Leishmania* species was not mentioned.

Recently, in an open-label trial in Kerman (another endemic area for Old World CL caused by *L tropica* in Iran), 99 patients with ACL were allocated to 3 treatment groups. The 3 groups received combined intralesional MA and imiquimod, imiquimod alone, or intralesional MA alone for 40 days; the authors reported 37%, 35%, and 23% response rates, respectively, indicating a better response in patients who received imiquimod alone or in combination with MA compared with patients treated

### Table 1. Baseline Demographics and Disease Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (N=119)†</th>
<th>Imiquimod Group (n = 59)‡</th>
<th>Placebo Group (n = 60)§</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>27.0 ± 13.2</td>
<td>27.4 ± 13.9</td>
<td>26.5 ± 12.4</td>
<td>.71</td>
</tr>
<tr>
<td>Female sex</td>
<td>66 (55.5)</td>
<td>35 (59.3)</td>
<td>31 (51.7)</td>
<td>.40</td>
</tr>
<tr>
<td>Location of lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face</td>
<td>39 (15.5)</td>
<td>20 (15.6)</td>
<td>19 (15.3)</td>
<td>.95</td>
</tr>
<tr>
<td>Trunk</td>
<td>2 (0.8)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>.98</td>
</tr>
<tr>
<td>Upper extremity</td>
<td>167 (69.3)</td>
<td>85 (66.4)</td>
<td>82 (68.1)</td>
<td>.96</td>
</tr>
<tr>
<td>Lower extremity</td>
<td>44 (17.5)</td>
<td>22 (17.2)</td>
<td>22 (17.1)</td>
<td>.91</td>
</tr>
<tr>
<td>No. of lesions per patient, mean ± SD</td>
<td>2.1 ± 1.2</td>
<td>2.2 ± 1.2</td>
<td>2.0 ± 1.2</td>
<td>.49</td>
</tr>
<tr>
<td>Duration of lesions, mean ± SD, wk</td>
<td>13.1 ± 6.0</td>
<td>13.0 ± 5.5</td>
<td>13.2 ± 6.5</td>
<td>.87</td>
</tr>
<tr>
<td>Size of lesions, median (minimum, maximum, interquartile range), mm²</td>
<td>208 (4, 4785, 467.7)</td>
<td>174 (4, 4785, 382.5)</td>
<td>237 (4, 3750, 542.3)</td>
<td>.28</td>
</tr>
<tr>
<td>Lesions without ulceration at baseline</td>
<td>55/252 (21.8)</td>
<td>32/128 (25.0)</td>
<td>23/124 (18.5)</td>
<td>.22</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) unless otherwise indicated.
†With 252 lesions.
‡With 128 lesions.
§With 124 lesions.

### Table 2. Intent-to-Treat Analysis of Rates of Complete Reepithelization, Clinical Cure, and Clinical Improvement at Weeks 4, 8, and 20 After Initiation of Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (N = 119)</th>
<th>Imiquimod-Treated Group (n = 59)</th>
<th>Placebo Group (n = 60)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete reepithelialization*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>2 (1.7)</td>
<td>(0.5-5.9)</td>
<td>1 (1.7) (0.3-9.0)</td>
<td>1 (1.7) (0.3-8.9)</td>
</tr>
<tr>
<td>Week 8</td>
<td>5 (4.2)</td>
<td>(1.8-9.5)</td>
<td>3 (5.1) (1.7-13.9)</td>
<td>2 (3.3) (0.9-11.4)</td>
</tr>
<tr>
<td>Week 20</td>
<td>50 (42.0)</td>
<td>(33.5-51.0)</td>
<td>26 (44.1) (32.2-56.7)</td>
<td>24 (40.0) (28.6-52.6)</td>
</tr>
<tr>
<td>Clinical cure†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>29 (24.4)</td>
<td>(17.5-32.8)</td>
<td>11 (18.6) (10.7-30.4)</td>
<td>18 (30.0) (19.9-42.5)</td>
</tr>
<tr>
<td>Week 8</td>
<td>55 (46.2)</td>
<td>(37.5-55.2)</td>
<td>26 (44.1) (32.2-56.7)</td>
<td>29 (48.3) (36.2-60.7)</td>
</tr>
<tr>
<td>Week 20</td>
<td>82 (62.1)</td>
<td>(43.2-60.9)</td>
<td>30 (50.8) (38.4-63.2)</td>
<td>32 (53.3) (40.9-65.4)</td>
</tr>
<tr>
<td>Clinical improvement‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>24 (20.2)</td>
<td>(13.9-28.3)</td>
<td>13 (22.0) (13.4-34.1)</td>
<td>11 (18.3) (10.6-29.9)</td>
</tr>
<tr>
<td>Week 8</td>
<td>18 (15.1)</td>
<td>(8.8-21.7)</td>
<td>6 (10.2) (4.7-20.5)</td>
<td>12 (20.0) (11.8-31.8)</td>
</tr>
<tr>
<td>Week 20</td>
<td>3 (2.5)</td>
<td>(0.9-7.2)</td>
<td>0 (0.0-6.1)</td>
<td>3 (5.0) (1.7-13.7)</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.
*Complete reepithelialization of all lesions without any induration.
†More than 75% reduction in the size of lesions compared with baseline.
‡A 50% to 75% reduction in the size of lesions compared with baseline.
with MA. These results discordant with the results of the present study in which the number of clinically cured patients was not significantly different between the 2 groups (Table 2). This incongruity may be owing to the use of different definitions for the primary outcome measure or to variations in the method of administration of MA (intraderal vs intramuscular injections).

Data on the efficacy of MA in the treatment of ACL caused by L tropica are scarce. In a randomized trial of ACL caused by L tropica in Syria, 20 patients with 38 lesions received 1 to 3 mL of MA, and 20 patients with 37 lesions received 25 μg of interferon gamma intradermally weekly for 5 consecutive weeks.19 Within 10 weeks, 29 of 38 lesions treated with MA and 1 of 37 lesions treated with interferon gamma were healed completely. In a recently published article from Israel, a complete response to treatment was observed in 6 of 9 patients treated with intraderal injections of sodium stibogluconate and in 4 of 5 patients treated with intravenous sodium stibogluconate.9 The development of drug-resistant L tropica species might be the reason for the low response to MA in the present study.18

It is recommended that patients with ACL receive treatment because of the prolonged course, potential scar formation, role of infected humans as reservoirs, and risk for development of leishmaniasis recidivans. No ideal therapy for CL is available, and the search for safer, more efficient, and cost-effective treatments is ongoing. Large-sampled, appropriately designed, and randomized controlled clinical trials are needed to evaluate therapeutic agents against CL. The clinical response to imiquimod cream in its FDA-approved indications (ie, genital warts) usually occurs after longer periods of treatment. For this reason, a more prolonged application of imiquimod may be more beneficial for the treatment of CL.9-10 Appropriately designed clinical trials are needed to test this hypothesis, although a more prolonged course of treatment with imiquimod presents the constraints of treatment cost and patient compliance.

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