MAJOR ARTICLE

Miltefosine for New World Cutaneous Leishmaniasis

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The oral agent miltefosine has demonstrated a >95% cure rate in Indian visceral leishmaniasis. We performed a large, placebo-controlled study of miltefosine therapy (2.5 mg/kg per day orally for 28 days) against cutaneous leishmaniasis in Colombia and Guatemala. In regions in Colombia where *Leishmania vianna panamensis* is common, the per-protocol cure rates for miltefosine and placebo were 91% (40 of 44 patients) and 38% (9 of 24). These values are similar to historic values for the antimony standard of care and placebo. In regions in Guatemala where *L. v. braziliensis* and *L. mexicana mexicana* are common, the per-protocol cure rates were 53% (20 of 38) for miltefosine and 21% (4 of 19) for placebo. The miltefosine rate was lower than historic antimony cure rates of >90%. Miltefosine was well tolerated. Miltefosine is a useful oral agent against cutaneous leishmaniasis due to *L. v. panamensis* in Colombia but not against leishmaniasis due to *L. v. braziliensis* in Guatemala.

Cutaneous leishmaniasis is endemic in the New World from around the United States–Mexico border, through Central America and the northern part of South America, and down to the region of Rio de Janeiro. The disease, which is characterized by papules that enlarge into ulcers, can be caused by a multitude of *Leishmania* species: members of the *Leishmania vianna* subgenus, such as *L. v. panamensis*, *L. v. braziliensis*, and *L. v. guayanensis;* and members of the *Leishmania mexicana* complex, such as *L. m. amazonensis* and *L. m. mexicana.* Parasite species were named for the regions of endemicity in which the parasites are found and subsequently were differentiated by biochemical and other parameters rather than by clinical characteristics of infected patients [1–3]. Thus, the clinical course of infections due to each species—the natural history of the ulcer and the response to chemotherapy—has to be laboriously investigated for each combination of species and chemotherapeutic agent.

It is generally thought that *L. v. panamensis* and *L. v. braziliensis* infections self-cure in >12 months and that 1%–3% of patients metastasize to the mucosa of the nose and mouth (mucosal disease), whereas *L. m. mexicana* infection self-cures much more rapidly and does not lead to mucosal metastasis. *L. v. panamensis* infection and *L. v. braziliensis* infection are treated to accelerate cure of the cutaneous lesion and to attempt to prevent mucosal metastasis, and *L. m. mexicana* infection may or may not be treated. Where *L. m. mexicana* coexists with *L. v. braziliensis* and cannot be distinguished by presentation, all presenting lesions are treated. Standard therapy consists of parenteral administration of pentavalent antimonials daily for 20 days [4], although both shorter courses [5] and longer courses with lower amounts of the drug per day [6] have been investigated. Depending on the region of endemicity, antimony cure rates are generally >80% and are frequently >90%.

Pentavalent antimonials have the disadvantages of multiple injections and mild-to-moderate clinical tox-

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icity, both of which are particularly vexing for a moderate clinical problem that almost always self-cures with time. The primary chemotherapeutic need is an oral agent that has acceptable toxicity and that competes with antimonials in efficacy. A 30-year search for an effective oral agent has so far been unrewarding. For example, the promising agent allopurinol had a cure rate of 33% in Colombia [7].

Miltefosine (hexadecylphosphocholine) inhibits phospholipid and sterol biosynthesis [8] of trypanosomids and is effective in vivo against *Leishmania,* including by the oral route [9, 10]. Phase 2 and then phase 3 trials against visceral leishmaniasis in India demonstrate a 97% cure rate in patients administered oral miltefosine at a dosage of 2.5 mg/kg per day for 4 weeks [11–14]. Although untreated visceral leishmaniasis results in death—in contrast with cutaneous leishmaniasis, which ultimately self-cures—very high efficacy against visceral disease does not guarantee similar efficacy against cutaneous disease. The antileishmanial agent paromomycin, for example, cured 73% of Indian patients with the visceral form of the disease at a dosage of 12 mg/kg per day for 3 weeks [15], but it cured 50%–60% of patients with cutaneous leishmaniasis in Colombia [16] and Central America [17], respectively, at approximately the same dose.

The program to investigate miltefosine for New World cutaneous leishmaniasis was initiated with an uncontrolled, open-label, dose-ranging study in regions in Colombia where *L. v. panamensis* predominated (hereafter, "*L. v. panamensis* regions"). The per-protocol cure rate for 32 patients who received ~2.5 mg/kg per day for 3–4 weeks was 94% [18]. The most frequent adverse effects were a feeling of motion sickness, vomiting, and transient elevations of liver transaminase levels. To investigate the therapeutic index for oral miltefosine more generally and in a controlled manner, we performed placebo-controlled trials of miltefosine in Colombia and Guatemala.

PATIENTS AND METHODS

Study design. This was a randomized, placebo controlled, double-blind multicenter trial of miltefosine. There were 2 study sites. In Colombia, the patients were both civilians and soldiers who acquired infection in the provinces of provinces of Urabá and Carmen de Chucurí and who were evaluated in local hospitals for diagnosis and treatment. In Guatemala, the patients were civilians who presented, received diagnoses, and were treated at 2 clinics operated by the Universidad del Valle de Guatemala, which is located in Poptun, El Peten, Guatemala.

Study population. Patients were of either sex, aged >12 years, had parasitologically confirmed cutaneous leishmaniasis, and did not have mucosal involvement. Previous treatment for the disease was permitted if therapy had stopped ⩾4 weeks earlier and the lesions were not improving. Significant concomitant diseases were excluded by history and by the requirement for approximately normal complete blood cell counts (i.e., WBC count, hemoglobin level, and platelet count), liver transaminase levels (i.e., aspartate aminotransferase and alanine aminotransferase levels), and kidney function test results (i.e., creatinine and blood urea nitrogen level). Pregnancy and lactation were also exclusion criteria.

Study treatments. Miltefosine (50 mg) or matching placebo capsules were administered orally for 28 days under the observation of study staff. To administer ~2.5 mg/kg per day, patients ⩾45 kg received 3 capsules per day (1 capsule in the morning, 1 capsule at lunch, and 1 capsule in the evening, after meals), and patients <45 kg received 2 capsules per day (1 capsule in the morning and 1 capsule in the evening, after meals).

Toxicity evaluation. Patients were interviewed for subjective adverse events daily during treatment. Blood samples were obtained for repeated blood cell counts, liver function tests, and kidney function tests weekly during therapy, at the end of therapy, and at 2 and 6 months after the end of therapy. Subjective and laboratory adverse events were graded according to the Common Toxicity Criteria (CTC) of the National Cancer Institute (http://ctep.cancer.gov/reporting/ctc.html).

Efficacy evaluation. For each lesion, the sizes of its ulcer and induration were measured at 2 weeks, 2 months, and 6 months after the end of therapy. Lesions for which there was incomplete reepithelialization of the ulcer or incomplete elimination of induration underwent repeated parasitological investigation.

A lesion was defined as a treatment failure if it enlarged by 50% or was positive for parasites 2 weeks to 6 months after the end of therapy, relapsed (enlarged) after previously diminishing in size, or did not completely reepithelialize by 6 months after the end of therapy. Appearance of a new lesion from which *Leishmania* could be demonstrated was also a criterion for failure.

Cure was defined as complete healing of all lesions by 6 months after the end of therapy. Thus, for a patient to be cured, no lesion could enlarge by 50%, be parasite positive, relapse, or heal incompletely, and no new *Leishmania*-positive lesion could appear.

Parasitology. Lesion samples were obtained by ⩾1 of the following methods: slit skin smear, aspirate, and biopsy. Proof of infection before treatment or of continued infection after treatment consisted of microscopic identification of *Leishmania* amastigotes in the direct Giemsa stain of the smear or the demonstration of motile promastigotes cultured from the aspirate and/or biopsy of a lesion. In Colombia, cultures of baseline lesion aspirates were identified to the species level via monoclonal antibody binding [2]. In Guatemala, cultures or

Table 1. Presenting characteristics of patients with cutaneous leishmaniasis in Colombia and Guatemala who received therapy with miltefosine.

Characteristic	Colombian site		Guatemalan site	
	Miltefosine recipients (n = 49)	Placebo recipients (n = 24)	Miltefosine recipients (n = 40)	Placebo recipients (n = 20)
Age, mean years ± SD	24 ± 10	25 ± 13	26 ± 10	28 ± 12
Male sex, %	86	75	98	100
Weight, mean kg ± SD	60 ± 13	57 ± 14	59 ± 8	60 ± 8
Median no. of lesions (range)	1 (1–8)	1 (1–5)	1 (1–10)	1 (1–3)
Ulcer size, median mm^2 (range)	171 (72–1775)	238 (6–2110)	165 (6–1650)	154 (6–3300)
No. (%) of patients with previous therapy failure	3 (6)	2 (8)	10 (25)	8 (40)

clinical samples preserved in absolute alcohol were identified to the species levels by PCR [19, 20].

Statistical analysis. The primary end point of the trial was the rate of cured patients. A 2-sided Cochran-Mantel-Haenszel (CHM) test stratified by site was performed to compare the cures after miltefosine and placebo. The comparison of cure rates within each site was performed by 2-sided χ^2 tests.

Ethics. The study protocol and amendments were approved by the responsible authority at the Colombian study site (Comite de Etica en Investigacion, Hospital Militar Central, Bogota, Colombia) and at the Guatemalan study site (Universidad del Valle Ethics Committee). The first visit of the first patient was in June 2000. The last visit of the last patient was in December 2002.

RESULTS

Patient characteristics. The presenting characteristics of the miltefosine and placebo recipients at the Colombian and Guatemalan sites are listed in table 1. On average, patients were in the third decade of life, weighed ~60 kg, and had 1 lesion. The ulcer size was ~200 mm^2. Of the 133 patients, 119 (89%) were men.

Compliance with therapy. Six of the 133 treated patients did not receive the full 28 days of medication. Two were miltefosine recipients in Colombia: one patient missed his last day of therapy and then was lost to follow-up; the other patient stopped therapy on day 27 because of intolerability. Two were miltefosine recipients in Guatemala; both did not appear for

Table 2. Efficacy of miltefosine used to treat cutaneous leishmaniasis in Colombian and Guatemalan patients.

Variable	Colombian site		Guatemalan site	
	Miltefosine recipients (n = 49)	Placebo recipients (n = 24)	Miltefosine recipients (n = 40)	Placebo recipients (n = 20)
No. of patients cured	40	9	20	4
No. of patients with treatment failure				
All	4	15	18	15
Parasite-positive lesions	0	15	12	12
Size of lesion doubled	2	5[a]	4[b]	2
Relapse	2	0	4	1
No. of unassessable patients				
All	5	0	2	1
Lost after therapy	2	0	1	1
Lost after 2 weeks	2	0	1	0
Lost after 3 months	1	0	0	0
Cure rate, n/N (%)				
Intent-to-treat	40/49 (82)	9/24 (38)	20/40 (50)	4/20 (20)
Per-protocol	40/44 (91)	9/24 (38)	20/38 (53)	4/19 (21)

[a] All 5 of these patients also had parasite-positive lesions.
[b] Two of these 4 patients also had parasite-positive lesions.

Table 3. *Leishmania* species infecting Guatemalan patients with cutaneous leishmaniasis.

	Miltefosine recipients			Placebo recipient		
Response	*Leishmania vianna braziliensis*	*Leishmania mexicana mexicana*	Unknown	*Leishmania vianna braziliensis*	*Leishmania mexicana mexicana*	Unknown
Cure	5	9	6	1	1	2
Treatment failure	10	5	3	11	2	2
Unassessable	1	0	1	1	0	0

NOTE. Data are no. of patients. *P* values for intent-to-treat cure rates for patients for whom species was identified: *L. v. braziliensis,* $P = .18$, by Fisher's exact test; and *L. m. mexicana,* $P = .54$, by Fisher's exact test.

treatment on day 21 and then were lost to follow-up. Two were placebo patients in Guatemala: one did not appear on day 21 and then was lost to follow up, and the other had his last week of treatment stolen and did appear for follow-up. All 6 partially treated patients are included with the 127 fully treated patients in the efficacy and tolerance evaluations below.

Efficacy. In Colombia, 40 miltefosine recipients were cured, 4 had illness that failed to respond to therapy, and 5 were lost to follow-up (table 2). The intent-to-treat cure rate was 82%. The per-protocol cure rate, in which the 5 patients who were lost to follow-up are not included, was 91%. There is no reason to hypothesize that the illness of these 5 patients would have failed to respond to therapy. For the 3 patients who were seen after treatment but not at 6 months, 1 patient had completely healed by 2 weeks after therapy, 1 patient had 1 completely healed lesion and one 85% healed lesion 2 weeks after therapy, and 1 patient had completely healed by 3 months after therapy.

There were 9 placebo recipients who were cured and 15 who had illness that failed to respond to therapy. The intent-to-treat and per-protocol cure rates were 38%. The difference between the intent-to-treat and per-protocol miltefosine cure rates and the respective placebo cure rates were statistically significant ($P < .001$, by the χ^2 test)

In Guatemala, 20 miltefosine recipients were cured, 18 had illness that failed to respond to therapy, and 2 were unassessable (table 2). Four placebo recipients were cured, and 15 had illness that failed to respond to the placebo. The intent-to-treat cure rates were 50% (miltefosine) versus 20% (placebo), and the per-protocol cure rates were close to these values, because few patients were lost to follow-up. The difference between the intent-to-treat and per-protocol miltefosine cure rates and the respective placebo cure rates was statistically significant ($P = .025$ for intent-to-treat cure rate and $P = .023$ for per-protocol cure rate, by χ^2 test). The overall treatment comparison of intent-to-treat and per-protocol cure rates stratified by site revealed significant differences ($P < .001$, by CMH test).

Parasitology. In Colombia, cultures of 7 baseline lesion aspirates were speciated by monoclonal antibody binding. All 7 parasites were *L. v. panamensis.* In Guatemala, 46 of the 60 infecting parasites were speciated by PCR (table 3). A total of 63% of speciated parasites were *L. v. braziliensis,* and 37% of speciated parasites were *L. m. mexicana.* The distribution of *L. v. braziliensis* and *L. m. mexicana* with cure and failure in response to miltefosine and placebo is shown in table 3. The rate of cure of *L. v. braziliensis* was low (33%), compared with the rate of cure of *L. m. mexicana* (60%).

Table 4. Tolerance to miltefosine therapy in patients with cutaneous leishmaniasis in Colombia and Guatemala.

	No. (%) of patients	
Variable	Miltefosine recipients ($n = 89$)	Placebo ($n = 44$)
Treatment-emergent adverse events		
Nausea	32 (36)	4 (9)[a]
Motion sickness	26 (29)	10 (23)
Headache	24 (27)	9 (20)
Vomiting		
⩾1	28 (31)	2 (5)[b]
1–2	22 (25)	1 (2)
3–4	3 (3)	1 (2)
>4	3 (3)	0 (0)
Diarrhea		
⩾1	5 (6)	1 (2)
1–2	4 (4)	1 (2)
>2	1 (1)	0 (0)
Laboratory parameters		
Creatinine level		
Increased	29 (33)	4 (9)[c]
CTC grade 1[d]	28 (31)	4 (9)
CTC grade 2[e]	1 (1)	0 (0)
Elevated aspartate aminotransferase level	7 (8)	8 (18)
Elevated alanine aminotransferase level	9 (10)	5 (11)

NOTE. CTC, Common Toxicity Criteria of the National Cancer Institute.
[a] $P < .001$, by χ^2 test.
[b] $P < .001$, by χ^2 test.
[c] $P = .003$, by χ^2 test.
[d] Less than 1.5 times the upper limit of normal.
[e] Between 1.5 and 3.0 times the upper limit of normal.

Table 5. Efficacy of standard antimonial therapy to treat cutaneous leishmaniasis in Colombia and Guatemala.

Study, therapy group	No. of subjects				Cure rate, %		Species	Reference(s)
	All	Cured	Had treatment failure	Lost to follow-up	ITT	PP		
Colombian studies								
Glucantime	66	52	4	10	79	93	84% *Leishmania vianna panamensis*	[7]
Placebo	56	17	29	10	30	37		
Allopurinol	60	18	37	5	30	33		
Glucantime	23	21	23	0	91	91	ND	[21]
Untreated	28	8	14	6	29	36	…	
Glucantime	31	26	5	0	84	84	ND	[22]
Guatemalan studies								
Glucantime	25	24	1	0	96	…	*Leishmania vianna braziliensis*	[23]
Placebo	15	1	14	0	6	…		
Glucantime	14	11	3	0	79	…	*L. v. braziliensis*	[24]
Placebo	11	0	11	0	0	…		
Glucantime	22	19	2	1	87	…	At least 50% *L. v. braziliensis*	[5]
Placebo	25	22	3	0	88	…	*Leishmania mexicana mexicana*	[25]

NOTE. ITT, intent to treat; ND, not determined; PP, per protocol.

Tolerance. Symptomatic and laboratory adverse events for all miltefosine and placebo recipients are summarized in table 4, which lists treatment-emergent subjective events that occurred in at least 10% of patients. The frequency of nausea and vomiting was significantly higher in miltefosine recipients. The large majority of patients who vomited did so on 1–2 occasions. For 3 patients, vomiting occurred 5, 6, or 7 times. No patient discontinued therapy prematurely for these reasons. The single premature discontinuation was due to persistent motion sickness and headache.

The creatinine level increased to more than the normal range in 32% of miltefosine recipients, compared with 4% of placebo recipients. In all cases but one, the increase was to CTC grade 1. There was no difference between miltefosine and placebo in the percentage of patients who experienced increases in the liver function tests. All increases were CTC grade 1 (i.e., <2.5 times the upper limit of normal).

DISCUSSION

This trial of miltefosine in Colombia and Guatemala is the largest placebo-controlled trial of chemotherapy for cutaneous leishmaniasis yet reported from the New World. Because New World cutaneous leishmaniasis almost always self-cures, and because the rate of self-cure varies with species and region of endemicity, data from uncontrolled trials are difficult to interpret. The present trial was planned after an initial open-label dose-ranging trial of miltefosine against Colombian cutaneous disease generated attractive results.

For miltefosine in Colombia, the 6-month intent-to-treat cure rate was 82%, and the 6-month per-protocol cure rate, considering that 5 patients were not assessable, was 91%. In contrast, for placebo, the 6-month intent-to-treat and per-protocol cure rates were 38%.

The miltefosine efficacy data in this study well parallels the data from the preceding open-label trial in the same regions of Colombia, for which the 6-month cure rates were 81% (30 of 37 cases) on an intent-to-treat basis and 94% (30 of 32 cases) on a per-protocol basis, with 5 patients being lost to follow-up [18].

A summary of cure rates due to standard therapy with antimony and with placebo is provided in table 5. In Colombia, the per-protocol glucantime cure rates in *L. panamensis* regions historically have been 84%–93%, and the placebo cure rates have been 36%–37%. The 91% and 38% miltefosine and placebo per-protocol cure rates, respectively, for the present study are very similar to those values. This comparison indicates that the efficacy of miltefosine is equivalent to historic values of standard therapy with glucantime with respect to *L. v. panamensis* disease in Colombia.

For miltefosine in Guatemala, the 6-month intent-to-treat cure rate for miltefosine was 50%, and for placebo, it was 20%.

Historic values of cure rates due to antimony and placebo for *L. v. braziliensis* and *L. m. mexicana* are also summarized in table 5. In Guatemala, *L. v. braziliensis* is highly susceptible to antimonial therapy, with a cure rate of generally >90%. Because 63% of speciated parasites were *L. v. braziliensis* in the present study, and because 67% were identified as *L. v. braziliensis* in previous work in this region [23], the species of *Leishmania* did not differ between the present and past work. Thus, the miltefosine cure rate of ~50% in the present study can be compared unfavorably to the historic antimony cure rates of >90% for Guatemalan patients infected with comparable species of *Leishmania.*

Patients with cutaneous leishmaniasis experience only a skin ulcer and are systemically healthy. Because this was the first blinded trial of miltefosine in an essentially normal population, our trial permits determination of the inherent clinical tolerance of this drug. Motion sickness and nausea were each reported by ~30% of patients, with nausea but not motion sickness being specifically attributable to the drug. Vomiting but not diarrhea was also specifically attributable to miltefosine and was experienced by 32% of patients. Nevertheless, approximately three-quarters of these patients had only 1–2 episodes of vomiting during the 28-day course of therapy, and no patient stopped therapy for this reason.

The creatinine level was more frequently elevated in the miltefosine group than in the placebo group, but almost all creatinine level elevations were mild (CTC grade 1). Aspartate aminotransferase and alanine aminotransferase levels were not more frequently elevated in the miltefosine group than in control subjects. The mild changes in laboratory parameters suggest that in the cutaneous leishmaniasis population, in contrast to visceral leishmaniasis patients who have systemic disease, routine recording of laboratory parameters need not be performed.

Chemotherapeutic agents are evaluated on the basis of efficacy, tolerance, and convenience and cost of administration. The standard agents for leishmaniases—pentavalent antimonials, pentamidine, and amphotericin B—all are effective for New World cutaneous leishmaniasis [26, 27], but they all have the disadvantages of repeated parenteral injection and of toxicity.

Miltefosine is an oral agent that this trial has revealed to have acceptable tolerance. The Colombian data from this trial show that miltefosine has demonstrable efficacy, and to a degree similar to historic values of antimony, against apparent *L. v. panamensis* disease in Colombia. This is the first firm demonstration that any oral agent is either an improvement over placebo or has efficacy comparable to historic values of standard therapy for a form of New World cutaneous leishmaniasis. For Old World cutaneous disease, the only placebo-controlled demonstration of efficacy of an oral agent is that of fluconazole for *L. major* disease in Saudi Arabia [28]. The efficacy of miltefosine against disease in Guatemala, although higher than that of placebo, was lower than historic values of antimony. The general value of miltefosine for New World cutaneous disease remains to be demonstrated by further studies against *L. v. braziliensis* and other endemic species.

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