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Treatment of New World cutaneous leishmaniasis with miltefosine

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Summary Miltefosine (2.5 mg/kg/day for 28 days) was investigated for treatment of New World cutaneous leishmaniasis in Colombia and Guatemala. The data from a controlled study was remarkably similar to the data of a prior uncontrolled pilot study. In the controlled study, the per-protocol 6-month cure rate *for Leishmania panamensis* disease was 91% compared with a concomitant placebo cure rate of 38%. In Guatemala, the cure rate for *L. braziliensis* and *L. mexicana* disease was ~50% compared with ~20% for placebo. In both countries, nausea but not 'motion sickness' and vomiting but not diarrhoea were experienced by approximately 30% more miltefosine patients than placebo patients. Mild elevation of creatinine, but not of aspartate aminotransferase or alanine aminotransferase, was also more frequently seen in the miltefosine group than in the placebo group. Miltefosine was well tolerated, and as effective as historic values of antimony for treatment of *L. panamensis* disease.

1. Initial dermatological use of miltefosine: treatment of skin cancer

Miltefosine was originally developed as an anticancer agent. Because oral administration was thought to lead to insufficient efficacy and side-effects in the cancer population (Dummer et al., 1993; Smorenburg et al., 2000), the drug was incorporated into a topical formulation, containing 6% miltefosine, for cutaneous cancers. In an open-label trial, topical miltefosine demonstrated improvement in approximately half of 18 cutaneous lymphoma lesions (Dummer et al., 1993). In later work, 20 breast cancer patients with progression of skin metastases were treated open-label with topical miltefosine in addition to systemic therapy. Modest efficacy for cutaneous lesions was seen (Smorenburg et al., 2000). In 1992, the 6% topical miltefosine formulation was registered in Germany as a treatment for cutaneous cancer.

2. Preclinical studies of miltefosine for cutaneous *Leishmania*

Investigation of miltefosine for cutaneous *Leishmania* occurred late in the product development sequence. In preclinical work, Escobar et al. (2002) reported the comparative in vitro efficacy of miltefosine against a range of *Leishmania* amastigotes within macrophages. As shown in Table 1, *L. donovani* is the most sensitive and an Old World cutaneous species (*L. major*) is the least sensitive of the *Leishmania* species within macrophages in vitro. As amastigotes within

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Table 1 In vitro efficacy of miltefosine against *Leishmania* species

Species	Promastigotes ED50 (uM)	Amastigotes in macrophages
L. donovani	0.5	3.9
L. tropica	1.1	8
L. aethiopica	2.0	3.8
L. panamensis	2.5	10
L. mexicana	7	8
L. major	9	34

Source: Data from Escobar et al. (2002).

macrophages, New World cutaneous species were approximately half as susceptible to miltefosine as was *L. donovani*. On the other hand, topical 6% miltefosine (Miltex®, Zentaris GmbH, Frankfurt/Main, Germany) reduced the lymph node burden of *L. major*, the species that was least susceptible in vitro, from 2000—59 000 parasites in control animals to 10—30 parasites in drug-treated animals (Schmidt-Ott et al., 1999). This dramatic reduction makes the relevance of in vitro susceptibilities to in vivo responsiveness unclear and suggests that clinical trials be undertaken even for *L. major*.

3. Overview of New World cutaneous leishmaniasis

Cutaneous leishmaniasis (CL) is endemic in the New World from approximately the US—Mexican border through Central America and the northern part of South America down to the level of Rio de Janeiro. The disease can be caused by a multitude of *Leishmania* species: members of the *L. vianna* subgenus such as *L. v. panamensis*, *L. v. braziliensis* and *L. v. guayanensis*; and members of the *L. mexicana* complex such as *L. m. amazonensis* and *L. m. mexicana*. Parasite species were named for the endemic regions in which the parasites are found, and were differentiated subsequently by biochemical and genetic methods, such as electrophoresis of isoenzymes of the glucose metabolic pathway, binding of monoclonals to parasite antigens and PCR (Chico et al., 1995; Kreutzer et al., 1983; Noyes et al., 1996) rather than by clinical characteristics of infected patients. Because these differentiating parameters are not clinically based, there is no inherent reason why clinical data should be associated with species. Clinical data from one species may, or may not, pertain to another species.

The typical evolution of New World cutaneous disease is that a papule at the site of a sandfly bite enlarges to a nodule and ulcerates over a period of a few months. At least for *L. v. braziliensis* and *L. v. guyanensis* disease in Brazil, the distribution of lesion types is typically 75% ulcer, 15% plaque, 10% papule-nodule (Palacios et al., 2001).

Cutaneous leishmaniasis is still typically diagnosed by classical methods. The parasites can be visualized within macrophages in Giemsa-stained aspirated or biopsied lesion material. Lesion material can also be cultured in the hope that parasites will multiply and be seen.

Cutaneous disease heals by re-epithelialization with scarring. Figure 1 shows a typical cutaneous lesion prior to treatment (Figure 1A), at the end of the 20-day period of standard

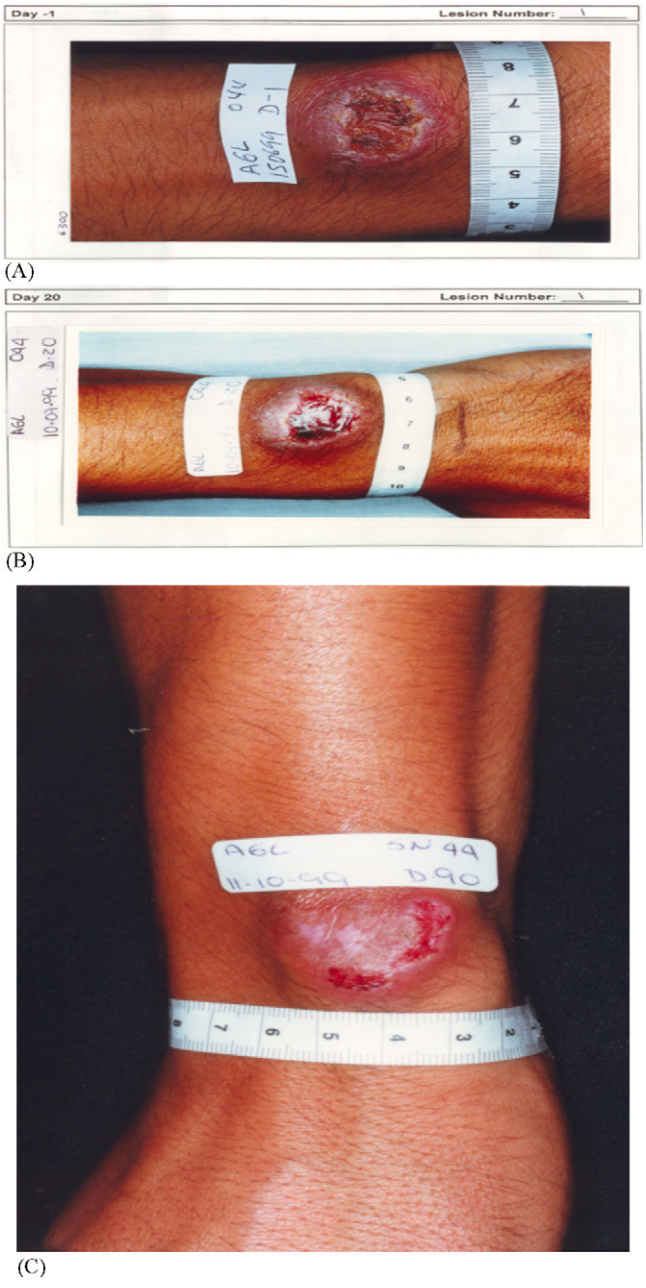

Figure 1 Typical ulcer of New World cutaneous leishmaniasis (A), after 20 days of standard antimonial treatment (B) and 3 months after treatment (C).

antimonial treatment when slight healing is seen (Figure 1B), and at 3-month follow-up when essentially complete re-epithelialization is evident (Figure 1C). Both the placebo and drug cure rates have been studied most thoroughly in Colombia and Guatemala. Data from those countries suggests that *L. m. mexicana* heals more rapidly than does *L. v. braziliensis* and *L. v. panamensis*. At least 75% of *L. m. mexicana* heals within 3 months, whereas the cure rates over 3 months for *L. v. panamensis* is 35% and that for *L. v. braziliensis* is 10% (Table 2).

Cutaneous leishmaniasis is treated to accelerate cure and to attempt to prevent dissemination to the mucosa. The classic treatment for cutaneous disease is pentavalent

Table 2 Efficacy of standard antimonial therapy and of placebo in Colombia and Guatemala

Group	No. of patients	No. cured/ failed/lost	Intent to treat cure rate	Per-protocol cure rate	Species	Study
Colombian studies						
Glucantime	66	52/4/10	79%	93%	84% *L. v. panamensis*	Velez et al. (1997)
Placebo	56	17/29/10	30%	37%		
Allopurinol	60	18/37/5	30%	33%		
Glucantime	23	21/23/0	91%	91%	ND	Soto-Mancipe et al. (1993)
Untreated	28	8/14/6	29%	36%		
Glucantime	31	26/5/0	84%	84%	ND	Soto et al. (1998)
Guatemalan studies						
Glucantime	25	24/1/0	96%	—	*L. v. braziliensis*	Navin et al. (1992)
Placebo	15	1/14/0	6%	—		
Glucantime	14	11/3/0	79%	—	*L. v. braziliensis*	Navin et al. (1990)
Placebo	11	0/11/0	0%	—		
Glucantime	22	19/2/1	87%	—	At least 50% *L. v. braziliensis*	Arana et al. (1994)
Placebo	25	22/3/0	88%	—	*L. m. mexicana*	Herwaldt et al. (1992)

ND: not determined.
Source: Data from Soto et al. (2004). ©2004 by the Infectious Diseases Society of America. Adapted with kind permission of The University of Chicago Press.

antimony (Sb^v) at 20 mg Sb/kg/day parenterally for 20 consecutive days. Treatment is generally successful with cure rates >90% (Table 2). The fundamental disadvantage of antimonial treatment regimens is that for a self-healing disease such as CL, the morbidity of 20 daily injections of a moderately toxic, costly drug approximates the morbidity of the presenting clinical problem itself. The routine chemotherapeutic requirements of effective, well-tolerated, non-parenteral and inexpensive agents are particularly cogent for CL.

With this information in mind, miltefosine was evaluated for treatment of CL in Colombia and Guatemala.

4. Pilot study of miltefosine for treatment of New World cutaneous leishmaniasis

The first study of miltefosine for treatment of CL was a pilot dose-ranging study with 16—20 patients in each dose group (Soto et al., 2001). The patients were male Colombian soldiers. Diagnosis was based on stain or culture of lesion material. Parasites that grew sufficiently in culture were speciated by isoenzyme electrophoresis.

Patients had to have at least one ulcerative lesion and, if previously treated with pentavalent antimony, antimony treatment had to have ended 4 weeks prior to the present study and the lesion had to be enlarging post-antimonial therapy.

Patients were treated with miltefosine according to a rising-dose scheme. Because it was thought that drug toxicity might be higher in the initial weeks of therapy, the dosage in week 1 was generally lower than in subsequent weeks, and the resulting regimens were complex. Group 1 patients received a low dosage of 50 mg (one capsule)/day, for the time period during which standard therapy is given (20 days). Group 2 received 50 mg/day on days 1—7 and then 100 mg/day (50 mg twice daily) on days 8—20. Group 3 received 100 mg/day on days 1—7, and then 150 mg/day (50 mg three times daily) on days 8—20. Group 4 received 150 mg/day for 28 days. The drug was administered with the morning, midday or evening meal.

After treatment with standard antimonials, lesions become parasite-negative, progressively heal up to 3 months after therapy and rarely relapse thereafter. To compare the results of the uncontrolled study with that standard, 'lesion cure' was defined as no parasites after therapy, complete re-epithelialization by 3 months after the end of therapy, and no relapse by 6 months after the end of therapy. For a patient to be cured, all lesions had to be healed. Note that if a patient's treatment was judged to have failed immediately after therapy or 3 months after therapy, rescue therapy was instituted at that time.

The 72 patients who received treatment were young men with a mean age of 23 years and mean weight of 67 kg. Patients had a mean of 2.0 lesions with a mean size of 278 mm^2 and a mean duration of the disease before study entry of 2.9 months. Parasites were cultured for 15 patients: 10 were *L. panamensis* and five were *L. amazonensis*.

Efficacy was modest in groups 1 and 2. In group 1, nine of 14 evaluable patients (64%) were cured. In group 2, in spite of an approximate doubling of dosage, efficacy did not increase and only twelve of 18 evaluable patients (67%) were cured (Table 3).

Group 3 was administered a larger dosage (mean dosage, 133 mg/day for 3 weeks); all 14 evaluable patients were cured. In group 4, who received 150 mg/day for 28 days, 89% of evaluable patients were cured (Table 3).

Table 3 Salient data from pilot study

Parameter	Group 1	Group 2	Group 3	Group 4
No. of patients	16	19	17	20
Entrance data				
Age (years)[a]	25	23	23	22
Weight (kg)[a]	66	67	67	67
No. lesions[a]	1.4	1.7	2.2	2.6
Lesion size (mm^2)[a]	172	304	307	315
No. previously failed Sb (%)	6 (38%)	7 (37%)	12 (71%)	4 (20%)
Efficacy data				
Lost to follow-up	2	1	3	2
No. evaluable	14	18	14	18
No. cured	9	12	14	16
% cured (per-protocol)	64%	67%	100%	89%

Source: Data from Soto et al. (2001). ©2001 by the Infectious Diseases Society of America. Adapted with kind permission of The University of Chicago Press.
[a] Mean value.

Vomiting and diarrhoea were experienced by 15 of 72 patients (21%) on no more than 5 days per patient. Liver transaminase levels were transiently elevated up to 2.5 times the upper limit of the normal range in groups 1—4 in 38, 42, 35 and 20% of patients respectively. Elevations to 2.6—5.0 times the normal upper limit were seen in one patient in each of groups 1, 2 and 4.

This pilot study was planned as an uncontrolled study, and efficacy data can only be compared to historic antimony data rather than to concomitant data. Nevertheless, when the two highest dose groups, who received 133—150 mg per day for 3—4 weeks, are combined per-protocol efficacy of evaluable patients was 94% (30 of 32 subjects), which is a high value. Also, the larger amount of drug appeared to be necessary, since the cure rate was 66% (21 of 32 subjects) for patients in the two lower dose groups who received 50—100 mg/day for 3 weeks.

Miltefosine regimens of 133—150 mg/day (2.0—2.3 mg/kg/day) for 3—4 weeks therefore seemed to be about as effective as standard therapy and much more effective than placebo. It therefore appeared that a dose of 2.5 mg/kg/day for 4 weeks could be used in further trials.

5. Controlled study

The second study in New World CL was a randomized, placebo-controlled double blind trial in Colombia and Guatemala (Soto et al., 2004). Patients differed from the pilot study in being both civilians and soldiers in Colombia and civilians in Guatemala.

The entrance criteria were similar to those of the pilot study although a broader age range and both sexes were included. Patients were aged ≥12 years, and prior treatment with antimony was permitted if therapy had stopped at least 4 weeks previously, the lesions were not improving and significant concomitant diseases were excluded by history and laboratory tests.

All patients received miltefosine at a dose of 2.5 mg/kg/day for 28 days or matching placebo. To administer approximately 2.5 mg/kg/day, patients ≥45 kg received three capsules per day (one in the morning, one at lunch, and one in the evening, following meals), and patients <45 kg received two capsules per day (one capsule in the morning and one capsule in the evening, following meals).

Patients were interviewed daily for subjective adverse events and blood was drawn weekly for repeat laboratory values. 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).

Cure was defined as complete healing of all lesions by 6 months after the end of therapy. For a patient to be cured, no lesion could enlarge by 50%, be parasite-positive after therapy, relapse or heal incompletely, and no new *Leishmania*-positive lesion could have appeared. As in the pilot study, if a patient was judged to have failed immediately after therapy or 3 months after therapy, rescue therapy was instituted at that time.

The entrance characteristics of patients were similar to those in the pilot study: patients were on average in the third decade of life, approximately 60 kg, and with one lesion. The ulcer size was approximately 200 mm^2. Of the 133 patients, 119 (90%) were male.

In Colombia, parasites from seven lesions were speciated by monoclonal antibody binding (Chico et al., 1995). All seven parasites were *L. v. panamensis*. In Guatemala, 46 of the 60 infecting parasites were speciated by PCR (Noyes et al., 1996). Sixty-three percent of speciated parasites were *L. v. braziliensis* and 37% of speciated parasites were *L. m. mexicana* (Soto et al., 2004).

5.1. Efficacy (Table 4)

As this study included completely different parasites at the two different sites, it was necessary to stratify the efficacy results by site.

Table 4 Efficacy data from controlled trial

	Colombian site		Guatemalan site	
	Miltefosine	Placebo	Miltefosine	Placebo
No. patients	49	24	40	20
No. cured	40	9	20	4
No. failed	4	15	18	15
Parasite-positive	0	15	12	12
Size doubled	2	5[a]	4[b]	2
Relapse	2	0	4	1
No. unevaluable	5	0	2	1
Lost after treatment	2	0	1	1
Lost after 2 weeks	2	0	1	0
Lost after 3 months	1	0	0	0
Intent to treat cure rate	40/49 = 82%	9/24 = 38%	20/40 = 50%	4/20 = 20%
Per-protocol cure rate	40/44 = 91%	9/24 = 38%	20/38 = 53%	4/19 = 21%.

Source: Data from Soto et al. (2004). ©2004 by the Infectious Diseases Society of America. Adapted with kind permission of The University of Chicago Press.
[a] All five of these patients also had parasite positive lesions.
[b] Two of these four patients also had parasite positive lesions.

In Colombia, 40 miltefosine patients were cured, four failed to respond and five were lost to follow-up. The per-protocol cure rate, in which the patients lost to follow-up are not included, was 91%. There were nine placebo patients who were cured and 15 who failed to respond. The per-protocol cure rates were 38% (*P* value for per-protocol cure rates <0.001 by χ^2 test).

In Guatemala, 20 miltefosine patients were cured, 18 failed to respond, and two were unevaluable; four placebo patients were cured and 15 failed to respond. The per-protocol cure rates for miltefosine and placebo were 53 and 21% respectively ($P = 0.023$). The distribution of *L. v. braziliensis* and *L. m. mexicana* with cure and failure in response to miltefosine and placebo was also investigated in patients whose species could be identified. For miltefosine, cure of known *L. v. braziliensis* was low (5 of 15 = 33%) compared to cure of known *L. m. mexicana* (9 of 14 = 67%).

5.2. Tolerance (Table 5)

Symptomatic and laboratory adverse events for all miltefosine patients and all placebo patients are summarized in Table 5. The frequency of nausea and vomiting was significantly higher in miltefosine patients. The large majority of patients who vomited did so on one or two occasions. For three patients, vomiting occurred five to seven times. No patient discontinued therapy prematurely because of these events. The one premature discontinuation was due to repeated 'motion sickness' and headache.

Creatinine increased above the normal range in 32% of miltefosine patients compared to 4% of placebo patients. 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 liver function tests. All increases were CTC grade 1 ($\leq$2.5 times the upper limit of normal).

Table 5 Tolerance data from controlled trial

	Miltefosine	Placebo
No. patients	89	44
Treatment emergent adverse events		
Nausea	32 (36%)	4 (9%)[a]
Motion sickness	26 (29%)	10 (23%)
Headache	24 (27%)	9 (21%)
Vomiting		
1 or more	28 (32%)	2 (5%)[b]
1—2	22 (25%)	1 (2%)
3—4	3 (3%)	1 (2%)
>4	3 (3%)	0 (0%)
Diarrhea		
1 or more	5 (6%)	1 (2%)
1—2	4 (5%)	1 (2%)
>2	1 (1%)	0 (0%)
Laboratory parameters		
Creatinine increased[d]	29 (33%)	4 (9%)[c]
CTC grade 1	28 (31%)	4 (9%)
CTC grade 2	1 (1%)	0 (0%)
AST increased	7 (8%)	8 (18%)
ALT increased	9 (10%)	5 (11%)

Source: Data from Soto et al. (2004). ©2004 by the Infectious Diseases Society of America. Adapted with kind permission of The University of Chicago Press.
[a] $P < 0.001$ (χ^2 test).
[b] $P < 0.001$ (χ^2 test).
[c] $P = 0.003$ (χ^2 test).
[d] For creatinine: CTC grade 1 signifies values less than 1.5 times the upper limit of normal. CTC grade 2 signifies values between 1.5 and 3.0 times the upper limit of normal.

6. Conclusions

Treatment of New World CL with miltefosine was investigated at two sites. Most data comes from Colombia, where the pilot study and the major portion of the controlled study were performed. The data from the controlled study were remarkably similar to the data from the uncontrolled pilot study. In the controlled study, the per-protocol cure rate was 91% compared to 94% in the pilot study. The controlled study also showed a concomitant placebo cure rate of 38%. In Colombia, the per-protocol Glucantime cure rates in *L. panamensis* regions historically ranged from 84 to 93% and the placebo cure rates ranged from 36 to 37%. The 91% and 38% miltefosine and placebo per-protocol cure rates 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 for *L. v. panamensis* disease in Colombia.

In Guatemala, *L. v. braziliensis* is highly sensitive to antimonial therapy with a cure rate generally >90%. The 6-month cure rate for miltefosine of ∼50% in the present study therefore compares unfavourably to the historic antimony cure rates.

Cutaneous leishmaniasis patients suffer only from a skin ulcer and are without systemic illness. One of the important features of the controlled trial was that it was the first blinded trial of miltefosine in any essentially normal human population, so the data of this trial gives our best estimate of the inherent clinical tolerance of miltefosine.

Nausea but not 'motion sickness,' and vomiting but not diarrhea were experienced by approximately 30% more miltefosine patients than placebo patients. Mild elevation of creatinine, but not of aspartate aminotransferase or alanine aminotransferase, was also seen more frequently in the miltefosine group than in the placebo group. The mild changes in laboratory parameters suggests that in the CL population, in contrast to visceral leishmaniasis patients who have systemic disease, routine recording of laboratory parameters may not be needed.

Miltefosine is an oral agent, which these trials show to have acceptable tolerance. The Colombian data from this trial show that miltefosine has demonstrable efficacy, which is 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 CL. One previous highly investigated oral agent — allopurinol — had a cure rate of 33% compared to a concomitant placebo cure rate of 37% (Velez et al., 1997). The efficacy of miltefosine against disease in Guatemala, although higher than that of placebo, was lower than historic values of antimony.

Although the Colombian data is highly encouraging, the general value of miltefosine in New World cutaneous disease remains to be demonstrated by further studies against the several endemic species in the New and Old Worlds. Given the lack of relevance of speciation with clinical features, studies in a large number of endemic regions may be needed before the potential of miltefosine for CL is clarified. An important design feature for these ultimately self-curing diseases is the inclusion of placebo-controls in the clinical trials.

Conflicts of interest statement

J. Berman is the Chair of the Miltefosine Product Development Team of TDR/WHO. J. Soto has received grant funding from Zentaris, the manufacturer of miltefosine.

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