

MILTEFOSINE, AN ORAL AGENT, FOR THE TREATMENT OF INDIAN VISCERAL LEISHMANIASIS

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ABSTRACT

Background There is no effective orally administered medication for any leishmania infection. We investigated miltefosine, which can be taken orally, for the treatment of Indian visceral leishmaniasis. Miltefosine is a phosphocholine analogue that affects cell-signaling pathways and membrane synthesis.

Methods The study was an open-label, multicenter, phase 2 trial in which four 30-person cohorts received 50, 100, or 150 mg of miltefosine per day for four or six weeks. The 120 patients, who ranged in age from 12 to 50 years, had anorexia, fever, and splenomegaly with at least moderate (2+) leishmania in a splenic aspirate. A parasitologic cure was defined by the absence of parasites in a splenic aspirate obtained two weeks after completion of treatment. The clinical response was assessed at six months.

Results In all 120 patients there was an initial parasitologic cure. Six patients had clinical and parasitologic relapses; the remaining 114 patients had not relapsed by six months after treatment, for a cure rate of 95 percent (95 percent confidence interval, 89 to 98 percent). With the regimen of 100 mg of miltefosine per day (approximately 2.5 mg per kilogram of body weight per day) for four weeks, 29 of 30 patients (97 percent) were cured. Gastrointestinal side effects were frequent (occurring in 62 percent of patients) but mild to moderate in severity, and no patient discontinued therapy because of gastrointestinal side effects. In two patients, treatment was discontinued because of elevated levels of aspartate aminotransferase or creatinine; in both patients the levels rapidly returned to normal. In 12 other patients, the level of aspartate aminotransferase increased to 100 to 150 U per liter during treatment.

Conclusions Orally administered miltefosine appears to be an effective treatment for Indian visceral leishmaniasis. (N Engl J Med 1999;341:1795-800.)

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VISCERAL leishmaniasis (kala-azar) is caused by infection of the spleen, liver, bone marrow, and reticuloendothelial system by leishmania that are acquired from sandfly bites. There are an estimated 500,000 cases per year¹ in Asia (primarily India), South America (primarily Brazil), and East Africa (primarily Sudan). There are also several hundred cases per year in the Mediterranean regions of southern Europe in immunocompetent persons and persons with human im-

munodeficiency virus (HIV) infection, and there are a few cases in the United States that have been acquired elsewhere. Patients with kala-azar present with hepatosplenomegaly and pancytopenia, and the disease is usually fatal if untreated. Until recently, it was treated with four weeks of injections of pentavalent antimonial agents, which were first used in the 1940s; amphotericin B was used as the secondary agent. In 1997, liposomal amphotericin B was licensed in the United States and other countries for this indication.^{2,3} Although liposomal amphotericin B is more than 95 percent effective and is generally well tolerated, it and the other new lipid formulations of amphotericin B are parenteral agents.³ The route of administration and its cost (\$5,000 for the drug alone) preclude its use in the developing world.

Decades of work — for example, on allopurinol,⁴ azoles,⁵⁻⁷ and other agents⁸ — have so far failed to identify an effective agent that can be given orally for this or any other form of leishmaniasis. Miltefosine (hexadecylphosphocholine) is a phosphocholine analogue that interferes with cell-signaling pathways but did not have clinical efficacy against tumors when administered orally.⁹ Because antileishmanial activity was found in vitro and in animal models,^{10,11} the agent was tried in a 30-patient pilot study of Indian kala-azar. When administered for four weeks, 100 mg of miltefosine every other day (approximately 50 mg per day) was partially effective; 200 mg per day was poorly tolerated; and the doses of 100 to 150 mg per day appeared highly effective and reasonably well tolerated.¹²

We undertook the present phase 2 study to determine whether there is a regimen of oral miltefosine that is highly effective and well tolerated in larger numbers of patients.

METHODS**Study Design**

The study was an escalating-dose, open-label, phase 2 trial of four regimens that were tested concurrently at three clinical cen-

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TABLE 1. BASE-LINE CHARACTERISTICS OF THE PATIENTS.*

CHARACTERISTIC	COHORT				
	1 (N=30)	2 (N=30)	3 (N=30)	4 (N=30)	ALL (N=120)
Dose	50 mg/day ×6 wk	50 mg/day ×1 wk, then 100 mg/day ×3 wk	100 mg/day ×4 wk	100 mg/day ×1 wk, then 150 mg/day ×3 wk	—
Age (yr)	28±13	24±12	29±13	27±10	27±12
Male sex (%)	77	70	70	67	71
Weight (kg)	39±12	36±10	40±10	40±8	39±10
Parasitologic grade	2.9±0.8	2.6±0.7	2.5±0.7	2.6±0.9	2.6±0.8
Temperature (°F)†	101±1.5	101±1.6	100±1.7	100±1.5	100±1.5
Size of spleen (cm)	8.5±2.7	7.7±3.0	7.3±3.2	8.8±3.0	8.1±3.0
White-cell count (×10 ⁻³ /mm ³)‡	2.9±1.1	3.3±0.85	3.3±1.2	2.9±1.0	3.1±1.1
Platelet count (×10 ⁻³ /mm ³)‡	114±47	126±31	132±63	150±57	130±51
Serum albumin (g/dl)§	3.4±0.5	3.3±0.8	3.1±0.7	2.7±0.4	3.1±0.6
Previous antileishmaniasis therapy (%)	40	25	50	50	41

*Data were obtained within seven days before the initiation of therapy. There were no significant differences among cohorts in presenting characteristics (by analysis of variance or chi-square test), except albumin level; the level of albumin in cohort 4 was significantly lower than that in cohorts 1, 2, and 3 (according to the Student–Newman–Keuls test). Plus–minus values are means ±SD.

†To convert degrees Fahrenheit to degrees Celsius, subtract 32 and multiply by 5/9.

‡The values are from centers 1 and 2, at which adherence to Good Clinical Practices guidelines was verified.

§The normal range is 3.8 to 5.0 g per deciliter.

ters in India. There were 10 patients in each of the 12 cohorts (four regimens at each of three centers), for a total of 120 subjects.

Study Patients

Potentially eligible patients were 12 to 50 years of age and had symptoms and signs compatible with a diagnosis of visceral leishmaniasis (fever, loss of appetite, and enlarged spleen) and had at least moderate (2+) leishmania on splenic aspiration performed within one week before they received the first dose of the drug (on a log scale of parasite counts per high-power field¹³). For consistency, parasitologic slides from centers 1 and 2 were read by the same reader, who also reevaluated the slides from center 3.

The criteria for exclusion were serious concomitant diseases, positivity for HIV, pregnancy, or severe kala-azar (spleen more than 12 cm below the left costal margin, hemoglobin level of less than 6.5 g per deciliter, white-cell count of less than 2000 per cubic millimeter, and platelet count of less than 75,000 per cubic millimeter).

Study Protocol

After informed consent was obtained and screening tests (ophthalmologic examination, complete blood count, and measurements of serum aspartate aminotransferase, creatinine, urea nitrogen, and albumin) were completed within one week before the first dose of drug was given, we sequentially assigned each patient at each of the three centers to one of four groups: cohort 1, which would receive 50 mg of miltefosine per day for six weeks (a total dose of 2100 mg); cohort 2, which would receive 50 mg per day for one week followed by 100 mg per day (50 mg twice a day) for three weeks (a total dose of 2450 mg); cohort 3, which would receive 100 mg per day for four weeks (a total dose of 2800 mg); or cohort 4, which would receive 100 mg per day for one week followed by 150 mg per day (50 mg three times per day) for three weeks (a total dose of 3850 mg). Miltefosine was administered as 50-mg capsules (Asta Medica, Frankfurt am Main, Germany) with a snack of bread and butter.

The administration of the drug was observed, and subjective side effects were recorded daily. Laboratory tests were repeated

weekly. Side effects and laboratory values were graded according to the Common Toxicity Criteria of the Southwest Oncology Group. We used a second splenic aspirate, obtained two weeks after the completion of therapy, to determine initial parasitologic cure or failure. We determined final cure or failure at the follow-up visit six months after therapy had ended. We defined final cure as an initial parasitologic cure (parasite count, 0) two weeks after the completion of therapy followed by clinical cure (a loss of fever and at least 33 percent improvement in spleen size, degree of cytopenia, and serum albumin level) at six months. Patients were also seen between two weeks and six months if they requested medical attention.

The study followed the Good Clinical Practices guidelines established by the Helsinki agreement, the Tropical Disease Research Program of the World Health Organization, and Asta Medica procedures. The protocol was reviewed by the institutional review boards of the Kala-azar Research Center, Banaras Hindu University, and Balaji Utthan Sanastan.

RESULTS

Characteristics of the Patients

The 120 patients were adolescents and adults (71 percent were men) who presented with moderate kala-azar (defined as a mean splenic-aspirate count of 2.6 on a log scale that extends up to 6.0; a mean spleen size of 8.1 cm below the left costal margin in a disease in which spleen sizes of 15 cm can be found; and moderate leukopenia and thrombocytopenia) (Table 1). Standard therapy with pentavalent antimony had failed in 41 percent of the patients.

Efficacy

All 120 patients had a parasitologic cure two weeks after the completion of therapy (Table 2). (For four

TABLE 2. RESPONSE TO THERAPY.*

EFFICACY VARIABLE	COHORT			
	1 (N=30)	2 (N=30)	3 (N=30)	4 (N=30)
Primary				
Parasitologic cure 2 wk after completion of therapy (no.)	30	30	30	30
Relapse (no.)	2	2	1	1
Clinical cure 6 mo after completion of therapy (no.)	28	28	29	29
Complete cure (%)†	93 (78–99)	93 (78–99)	97 (83–100)	97 (83–100)
Secondary				
Spleen size (cm below the costal margin)				
Before therapy	8.5±2.7	7.7±3.0	7.3±3.2	8.8±3.0
2 Wk after completion of therapy	1.2±1.8	1.1±1.5	1.2±1.3	1.2±1.3
6 Mo after completion of therapy‡	0.2±0.5	0.1±0.2	0.4±0.7	0.2±0.3
White-cell count (×10 ⁻³ /mm ³)§				
Before therapy	2.9±1.1	3.3±0.85	3.3±1.2	2.9±1.0
2 Wk after completion of therapy	7.0±2.1	8.0±2.9	8.3±1.4	7.3±1.9
Platelet count (×10 ⁻³ /mm ³)§				
Before therapy	114±47	126±31	132±63	150±57
2 Wk after completion of therapy	242±56	304±96	299±75	337±100

*Plus-minus values are means ±SD.

†Values in parentheses are 95 percent confidence intervals.

‡This calculation includes data from the 114 patients who did not have a relapse.

§The values are from centers 1 and 2, at which adherence to Good Clinical Practices guidelines was verified.

patients, the parasitologic grade two weeks after therapy was read as 1+ by one reader and as 0 by a second reader.) Six patients relapsed within six months after completing therapy, for a cure rate of 95 percent (95 percent confidence interval, 89 to 98 percent). The final rate of cure for the cohorts was therefore between 93 and 97 percent; the cure rate for cohort 3 was 97 percent (95 percent confidence interval, 83 to 100 percent) (Table 2). The secondary efficacy variables — spleen size, white-cell count, and platelet count — changed in ways implying a cure of kala-azar (Table 2).

The six patients who did not have a clinical cure six months after completing therapy had spleen sizes of 4 to 10 cm below the costal margin (mean, 6.5) and parasitologic grades of 1 to 4 (mean, 2.5) on relapse. Patients who eventually relapsed, three of whom had had no response to previous therapy, could not be distinguished from those who were eventually cured on the basis of mean base-line characteristics (parasitologic grade for the patients who relapsed, 2.8; spleen size, 8.9 cm; white-cell count, 3300 per cubic millimeter; platelet count, 122,000 per cubic millimeter) or mean characteristics two weeks after completing therapy (spleen size, 0.8 cm; white-cell count, 6000 per cubic millimeter; and platelet count, 225,000 per cubic millimeter). Whereas the mean (±SD) daily dose for all 120 patients was 2.8±1.3 mg per kilogram, the mean daily dose for 5 of the 6 patients in whom therapy failed was 1.7 mg per kilogram (range, 1.0 to 2.3). The sixth patient received

the third-highest daily dose, 4.8 mg per kilogram, and was one of the two patients who prematurely discontinued therapy after two weeks because of side effects. All patients in whom therapy failed were successfully treated with liposomal amphotericin B.

Toxicity

Side effects and laboratory evidence of toxicity of miltefosine were monitored particularly closely at centers 1 and 2. Gastrointestinal side effects, almost all of which consisted of vomiting and diarrhea, were frequent, and at a center at which the effects of drug administration were recorded precisely, the episodes occurred 0.5 to 2 hours after the drug was administered. For the eight patient cohorts at centers 1 and 2, side effects were reported on a mean of 8 percent of days (range, 2 to 17 percent) and by 62 percent of patients (range, 20 to 100 percent).

Patients who had side effects had symptoms on 13 percent of the days on which they received the drug — that is, on an average of 4 of the 28 days of their therapy. There was an average of 1.7 (range, 1.0 to 1.9) gastrointestinal episodes per day with side effects. The maximal number of gastrointestinal episodes per day was six, reported on two days.

There was no significant relation between the cohort (i.e., the dose administered each day) and the total number of gastrointestinal episodes, number of gastrointestinal episodes per day, or percentage of patients who had gastrointestinal episodes. Furthermore, there was no significant relation between the

dose of miltefosine in milligrams per kilogram per day and the total number of gastrointestinal episodes. There was no significant trend in the relation between the number of weeks of therapy and the number of gastrointestinal side effects for cohort 3 (which received 100 mg per day for four weeks), the cohort of greatest interest.

In spite of the gastrointestinal side effects, the mean weight of the patients before therapy was 39 ± 10 kg and the mean weight at the end of therapy was 40.4 kg. Before starting therapy, patients were told to expect gastrointestinal side effects of mild-to-moderate severity, and no patient discontinued therapy because of gastrointestinal side effects.

Excluding 1 patient who prematurely discontinued therapy because of elevations in serum aspartate aminotransferase levels, 12 of 80 patients at centers 1 and 2 had serum aspartate aminotransferase values between 100 and 150 U per liter during therapy (upper limit of normal, 40). For all 12 patients, the values reverted to less than 100 U per liter within one to two weeks after beginning therapy. At center 2, one patient had a base-line value of 243 U per liter that decreased to 205 U per liter one week later and 73 U per liter after two weeks. Another patient had a base-line value of 145 U per liter that rose to 290 U per liter one week later (the end of therapy) and was 70 U per liter two weeks after the completion of therapy. At center 1, there was no significant relation ($P=0.30$ by *t*-test) between the dose in milligrams per kilogram per day and the number of patients who had elevations in serum aspartate aminotransferase levels to values greater than 100 U per liter, but at center 2 there was a significant relation ($P=0.04$).

Other than one patient who prematurely discontinued therapy because of elevations in serum urea nitrogen and creatinine levels, only two patients at centers 1 and 2 had elevations above the normal range (20 mg per deciliter [7 mmol per liter] for serum urea nitrogen and 1.5 mg per deciliter [133 μ mol per liter] for serum creatinine). In both cases, the serum urea nitrogen level was less than 25 mg per deciliter (9 mmol per liter), and the serum creatinine level was less than 2.0 mg per deciliter (177 μ mol per liter).

Two patients prematurely discontinued therapy because of laboratory evidence of drug toxicity. One patient in cohort 1 at center 1, who received 2.4 mg of miltefosine per kilogram per day, had elevated levels of serum aspartate aminotransferase (640 U per liter) during the second week of therapy in spite of having a base-line value that was only slightly high (49 U per liter). Therapy was discontinued on day 15. The level of aspartate aminotransferase then fell rapidly to 37 U per liter two weeks later. Even though the patient received therapy for only two weeks, the patient did not relapse. A patient in cohort 4 at center 1, who received 4.8 mg of miltefosine per kilogram per day (the third-highest dose), had three ep-

isodes of vomiting on day 10, two episodes on day 11, three on day 12, two on day 14, and four on day 15. Some of the episodes preceded rather than followed administration of the drug. On day 14, the serum creatinine level was 5.2 mg per deciliter (460 μ mol per liter, as compared with 1.0 mg per deciliter [88 μ mol per liter] before and one week after beginning therapy) and the serum urea nitrogen level was 58 mg per deciliter (21 mmol per liter, as compared with less than 16 mg per deciliter [5.7 mmol per liter] before and one week after beginning therapy). Therapy was discontinued on day 15. One week after the completion of therapy, the serum creatinine level fell to 1.7 mg per deciliter (150 μ mol per liter), and the serum urea nitrogen level fell to 27 mg per deciliter (9.6 mmol per liter). The values returned to base-line levels by two weeks after the discontinuation of therapy. This patient relapsed.

Ophthalmologic abnormalities have been found in rodents after treatment with miltefosine.⁹ No ophthalmologic abnormalities due to treatment were found in our patients.

DISCUSSION

Soon after humans are bitten by leishmania-infected sandflies, parasites are found in macrophage phagolysosomes. Humans frequently have efficient cellular immune responses to this obligate intraphagolysosomal parasite and do not have more than mild and reversible symptoms.¹⁴ However, marked enlargement of the spleen and substantial suppression of marrow indicate that the host is unable to control the parasite immunologically, that clinical disease has begun, and that death, often from pancytopenia, will ensue. Even with standard treatment, there is a substantial death rate once clinical disease is evident. Eleven percent of 3076 treated patients in Sudan died of unspecified causes.¹⁵ In a smaller series of 83 Sudanese patients, 5 died of anemic heart failure, gastrointestinal bleeding, or liver failure or died suddenly during antimonial therapy.¹⁶

Miltefosine, a close analogue of lecithin (phosphatidylcholine) in which phosphorylcholine is attached by an ether bond rather than an ester bond to a carbohydrate backbone, modifies cell-signaling pathways and membrane synthesis. Leishmania contain high levels of ether lipids,¹⁷ but the exact mechanisms of miltefosine's cytotoxicity with respect to the parasite are unknown.

In this open-label, dose-escalating, multicenter clinical trial, miltefosine was administered orally to four 30-member cohorts of Indian patients (12 to 50 years of age) with moderate kala-azar. All 120 patients had an initial parasitologic cure after two weeks of therapy. A total of 114 patients had not had a clinical or parasitologic relapse by six months after the completion of therapy, so the cure rate was 95 percent. The cure rate for patients who had never received

therapy with pentavalent antimony was virtually identical to that for patients in whom previous therapy had failed.

These regimens caused frequent but mild-to-moderate gastrointestinal toxicity. Sixty-two percent of the patients had gastrointestinal side effects, which averaged 1.7 episodes on each of 4 of the 28 days of therapy for these patients. Serum aspartate aminotransferase values were occasionally abnormal, and creatinine values were abnormal only rarely. When laboratory values increased to an unacceptable level (for serum aspartate aminotransferase in one patient and creatinine in one patient), therapy was discontinued, and the values rapidly normalized.

The overall results of this large phase 2 trial indicate that the total dose of miltefosine can be varied by a factor of approximately two without efficacy or toxicity being changed significantly. The cure rate for cohort 1, which received 50 mg of miltefosine per day for six weeks (a total dose of 2100 mg), was similar to the cure rate for cohort 4, which received 100 mg per day for one week followed by 150 mg per day for three weeks (a total dose of 3850 mg). Also, the side effects generally did not differ significantly between cohorts 1 and 4. The pilot study¹² suggested, however, that lower doses may be ineffective and that higher doses may not be tolerated, and the data on relapses in the present study support this conclusion. The mean daily dose in the present study was 2.8 mg per kilogram, but five of the six patients who relapsed received 1.0 to 2.3 mg per kilogram per day, and one of the two patients who prematurely discontinued therapy because of side effects received 4.8 mg per kilogram per day.

We recommend the regimen given to cohort 3 (100 mg per day [approximately 2.5 mg per kilogram per day] for four weeks) for phase 3 trials and eventual regulatory approval because of its high absolute efficacy (97 percent) and simplicity of administration. However, the dose should be adjusted to the patient's weight so that a dose of 4 mg per kilogram per day is not exceeded.

Therapeutic agents may be compared on the basis of efficacy, tolerance, and practical issues such as route of administration and cost. Classic therapy with pentavalent antimonial agents is limited by a clinical failure rate of 40 percent in some parts of India¹⁸ and is commonly¹⁹ associated with moderate toxicity (a 97 percent incidence of chemical pancreatitis; a 33 percent incidence of myalgias, gastrointestinal pain, and abnormalities of liver enzymes; a 20 percent incidence of incidental T-wave abnormalities; and in rare cases, death from cardiac causes with generic formulations²⁰). This treatment also requires daily injections for 28 to 40 days. Amphotericin B is greater than 95 percent effective but is almost always associated with fever,²¹ frequently causes renal dysfunction, and requires intravenous injections for 15 to 20

days. The most widely used new formulation of amphotericin B (liposomal amphotericin B) is also more than 95 percent effective but requires injections on six days over a three-week period of medical attention, it may have immediate side effects, and its cost precludes its use in more than 99 percent of patients.

Miltefosine, at a dose of 100 mg per day for four weeks, was 97 percent effective in one 30-patient cohort in our study of 120 patients. Because the infection was cured in 5 of 5 patients in the pilot study,¹² 29 of 30 patients in our study, and 16 of 16 patients in another recent study,²² the total cure rate to date with this regimen is 50 of 51, or 98 percent (95 percent confidence interval, 90 to 100 percent). In comparison, liposomal amphotericin B was approved in the United States on the basis of experience in 108 patients with kala-azar; the largest cohort had 42 patients, and efficacy was 97 percent.²

Although miltefosine often causes gastrointestinal side effects, may cause elevations in serum aspartate aminotransferase and creatinine levels, and should not be used in pregnancy because it is teratogenic in animals,⁹ these problems are tolerable, reversible, and avoidable, respectively. Miltefosine is easily synthesized, and its cost is predicted to be acceptable. Its effectiveness in countries with leishmania that might be less susceptible to miltefosine, in children, in HIV-infected patients, and in persons with more severe disease remains to be determined in phase 3 and 4 trials. Nevertheless, this large phase 2 trial shows that miltefosine is the first oral agent that is clearly effective and tolerated for any major leishmania-infected population. For the first time, there may be the possibility of outpatient treatment of large populations and the eradication of a visceral disease for which the only known reservoir is human.

Supported by grants from the United Nations Development Program–World Bank–World Health Organization Special Program for Research and Training in Tropical Diseases (960326, to Dr. Thakur, and 980326, to Dr. Jha).

We are indebted to the many people whose participation made this study possible, particularly Dr. A.D.M. Bryceson and Dr. W.E. Gutteridge for intellectual advice, Dr. N. Kshirsager and Dr. P. Naik for clinical monitoring, and the clinical staff at the three study centers for patient care.

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