Phase IV Trial of Miltefosine in Adults and Children for Treatment of Visceral Leishmaniasis (Kala-Azar) in Bangladesh

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Abstract. Miltefosine (target dose of 2.5 mg/kg/day for 28 days) is the recommended treatment for visceral leishmaniasis (kala-azar) in Bangladesh on the basis of data from India. We evaluated miltefosine in a phase IV trial of 977 patients in Bangladesh. At the six-month final follow up, 701 were cured. 24 showed initial treatment failure, and 95 showed treatment failure at 6 months, although 73 of the 95 showed treatment failure solely by the criterion of low hemoglobin values. One hundred twenty-one patients were not assessable. With the conservative assumption that all low hemoglobin values represented treatment failure, the final per protocol cure rate was 85%. Of 13 severe adverse events, 6 led to treatment discontinuation and 7 resulted in deaths, but only 1 death (associated with diarrhea) could be attributed to drug. Nearly all non-serious adverse events were gastrointestinal: vomiting in 25% of patients and diarrhea in 8% of patients. Oral miltefosine is an attractive alternative to intramuscular antimony and intravenous amphotericin B for treatment of kala-azar in Bangladesh.

INTRODUCTION

Most cases of visceral leishmaniasis (VL) (kala-azar) worldwide occur in contiguous regions of India, Nepal, and Bangladesh.¹ In Bangladesh, VL epidemics occur approximately every 15–20 years and last for approximately one decade. The prevalence of the disease in Bangladesh has increased sharply in recent years. Approximately 10,000 patients are reported annually from government health facilities and many more go unreported. The current prevalence is estimated to be 45,000 cases with more than 40.6 million persons at risk. Of 64 districts in Bangladesh, at least 34 districts have been reportedly affected (Ahmed B, unpublished data).

As in other countries, VL in Bangladesh is a disease of the poorer sections of society residing in rural areas. Mortality from kala-azar when untreated is nearly 100%, and most patients die of intercurrent infections after reduced immunity and undernutrition. Although treatment for this disease is needed, conventional treatment of VL with parenteral pentavalent antimony has multiple side effects, and resistance to this drug is increasing in the Indian subcontinent.² Amphotericin B deoxycholate, although effective, is given intravenously 15 times over 30 days. Liposomal amphotericin B is administered less frequently than amphotericin B deoxycholate and shows excellent effectiveness in India,³ but is the more expensive option for treatment of VL⁴ and is not presently available in Bangladesh.

Miltefosine is an orally administrable agent that has completed phase III and phase IV evaluation for treatment of VL in India.^{5,6} and is now recommended as the treatment of choice in Bangladesh. The aim of the present study was to evaluate the effectiveness of miltefosine in outpatients in this country.

PATIENTS AND METHODS

Study design. The study was an open-label, single-group trial of miltefosine at 12 outpatient centers in VL-endemic regions of Dhaka, Rajshahi, and Mymensingh, Bangladesh.

Patients. Patients were 2–65 years of age. Both male and female patients participated, and patients were enrolled during October 2006–September 2007. Inclusion criteria were signs and symptoms compatible with VL (fever for at least two weeks, palpable splenomegaly, weight loss by history), positive serologic test result for leishmaniasis (rK39),⁷ hemoglobin level ≥ 6 g/dL, no infection with HIV, not pregnant or lactating, not being currently treated with an antileishmanial compound, and no significant concomitant medical condition. Malaria and enteric fever were ruled out by blood smears and the Widal test, respectively. If present, malaria and enteric fever were treated. These patients could then be admitted into the study. If tuberculosis was suspected on clinical grounds, the patient was not eligible for this study.

Study procedures. Enrolled patients were given sufficient drugs for treatment for one week. Patients returned to the outpatient clinic once a week for three successive weeks for assessment of compliance and adverse events (AEs) and to receive drug for the next week's treatment. At the end of 28 days of treatment, patients were assessed for initial cure and were given instructions for follow-up at two months and six months after the end of treatment.

Drug treatment. Miltefosine was administered daily for 28 days after meals. For children (≤ 12 years of age), the daily dose was 2.5 mg/kg body weight using 10-mg capsules split into twice-a-day doses. For adults (> 12 years of age) weighing < 25 kg, the daily dose was 50 mg of miltefosine/day as one capsule (50 mg) in the evening. For adults (> 12 years) weighing ≥ 25 kg, the daily dose was 100 mg of miltefosine as one capsule (50 mg) in the morning and one capsule in the evening.

Assessments. Efficacy parameters (temperature and weight, spleen size, and hemoglobin level) were evaluated at initial screening and on days 7, 14, 21 and 28 of treatment and 2 months

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and 6 months after the end of treatment. If the patient showed signs of VL during follow-up, the patient was to be referred to the corresponding medical center for spleen/bone marrow aspiration for parasitologic evaluation of possible VL.

Safety parameters. The type, duration, and common toxicity criteria (CTC) severity grade of AEs were determined during treatment. The CTC grades for some safety parameters are quantitative.⁸ These grades for non-quantifiable parameters are 1 = mild, 2 = moderate, 3 = severe, and 4 = life-threatening.

Response criteria. Initial cure at the end of therapy or up to two months after the end of treatment was defined as loss of fever and a spleen size at least 30% smaller than at pretreatment, and hemoglobin level of at least 10.0 g/dL or an increase with respect to the pretreatment value of at least 10%.

Final cure six months after the end of therapy was defined as loss of fever; a spleen size at least 70% smaller than at pretreatment or, if the spleen size was < 1 cm at pretreatment, not > 1 cm; weight gain of at least 0.5 kg; and a hemoglobin level of at least 10.0 g/dL or an increase with respect to the pretreatment value of at least 10%.

Analytic populations. The per-protocol population was defined as patients who received at least 90% of the planned treatment (i.e., at least 25 days of treatment). The intent-to-treat 1 group was defined as patients who received 14–24 days of therapy. The intent-to-treat 2 group was defined as patients who received 1–13 days of therapy. Non-evaluable patients were defined as patients who were lost to follow-up after receiving at least one dose of drug.

Sample size. For this single-group trial, the sample size was selected to be comparable with that of the phase IV Indian trial (approximately 1,000 patients).⁶

Ethical review. The protocol was reviewed by the Bangladesh Medical Research Council and the Ethical Review Committee of the World Health Organization.

RESULTS

Patients and compliance. Of 1,007 patients, 977 (97.0%) patients received at least one dose of drug. Approximately

60% of patients were males and 60% were adults. Adult patients weighed a mean $\pm$ SD of $51 \pm$ kg.

It is standard practice in the Bangladesh outpatient setting in which this trial was conducted to treat suspected VL patients who are rK39 positive whether or not splenomegaly is present. Because it is unclear whether rK39-positive patients without splenomegaly are infected with *Leishmania*, efficacy analysis was performed separately on 817 patients who were rK39 positive and had splenomegaly (splenomegaly group) and 160 patients who were rK39 positive and did not have splenomegaly (no splenomegaly group).

799 (98%) patients in the splenomegaly group and 141 (88%) patients in the no splenomegaly group received 25–28 days of treatment and were included in the per-protocol population (Table 1). Only 37 patients were in the other prospectively defined analytic groups. The intent-to-treat 1 group, the intent-to-treat 2 group, and the non-evaluable group contained 8, 0, and 29 patients, respectively. All but one of intent-to-treat 1 patients and non-evaluable patients withdrew from the protocol because of AEs (10 patients), patient wish (9 patients), or other reasons (17 patients). The remaining patient was in the intent-to-treat 1 splenomegaly group and was cured. For clarity of presentation, the one intent-to-treat 1 splenomegaly group patient was combined with the 799 per-protocol splenomegaly patients to give a total of 800 per-protocol patients for analysis of efficacy.

Efficacy in the per-protocol population. In the splenomegaly group, initial cure was seen in 733 (92%) of the 800 patients. Final cure was seen in 591 of the 733 initially cured patients. The total number of patients who showed treatment failure was 21 at the initial periods and 84 at the final six-month evaluation. The overall cure rate in the splenomegaly group was 591 (74%) of 800 treated patients and 591 (85%) of 696 patients who were evaluable at the initial and final follow-up visits.

Interestingly, the degree of splenomegaly at presentation did not predict patients who would eventually be cured or showed treatment failure. Pretreatment mean spleen size was 8.6 cm. Mean spleen size was 9.6 cm in patients who were cured, 10 cm in patients who initially showed treatment failure, and 5.7 cm in patients who showed treatment failure at 6 months.

TABLE 1
Efficacy outcomes of different patient populations treated with miltefosine for visceral leishmaniasis (kala-azar), Bangladesh*

Characteristic	Per protocol		Intent to treat (1)		Non-evaluable patients		Total
	Splenomegaly	No splenomegaly	Splenomegaly	No splenomegaly	Splenomegaly	No splenomegaly	
No. patients	799	141	5	3	13	16	977
M	501	87	3	2	10	9	612
F	298	54	2	1	3	7	365
Child	367	26	3	0	2	2	400
Adult	432	115	2	2	11	14	577
No. patients withdrawn	0	0	4	3	13	16	36
AE	0	0	0	0	2	2	4
SAE	0	0	1	0	3	2	6
Patient wish	0	0	0	0	3	6	9
Other	0	0	3	3	5	6	17
Initial results at 4 weeks and/or 2 months							
Cure	732	132	1	NA	NA	NA	865
Failure	21	3	0	NA	NA	NA	24
Not assessable	46	6	0	NA	NA	NA	52
Final results at 6 months							
Cure	590	110	1	NA	NA	NA	701
Failure	84	11	0	NA	NA	NA	95
Not assessable	58	11	0	NA	NA	NA	69

*AE = adverse event; SAE = severe adverse event; NA = not available.

In the no splenomegaly group, initial cure was seen in 132 (94%) of 141 patients. Final cure was seen in 110 of the 132 initially cured patients. The total number of patients who failed to respond to treatment was 3 initial treatment failures and 11 final treatment failures. The overall cure rate in the no splenomegaly group was 110 (78%) of 141 treated patients and 110 (89%) of 124 patients who were seen at follow up.

For per-protocol patients, because cure rates and failure rates for the splenomegaly group and the no splenomegaly group were similar, we merged the two groups and calculated efficacy rates for all per-protocol patients combined. Of 941 per-protocol patients, 865 (92%) were initially cured and 24 (2.6%) initially failed to respond to treatment. At the six-month final follow-up, 701 patients were cured and an additional 95 patients failed to respond to treatment. The overall cure rate for the per-protocol population was 701 (74%) of 941 treated patients and 701 (85%) of 820 evaluated patients.

Tolerance. Drug tolerance in the 977 patient safety population was determined by analysis of severe AEs (SAEs) and AEs. There were 13 SAEs: 6 (Table 1) that led to treatment discontinuation and 7 in the per-protocol population that did not lead to treatment discontinuation.

The 13 SAEs consisted of intestinal tuberculosis (1), enteric fever (2), vomiting with or without nausea and cough (3) leading to deaths of 2 patients, diarrhea (1) leading to death of 1 patient, hemoptysis/dyspnea/cough (2) leading to death of 1 patient, edema and severe fatigue (1), edema and anemia (1), severe anemia (1) leading to death of 1 patient, and death of a person 2 months after taking medication for 1 week, which was the last trial visit (1).

Cases of tuberculosis and enteric fever were judged to be unrelated to study medication. The two deaths associated with vomiting occurred 19 and 33 days after the end of treatment; the third case of vomiting started before the study drug was given. The death caused by diarrhea occurred on day 21 of treatment, and, because miltefosine is known to have gastrointestinal side effects, might have been related to the drug. Cases of hemoptysis/cough and edema occurred during treatment with miltefosine but these side effects are not known side effects of miltefosine. The case of severe anemia that resulted in death could have been a case of clinical failure. Clear attribution of SAEs to miltefosine was not possible in any case, but the death caused by diarrhea may have been attributable to a drug effect.

The AEs of the gastrointestinal system, the most common site of clinical intolerance, are shown in Table 2. Vomiting

and diarrhea were common and occurred in 241 (25%) and 77 (8%) of the patients, respectively. Each episode lasted approximately 3 days, and was CTC grade 1 in 61% of cases, CTC grade 2 in 32% of cases, and CTC grade 3 in 4% of cases. The AEs events related to the respiratory system were rare. A total of 17 patients (2%) had either cough, rhinitis, hemoptysis, upper respiratory tract infection, dyspnea, or pharyngitis.

In terms of other clinical side effects, a total of 44 patients reported epistaxis, headache, fever, malaise, edema, rash, vertigo, motion sickness, myalgia, asthenia, varicella, influenza-like symptoms, increase in appetite, decrease in weight, skin depigmentation, amenorrhea, back pain, allergic reaction, otitis media, or dysaesthesia.

DISCUSSION

Miltefosine has been the subject of formal drug development for treatment of VL in India. Phase II, phase III, and phase IV trials showed 97%, 97% and 95% per protocol cure rates, respectively, in India.^{5,6,9}

Because Bangladesh borders India and there is an immediate need for orally administrable agents for VL, miltefosine was not evaluated in Bangladesh according to this strict phase II–III–IV sequence. Instead, this first trial in Bangladesh was an outpatient phase IV evaluation. The advantage of such a trial is that efficacy and tolerance under real world conditions are seen.

The limitations of this trial originate from the same real-world factors. Under outpatient clinic conditions, the presence of *Leishmania* is implied by rK39 seropositivity, rather than by visualizing parasites in splenic aspirates, because this latter technique may lead to fatal complications. The rK39 test has a false-positive rate of approximately 10%,⁷ especially if splenomegaly is absent. It is standard practice in the outpatient setting in Bangladesh to treat patients who are rK39 positive whether or not splenomegaly is present. In a protocol violation, such no splenomegaly patients were entered into this trial. Possible inclusion of non-VL cases could artifactually increase or decrease the cure rate if the concomitant disease remitted by itself or continued unabated. Nevertheless, the efficacy rates for patients with splenomegaly and without splenomegaly were similar. When data from splenomegaly and no splenomegaly patients were combined, there were 701 patients who were cured and 119 initial plus final patients who did not respond to treatment, giving a cure rate of 85% for evaluable patients.

TABLE 2

Incidence of gastrointestinal adverse events that emerged during treatment of patients with miltefosine for visceral leishmaniasis (kala-azar), Bangladesh*

Adverse event	No. patients with adverse event	Duration of adverse event, days†	CTC 1 (mild)‡	CTC 2 (moderate)‡	CTC 3 (severe)‡	CTC 4 (life-threatening)‡
Vomiting	241	2.7 (2.0)	148	87	6	0
Diarrhea	77	2.9 (2.4)	55	16	6	0
Abdominal pain	3	6.7 (9.0)	2	1	0	0
Anorexia	8	4.1 (5.8)	4	2	0	0
Nausea	2	5.0 (5.7)	2	0	0	0
Dyspepsia	1	4	1	0	0	0
Gastritis	3	7.3 (1.2)	3	0	0	0
Gastric ulcer	2	3.0 (0)	2	0	0	0
Dysentery	1	2	1	0	0	0

* CTC = common toxicity criteria.

† Values are mean (SD).

‡ Values are no. patients in each CTC category.

Interpretation of the 85% cure rate was hampered by the inability to completely determine the causes of low hemoglobin levels at six-months of follow up. A total of 73 of the 95 patients who showed treatment failure at six months did so for this reason. Low hemoglobin levels for reasons other than VL are common in this patient population, and it is a present source of controversy whether low hemoglobin levels should be a criterion for lack of cure. In the phase III trial in India, for example, only one of three of such patients were eventually determined to have relapsing VL.⁵ If 25 of the 73 patients with low hemoglobin levels at 6 months were patients who failed to respond to treatment, the cure rate in this trial would have been 92%. Nevertheless, the protocol specified that low hemoglobin levels constituted clinical treatment failure. Thus, we conclude that all 73 patients with low hemoglobin levels at 6 months were patients who failed to respond to treatment and report a conservative cure rate of 85%.

Tolerance of miltefosine was similar to what has been reported from India.⁶ The number of deaths (7) in this trial with 1,000 patients was higher than the 3 deaths in the similarly sized phase IV trial in India, but the number of deaths possibly related to gastrointestinal side effects of miltefosine was 1 in each study. Nearly all non-serious AEs were gastrointestinal: vomiting in 25% of patients and diarrhea in 8% of patients. The rate of vomiting was somewhat higher than the rate of 14% in the phase IV study in India.⁶

In conclusion, the overall cure rate was 85% in evaluated patients. If only one of three patients with low hemoglobin levels were considered patients who failed to respond to treatment, an in India, the cure rate would increase to 92%. In terms of drug tolerance, perhaps 1 of the approximately 1,000 patients died of gastrointestinal side effects and approximately 30% of patients had vomiting and/or diarrhea of low-to-moderate grade. Data from this study convey an understanding of the benefits and risks for what is presently first-line therapy for treatment of VL in Bangladesh. We cannot find reliable data on the other alternatives, parenteral pentavalent antimony and intravenous amphotericin B deoxycholate, in a phase IV setting in Bangladesh. In formal phase II clinical trials in (antimony-sensitive) regions of India, these difficult-to-tolerate agents had cure rates of approximately 85%² and 95%,¹⁰ respectively.

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REFERENCES

1. Guerin PJ, Olliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P, Wasunna MK, Bryceson AD, 2002. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and proposed research and development agenda. *Lancet Infect Dis* 2: 494–501.
2. Sundar S, More DK, Singh MK, Singh VP, Sharma S, Makharia A, Kumar PC, Murray HW, 2000. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. *Clin Infect Dis* 31: 1104–1107.
3. Sundar S, Chakravarty P, Agarwal D, Rai M, Murray HM, 2010. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. *N Engl J Med* 362: 504–512.
4. Meheus F, Balasegaram M, Olliaro P, Sundar S, Rijal S, Faiz MA, Boelaert M, 2010. Cost-effectiveness analysis of combination therapies for visceral leishmaniasis in the Indian subcontinent. *PLoS Negl Trop Dis* 4: e818.
5. Sundar S, Jha TK, Thakur CP, Engel J, Sindermann H, Fischer C, Junge K, Bryceson A, Berman J, 2002. Oral miltefosine for Indian visceral leishmaniasis. *N Engl J Med* 347: 1739–1746.
6. Bhattacharya SK, Sinha PK, Sundar S, Thakur CP, Jha TK, Pandey K, Das VR, Kumar N, Lal C, Verma N, Singh VP, Ranjan A, Verma RB, Anders G, Sindermann H, Ganguly NK, 2007. Phase 4 trial of miltefosine for the treatment of Indian visceral leishmaniasis. *J Infect Dis* 196: 591–598.
7. Boelaert M, El-Safi S, Hailu A, Mukhtar M, Rijal S, Sundar S, Wasunna M, Aseffa A, Mbui J, Menten J, Desjeux P, Peeling R, 2008. Diagnostic tests for kala-azar: a multi-centre study of the freeze-dried DAT, rK39 strip test and KAtex in east Africa and the Indian subcontinent. *Trans R Soc Trop Med Hyg* 102: 32–40.
8. *Common Toxicity Criteria of the NCI*. Version 2.0, 1999. Bethesda, MD: National Cancer Institute.
9. Jha TK, Sundar S, Thakur CP, Bachmann P, Karbwang J, Fischer C, Voss A, Berman J, 1999. Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. *N Engl J Med* 341: 1795–1800.
10. Sundar S, Chakravarty J, Rai VK, Agrawal N, Singh SP, Chauhan V, Murray HW, 2007. Amphotericin B treatment for Indian visceral leishmaniasis: response to 15 daily versus alternate-day infusions. *Clin Infect Dis* 45: 556–561.