Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial

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Summary

Background **Improved treatment approaches are needed for visceral leishmaniasis. We assessed the efficacy and safety of three potential short-course combination treatments compared with the standard monotherapy in India.**

Methods **Standard treatment (1 mg/kg amphotericin B infusion on alternate days for 30 days, total dose 15 mg/kg) was compared with three drug combinations (single injection of 5 mg/kg liposomal amphotericin B and 7-day 50 mg oral miltefosine or single 10-day 11 mg/kg intramuscular paromomycin; or 10 days each of miltefosine and paromomycin) in an open-label, parallel-group, non-inferiority, randomised controlled trial in two hospital sites in Bihar, India. Patients aged 5–60 years with parasitologically confirmed visceral leishmaniasis were randomly assigned one of the four treatments by the trial statistician by use of a computer-generated list. Clinical assessments were done at the end of treatment (15 days on combination treatment; 31 days for standard treatment) and after 45 days and 6 months. The primary endpoint was definitive cure (defined as no sign or symptom of visceral leishmaniasis and parasitologically cured to the last follow-up). Analyses were done both by intention to treat and per protocol. This trial is registered with ClinicalTrials.gov, number NCT00696969.**

Findings **Between June, 2008, and July, 2009, 634 patients were assigned amphotericin B (n=157), liposomal amphotericin B with miltefosine (n=160) or paromomycin (n=158), or miltefosine and paromomycin (n=159). 618 patients were in the per-protocol population. There were two relapses in each group. The numbers with definitive cure at 6 months for the intention-to-treat population were 146 (cure rate 93·0%; CI 87·5–96·3) for amphotericin B, 156 (97·5%; 93·3–99·2) for liposomal amphotericin B and miltefosine, 154 (97·5%; 93·24–99·2) for liposomal amphotericin B and paromomycin, and 157 (98·7%; 95·1–99·8) for miltefosine and paromomycin. All combinations were non-inferior to the standard treatment, in both the intention-to-treat and per-protocol populations. Patients in the combination groups had fewer adverse events than did those assigned standard treatment.**

Interpretation **Combination treatments for visceral leishmaniasis are efficacious and safe, and decrease the duration of therapy, thereby encouraging adherence and reducing emergence of drug-resistant parasites.**

Funding **Drugs for Neglected Diseases initiative and the Indian Council of Medical Research.**

Published **Online** January 20, 2011 DOI:10.1016/S0140-6736(10)62050-8

See **Online/Comment** DOI:10.1016/S0140-6736(10)62237-4

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Introduction

Visceral leishmaniasis (also known as kala-azar) affects populations in Asia, east and north Africa, South America, and southern Europe, and is a major public health problem in south Asia.[1] This disease is caused by the protozoan parasites *Leishmania donovani* and *L infantum* (*L chagasi* in Latin America). The parasites are transmitted through the bite of female phlebotomine sandflies, and they infect and multiply in different macrophage populations in the human host. Fever, often associated with rigor and chills, splenomegaly, pancytopenia, weight loss, and weakness are the main clinical features of visceral leishmaniasis, which is fatal if untreated. Pentavalent antimonials are the most common treatment worldwide; however, in the Indian state of Bihar, they are now ineffective because of the development of resistance,[2,3] leading to the use of amphotericin B as the standard treatment for visceral leishmaniasis.[4] Resistance to pentavalent antimonials is also being reported in neighbouring Nepal.[5]

Nevertheless, progress has been made during the past two decades; in addition to amphotericin B deoxycholate, liposomal amphotericin B, miltefosine, and paromomycin have been developed and registered in various countries for the treatment of visceral leishmaniasis. Recent advances from studies in Bihar give options for single-dose and combination treatments. A single high dose (10 mg/kg) of liposomal amphotericin B was highly effective (96% definitive cure rate at 6 months),[6] whereas a lower dose (5 mg/kg) was about 90% effective.[7] However, development of parasite resistance is a concern with monotherapies, especially miltefosine and paromomycin because they need long treatment courses (miltefosine 28 days; paromomycin 21 days), which can result in poor adherence. Studies are underway to test combinations of various antileishmanial drugs[8] to maintain high efficacy,

delay the development of drug resistance, decrease treatment duration, and possibly reduce toxicity and cost. Although use of miltefosine and paromomycin is a recent treatment approach, laboratory evidence indicates that resistance to these drugs might emerge quickly if they are used as monotherapy.[9,10]

The efficacy and safety of combinations of approved antileishmanial drugs in animals have lent support to the combined use of these drugs.[11] In a phase 2 study of a single dose of liposomal amphotericin B (3·75–5 mg/kg) followed by 7, 10, or 14 days of miltefosine, cure rates were 96% or higher in all groups.[12] In this trial, we aimed to identify an effective and safe short-course combination treatment of visceral leishmaniasis in India (liposomal amphotericin B and miltefosine *vs* liposomal amphotericin B and paromomycin *vs* miltefosine and paromomycin) that is not less than 7% inferior to standard treatment with amphotericin B.

Methods

Study design and patients

For the **study protocol** see http://www.dndi.org/index.php/clinical-trial-protocols.html?ids=2

This open-label, parallel-group, randomised controlled trial was based on a non-inferiority design. This study was done at two sites in Bihar: Muzaffarpur (site of Kala-Azar Medical Research Center of Banaras Hindu University, Varanasi) and Patna (Rajendra Memorial Research Institute of Medical Sciences).

Patients of both sexes aged between 5 years and 60 years with symptoms (particularly a history of fever) and signs (eg, splenomegaly) of visceral leishmaniasis with presence of parasites confirmed by microscopy of spleen or bone-marrow aspirates were enrolled into the study. Because miltefosine has teratogenic potential, women of child-bearing age were included if they had a negative pregnancy test and were using an assured method of contraception or agreed to receive an injection of medroxyprogesterone acetate (DepoProvera, Pfizer, NY, USA). Reasons for exclusion were haemoglobin concentrations less than 50 g/L, total leucocyte count less than 1×10^9/L, serum creatinine concentration outside the normal range of 55–140 µmol/L, platelet count less than 40×10^9/L, serum aminotransferase concentration higher than three times the upper limit of the normal ranges, bilirubin concentration more than 34·2 µmol/L, prothrombin time more than 5 s longer than control, positive serology for HIV or hepatitis B or C viruses, severe concurrent illness, and receipt of any antileishmanial or antifungal drug in the previous 45 days. Pregnant and breast-feeding women, patients with known hypersensitivity to the study drugs, and those with diabetes, hypertension, or tuberculosis were also excluded.

The protocol was approved by the institutional ethics committees, the Indian Council of Medical Research, the Drug Controller General of India, and the Ethics Committee of Basel University, Basel, Switzerland. The protocol was designed and completed in accordance with the general ethical principles outlined in the Declaration of Helsinki, 2000, and International Conference on Harmonization guidelines for good clinical practice. The protocol was first approved to enrol adults only (n=120), then extended to children older than 5 years after a safety and initial efficacy assessment (up to day 45 of follow-up) was reviewed by the independent Data Safety Monitoring Board and the Drug Controller General of India. At that time, 293 adults had already been enrolled. Thereafter, an additional 169 adults and 173 children (aged 5–18 years) were enrolled. Children were equally distributed between the four groups.

Randomisation and masking

A computer-generated, randomisation code was generated by the trial statistician by use of SAS 9.1 (SAS Institute, Cary, NC, USA). To ensure maximum balance of the numbers in each group at any time and to minimise bias, block sizes of 16 were generated for treatment allocation of patients to one of the four treatment groups, with equal allocation ratio and independently for each site. The treatments were 1 mg/kg amphotericin B infusion on alternate days for 30 days (standard treatment), single injection of 5 mg/kg liposomal amphotericin B and 7-day 50 mg oral miltefosine or 10-day 11 mg/kg intramuscular paromomycin, or 10 days each of miltefosine and paromomycin. Individual, opaque, sealed, and sequentially numbered envelopes were provided to each trial site, one envelope per patient, to indicate the allocation of individual patients to treatment. Microscopists were masked to the treatment given, and 10% of the slides were examined by an external microscopist for quality control during the early phases of the study. Concordance was reported in 112 of 113 slides.

Procedures

Patients were screened by clinical examination and rk39 (the recombinant K39 antigen of *Leishmania*) rapid immunochromatographic test (InBios International, WA, USA).[13] Patients with presumptive visceral leishmaniasis on the basis of that test underwent spleen or bone-marrow biopsy for definitive diagnosis based on the detection of parasites by microscopic examination.[14] These procedures are routinely used for the management of patients with visceral leishmaniasis at the study sites. Eligible patients were then informed of the trial objectives and procedures, and were asked to give voluntary consent and sign the consent form. For children, the consent of parents or guardian was sought. Patients who met the entry criteria were enrolled and randomly assigned to treatment at each trial site. Treatment was started within 7 days of baseline evaluation, which included clinical assessment (ie, body temperature, weight, and liver and spleen size) and laboratory tests. Additionally, electrocardiography and chest radiography were done if clinically indicated. Splenic smears were graded from 0 to 6+ on a logarithmic scale according to Chulay and Bryceson;[15] bone-marrow smears were obtained if splenic aspiration was not possible.

www.thelancet.com Published online January 20, 2011 DOI:10.1016/S0140-6736(10)62050-8

In the control group, patients were given the standard treatment, 1 mg/kg amphotericin B deoxycholate (Piramal Healthcare, Mumbai, India) by intravenous infusion over 6 h in 5% dextrose on alternate days for 30 days (total dose of 15 mg/kg). Patients allocated liposomal amphotericin B combined with miltefosine received 5 mg/kg liposomal amphotericin B (AmBisome, Gilead Sciences, CA, USA) by intravenous infusion over 2 h in 5% dextrose on day 1 followed by 7 days (days 2–8) of oral miltefosine (Paladin, Montreal, Canada), 50 mg once a day if below 25 kg bodyweight, twice a day if 25 kg or more, or 2·5 mg/kg for children younger than 12 years. Patients allocated liposomal amphotericin B combined with paromomycin received 5 mg/kg liposomal amphotericin B by intravenous infusion over 2 h on day 1 and 11 mg/kg paromomycin base (Gland Pharma, Hyderabad, India) per day intramuscularly (equivalent to 15 mg/kg per day of paromomycin sulphate) for 10 days, on days 2–11. Patients assigned miltefosine and paromomycin received oral miltefosine (doses as in the liposomal amphotericin B and miltefosine group) for 10 days (instead of 7 days) and 11 mg/kg per day paromomycin intramuscularly for 10 days, on days 1–10. Before treatment with amphotericin B or liposomal amphotericin B, a test infusion of 1 mg was given and patients were observed for 30 min for any allergic response before the rest of the infusion was given. All assigned treatments were given by a trained study nurse to ensure adherence.

Once treatment was started, patients underwent daily clinical and scheduled laboratory assessments on day 7 and day 15 for all groups, and additionally on day 31 for the control group. Patients were in hospital for 31 days in the control group and for 15 days in the other groups. The parasitological examination (splenic or bone-marrow smears) was repeated on day 31 in the control group and on day 15 for the other groups. Patients on combination treatments with a score of 1+ (1–10 parasites/1000 microscopic fields; 10×100 magnification under oil immersion) on day 15 were again tested on day 45 for parasitological assessment.

The primary objective was to compare the efficacy of the combination treatments (ie, definitive cure rate) versus the standard treatment. Initial cure was defined as absence of fever, clinical improvement, reduction in spleen size, and no parasites in spleen or bone-marrow smears on day 45. For a definitive cure, assessed at 6 months (defined as 2 weeks before the 6-month point to 6 weeks afterwards) from the beginning of treatment, initial cure was needed with no signs or symptoms of relapse. The secondary objectives were to compare safety and tolerability of the different combination treatments with the recommended standard treatment during treatment and follow-up. To assess safety of the treatments, the Common Terminology Criteria for Adverse Events version 3.0 was used. Patients with adverse events of grade 3 or higher were withdrawn from the study. Those withdrawn because of adverse events or relapses after initial cure were given rescue treatment with liposomal amphotericin B in doses of 3 mg/kg per day for 5 days or another recommended treatment (paromomycin in one case) if liposomal amphotericin B was contraindicated.

Data handling and statistical analysis

We assumed a definitive cure rate of 97% with the reference drug (amphotericin B) and a non-inferiority margin of 7% for the test groups. With a power of 90%

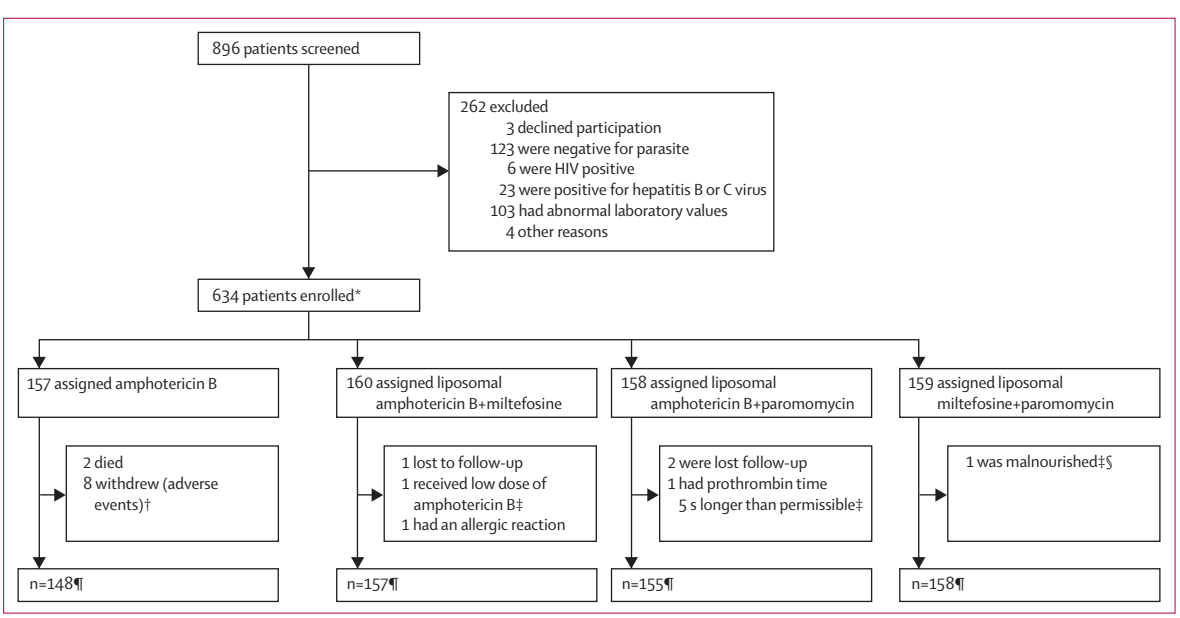

Figure 1: **Trial profile**

*Intention-to-treat population. †One of whom subsequently died. ‡Major deviation from protocol. §Body–mass index less than 15 kg/m². ¶Per-protocol population.

and equal allocation ratio, the sample size per group was 148; on the assumption of a drop-out rate of 5%, 156 patients per group were needed, with a total sample size for four groups of 624 patients. A non-inferiority margin of 7% was chosen because 90% was thought to be the minimum acceptable rate of definitive cure; it was the rate attained by a single 5 mg/kg dose of liposomal amphotericin B in a previous trial.[7]

Data were collected from hospital records of each centre and transferred to specially designed case report forms, which were collected and processed by a contract research organisation. The contract research organisation and the sites were audited by an independent international auditor, who reported no major faults. The case report forms were monitored on site by independent clinical monitors of the contract research organisation, initially twice a month, then later once a month (as the rate of enrolment decreased and stopped later in the study, only follow-up data were monitored and thus the frequency of monitoring decreased). A double data entry was done with an Oracle Clinical Database and tools for data cleaning, including queries to the investigators in case of inconsistencies. Adverse events and concomitant treatment were coded by the Medical Dictionary for Regulatory Activities and the WHO Drug Dictionary. The trial statistician was responsible for the quality control of data handling and participated in analyses.

The primary endpoint of definitive cure rate was analysed for all randomly assigned patients (intention to treat [ITT]) and for the per-protocol patients. The per-protocol group consisted of all patients who were enrolled, had no major protocol deviation, received the full treatment, and were assessed at day 15 or 31, day 45, and 6 months (–2 to +6 weeks). The cure rate comparisons are presented as the difference in proportion cured and the one-sided 97·5% CI for the difference in proportions using normal approximation to the binomial law. Each combination treatment was compared with the reference drug and, if the difference was not statistically significant, compared with the two other combination regimens based on a non-inferiority margin difference higher than –7%. The Bonferroni-adjusted alpha risk for multiple comparisons between combination regimens and the reference group was 0·0125. The decision rule stated that, if the lower limit of the CI was more than –7%, non-inferiority could be concluded.

The frequency of patients with at least one adverse event or serious adverse event was calculated along with the number and frequency of adverse events (including serious adverse events) per body system. Laboratory values were evaluated by ANOVA, and groups were further compared by post-hoc pairwise comparisons with the Tukey method (which accounts for unequal groups and multiplicity). The homogeneity of variance was verified

	Amphotericin B (n=157)	Liposomal amphotericin B+ miltefosine (n=160)	Liposomal amphotericin B+ paromomycin (n=158)	Miltefosine+ paromomycin (n=159)
Demographic characteristics				
Mean age (years [SD])	28 (14)	27 (13)	25 (14)	28 (16)
Age range (years)	6–60	6–58	5–58	6–60
Aged ≤18 years (%)	41	39	45	46
Male	98 (62%)	117 (73%)	100 (63%)	107 (67%)
Recruitment site				
Kala-Azar Medical Research Centre (%)	119 (76 %)	120 (75%)	119 (75%)	120 (75%)
Rajendra Memorial Research Institute of Medical Sciences (%)	38 (24%)	40 (25%)	39 (25%)	39 (25%)
Clinical characteristics				
Patients with history of previous treatment (>45 days before enrolment)	3	2	3	0
Splenic aspirate score	2·2 (1·1)	2·2 (1·2)	2·1 (1·0)	2·1 (1·1)
Weight (kg)	40·5 (11·7)	40·5 (11·3)	39·0 (11·7)	39·9 (12·1)
Spleen size (cm) below left costal margin	5·0 (3·3)	5·0 (3·6)	6·0 (3·8)	5·0 (3·2)
Haemoglobin (g/L)	81 (17)	84 (17)	82 (19)	83 (19)
White-cell count (median [IQR]; $\times10^9$/L [min–max])	2·9 (2·2–3·7)	2·9 (2·1–3·9)	3·1 (2·2–3·9)	3·0 (2·2–3·9)
Platelet count (median [IQR]; $\times10^9$/L [min–max])	112 (78·0–150·0)	117 (80·5–154·0)	110 (79·0–143·0)	110 (80·0–156·0)
Creatinine (μmol/L)	71 (11)	73 (11)	72 (11)	72 (11)
Blood urea nitrogen (mmol/L)	64·4 (23·2)	61·6 (23·0)	66·9 (24·4)	64·7 (22·1)
Alanine aminotransferase (median [IQR]; U/L [min; max])	31 (19·0–50·0)	34 (20·5–59·7)	30 (18·0–45·6)	33 (20·0–51·0)
Aspartate aminotransferase (median [IQR]; U/L [min; max])	48 (32·0–74·0)	49 (32·4–78·1)	45·6 (32·0–66·0)	48 (33·0–71·0)

Mean values (SD) unless otherwise specified. ITT=intention to treat. Min=minimum. Max=maximum.

Table 1: **Characteristics of all patients at baseline (ITT)**

www.thelancet.com **Published online January 20, 2011 DOI:10.1016/S0140-6736(10)62050-8**

with Levene's test, and an adjustment with the Welch *t* test was made if there was heteroscedasticity. Welch's method is robust for unequal variances.[16] The shape of the distribution of residuals was studied and, if not normal, a log transformation was used; otherwise, a non-parametric test based on Savage scores enabling multiple comparison of the treatment groups was used. Cure rate tests were one tailed, and all other analyses were two tailed. In the two-tailed analyses, significance was set at a p value 0·05 or less unless otherwise stated. All analyses were done with the SAS system software version 9.2. The trial is registered with ClinicalTrials.gov, number NCT00696969.

Role of the funding source

Study design was finalised with input from the principal investigators and their collaborators, external experts, and representatives of the sponsors. The sponsors of the study had no contact with the patients and had no role in data collection, analysis, interpretation, or the decision to publish the results, although they did contribute to the writing of the report. SS had full access to all the data of the study at the end of the trial and had final responsibility for the decision to submit for publication. All authors are responsible for the completeness and accuracy of the data presented.

Results

Between June, 2008, and July, 2009, we assessed the eligibility of 896 patients who were serologically positive by rK39 rapid test (figure 1). 262 were excluded because they did not meet the entry criteria. The 634 patients who were enrolled and randomly assigned to one of the four treatment groups constituted the ITT population.

618 patients were enrolled and completed treatment and follow-up visits according to the protocol; they constituted the per-protocol population. Eight patients in the amphotericin B group did not complete treatment because of adverse events (one of whom had a cardiac infarct and subsequently died) and were removed from the per-protocol population (there were three other serious adverse events). One patient had a systemic allergic reaction to the test injection of liposomal amphotericin B and was withdrawn. Two patients who did not meet the entry criteria were included and randomised erroneously; one had deviation of prothrombin time and the other was a malnourished child. Another patient was underdosed with liposomal amphotericin B (2·67 mg/kg instead of 5 mg/kg) in the liposomal amphotericin B and miltefosine group. Although all these three patients were cured, they were regarded as having major protocol deviations and were not included in the per-protocol population analyses in accordance with the protocol. In the amphotericin B group, one patient died in a car accident and another died of an infarct during treatment; three patients were lost to follow-up at day 180 (figure 1) and were also excluded from the per-protocol population.

	Amphotericin B	Liposomal amphotericin B+ miltefosine	Liposomal amphotericin B+ paromomycin	Miltefosine+ paromomycin
Intention-to-treat population				
Number of patients (634)	157	160	158	159
Number of patients cured (%, 95% CI)	146 (93·0, 87·5–96·3)	156 (97·5, 93·3–99·2)	154 (97·5, 93·2–99·2)	157 (98·7, 95·1–99·8)
Per-protocol population				
Number of patients (618)	148	157	155	158
Number of patients cured (%, 95% CI)	146 (98·6, 94·7–99·8)	155 (98·7, 95·0–99·8)	153 (98·7, 94·9–99·8)	156 (98·7, 95·0–99·8)

Table 2: **Definitive cure at 6 months, by treatment group**

	Intention-to-treat population		Per-protocol population	
	Difference in proportion (%)	97·5% CI	Difference in proportion (%)	97·5% CI
Liposomal amphotericin B+miltefosine *vs* amphotericin B	4·5	–0·83 to 9·84	0·1	–2·84 to 3·00
Liposomal amphotericin B+paromomycin *vs* amphotericin B	4·5	–0·88 to 9·83	0·1	–2·88 to 3·00
Miltefosine+paromomycin *vs* amphotericin B	5·7	0·77 to 10·72	0·1	–2·83 to 3·00
Liposomal amphotericin B+miltefosine *vs* liposomal amphotericin B+paromomycin	0	–3·90 to 3·97	0	–2·84 to 2·87
Miltefosine+paromomycin *vs* liposomal amphotericin B+miltefosine	1·2	–2·16 to 4·64	0	–2·82 to 2·83
Miltefosine+paromomycin *vs* liposomal amphotericin B+paromomycin	1·2	–2·15 to 4·70	0	–2·82 to 2·87

Table 3: **Differences in cure rates between treatment groups and the standard group, and between groups**

Baseline characteristics were similar in the four groups (table 1). Overall, there were more male than female participants and more adults than children (293 adults were enrolled before children could be included). Eight patients with previous history of treatment of visceral leishmaniasis were enrolled (table 1); all but one of these patients were cured at 6-month follow-up.

Relapse rates in all groups were equal (two per group). Three patients in the liposomal amphotericin B and miltefosine group, two in the liposomal amphotericin B and paromomycin group, and one in the miltefosine and paromomycin group had detectable (score 1+) parasites in their splenic smears on day 15; on repeat examination, all were negative on day 45.

Table 2 shows the definitive cure rates at the final follow-up visit based on the ITT and per-protocol populations. In the ITT analyses, the patients who were withdrawn because of adverse events, those who died, and those lost to follow-up are regarded as failures. Table 3 shows the difference between amphotericin B treatment and the combinations. The lower limit of the CI of the difference in the per-protocol population did not reach –7% for any group, indicating non-inferiority of the three combination groups to the control group.

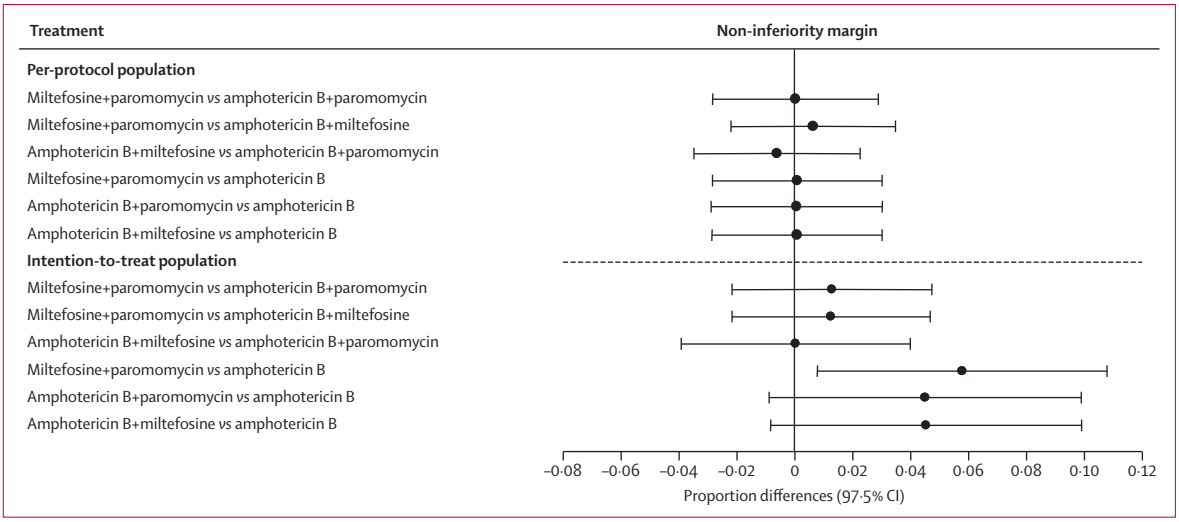

Figure 2: **Comparison of efficacy rates of different groups, by differences in proportion of cure rates**

	Amphotericin B (n=157)	Liposomal amphotericin B+ miltefosine (n=160)	Liposomal amphotericin B+ paromomycin (n=158)	Miltefosine+ paromomycin (n=159)
Patients with at least one adverse event	143 (91%)	83 (52%)	73 (46%)	72 (45%)
Serious adverse events reported	2*	1†	0	0
Deaths (one unrelated)	2 (1%)	0	0	0
Discontinuations because of adverse events	8 (5%)‡	1	0	0
Cardiac infarct (serious adverse event)	1	0	0	0
Ascites (serious adverse event)	1	0	0	0
Diarrhoea	3 (2%)	3 (2%)	1	5 (3%)
Vomiting	30 (19%)	25 (16%)	5 (3%)	16 (10%)
Asthenia	1	3 (2%)	2 (1%)	3 (2%)
Chills	113 (72%)	20 (13%)	20 (13%)	0
Injection site pain	0	0	10 (6%)	14 (9%)
Pyrexia	37 (24%)	31 (19%)	33 (21%)	32 (20%)
Increased creatinine	15 (10%)	1	6 (4%)	6 (4%)

ITT=intention to treat. *Ascites and cardiac infarct leading to death during treatment, possibly related. †Urticaria during test injection of amphotericin B (related). ‡One of whom subsequently died.

Table 4: **Serious adverse events and most common adverse events related or possibly related to treatment (ITT population)**

Pair-wise comparisons between control and the three combination groups showed non-inferiority of the combination treatments analyses using the ITT population (figure 2).

There were four serious adverse events. The Data Safety Monitoring Board regarded one death (cardiac infarct) during the first infusion in the amphotericin B group as probably related to treatment and one generalised allergic reaction (urticaria) during liposomal amphotericin B testing in the liposomal amphotericin B and miltefosine group as related to treatment. There were two serious adverse events after completion of treatment: one possible Guillain-Barré syndrome (in the amphotericin B group) was possibly related to treatment and the other (death caused by car accident) was unrelated.

In addition to the two patients whose treatment was discontinued because of a serious adverse event, mentioned above and one of whom died, seven other patients (all in the amphotericin B group) were withdrawn and given rescue treatment because of treatment-related adverse events (five patients had increased serum creatinine concentrations, one with raised concentrations of liver enzymes, and one with pedal oedema and ascites). These patients were followed to the resolution of the adverse events, which were all reversible.

Most adverse events were reported in the control group, in which 91% of patients had at least one adverse event. In the other three groups, 45–52% of patients had at least one adverse event (table 4). The total number of treatment-related adverse events was small (mostly chills, pyrexia, pain at the injection site, and vomiting). These adverse events were mild or moderate in severity. Vomiting was most common in patients receiving amphotericin B. Pain at the injection site (mild and moderate) was reported with paromomycin; chills and rigor were reported during infusion, mostly with amphotericin B and some with liposomal amphotericin B, but there were no reports in the group that did not have amphotericin B or liposomal amphotericin B (table 4).

Table 5 provides the selected laboratory measurements before treatment, during treatment, and on day 45 of follow-up. The values did not differ significantly between the four groups at baseline. The increases in many values during treatment were significantly different in the drug combination groups compared with those in the control (ie, haemoglobin, white blood cell and platelet counts,

	Amphotericin B (n=157)	Liposomal amphotericin B+ miltefosine (n=160)	Liposomal amphotericin B+ paromomycin (n=158)	Miltefosine+ paromomycin (n=159)	p value
Haemoglobin (g/L)					
Baseline	81 (17)	84 (17)	82 (19)	83 (19)	0·58*
Day 15 or 31	85 (13)	97 (17)	95 (16)	97 (18)	<0·0001* (1,2,3)
Day 45	95 (13)	110 (18)	109 (18)	108 (20)	<0·0001† (1,2,3)
White-cell count (×10⁹/L)					
Baseline	3·1 (1·3)	3·3 (1·7)	3·4 (1·8)	3·4 (2·0)	0·32*
Day 15 or 31	7·0 (3·0)	6·5 (2·5)	6·1 (2·2)	6·8 (2·2)	0·0002* (2,6)
Day 45	7·9 (3·2)	8·0 (2·8)	8·6 (2·6)	8·3 (2·8)	0·36*
Platelet count (×10⁹/L)					
Baseline	125·8 (70·8)	131·0 (74·8)	120·1 (64·2)	129·6 (76·9)	0·54*
Day 15 or 31	263·7 (100·6)	263·1 (107·8)	243·9 (83·4)	303·1 (116·3)	<0·0001* (3,5,6)
Day 45	290·6 (104·4)	268·6 (97·7)	266·9 (99·7)	278·9 (106·3)	0·16*
Blood urea nitrogen (mmol/L)					
Baseline	64·4 (23·4)	61·6 (23·0)	66·9 (24·4)	64·7 (22·1)	0·22*
Day 15 or 31	99·7 (41·2)	66·6 (22·7)	69·2 (27·7)	68·6 (26·0)	<0·0001† (1,2,3)
Day 45	81·2 (29·1)	63·6 (20·2)	67·5 (27·2)	68·3 (28·3)	<0·0001* (1,2,3)
Creatinine (µmol/L)					
Baseline	71·3 (11·4)	73·4 (10·8)	72·2 (11·9)	72·7 (10·3)	0·39*
Day 15 or 31	21·8 (33·7)	−5·3 (16·4)	−2·3 (26·9)	−0·4 (26·6)	<0·0001* (1,2,3)
Day 45	4·00 (17·1)	−5·8 (14·1)	−3·4 (16·2)	−4·5 (15·2)	<0·0001* (1,2,3)
Aspartate aminotransferase (U/L)					
Baseline	54·7 (25·5)	56 (28·4)	50·9 (25·3)	52·7 (24·2)	0·32*
Day 15 or 31	33·9 (42·2)	37·8 (19·4)	38·5 (17·1)	42·9 (22·5)	0·0085* (3)
Day 45	31·3 (14·9)	32·5 (15·7)	33·2 (14·8)	32·4 (13·4)	0·2*
Alanine aminotransferase (U/L)					
Baseline	39·6 (27·3)	42·5 (28·4)	35·7 (23·8)	39·1 (26·3)	0·15
Day 15 or 31	31·8 (42·7)	39·6 (24·9)	37·2 (25·4)	43·7 (27·3)	0·0129* (3)
Day 45	28·8 (16·8)	28·5 (15·0)	27·7 (12·8)	28·8 (15·6)	0·36†

Post-hoc comparisons are as follows: (1) amphotericin B *vs* liposomal amphotericin B+miltefosine; (2) amphotericin B *vs* liposomal amphotericin B+paromomycin; (3) amphotericin B *vs* miltefosine+paromomycin; (4) liposomal amphotericin B+miltefosine *vs* liposomal amphotericin B+paromomycin; (5) liposomal amphotericin B+miltefosine *vs* miltefosine+paromomycin; and (6) liposomal amphotericin B+paromomycin *vs* miltefosine+paromomycin. Change is calculated only for patients available on screening and respective test day. *p value was calculated using ANOVA with pairwise post-hoc comparison between groups (comparison of raw value at baseline and of change with baseline for day 15 or 31 and day 45 at 0·05 level). †p value was calculated using a Welch adjustment of ANOVA (heteroscedasticity) with pairwise post-hoc comparison between groups.

Table 5: **Changes in laboratory measurements during treatment (mean values [SD])**

blood urea nitrogen, creatinine, alanine and aspartate aminotransferases), and in some measurements in different combination groups (white blood cells, platelets). Most measurements returned to baseline or normal values by day 45 (ie, liver enzymes, platelets), but some remained high in the control group (ie, blood urea nitrogen). None of these changes were regarded as clinically concerning and were not at the cutoffs defined in the protocol for withdrawal.

Improvement of anaemia, measured by haemoglobin concentrations, was slower in patients assigned amphotericin B than in those assigned the drug combinations. Creatinine concentrations were higher throughout treatment in the control group than in the other groups (table 5 and figure 3 A and B).

Although the liver enzyme concentrations tended to be higher (not significantly) during treatment with combination therapy than amphotericin B therapy (table 5), none of these increases were regarded by the treating physicians to be clinically important to warrant withdrawal from treatment or any intervention.

Discussion

All three combination treatments were effective, and they were less toxic (particularly for the kidneys) and better tolerated than was the standard treatment with amphotericin B. The non-significant difference between cure rates of combinations (>97%) and control (93%) was attributable to poor tolerance to amphotericin B treatment, which led to a lower than expected cure rate (93·6% *vs* 97·0% assumed for the design of the trial) in the ITT analyses. For the per-protocol population, after exclusion of the nine patients withdrawn because of serious and non-serious adverse events (regarded as

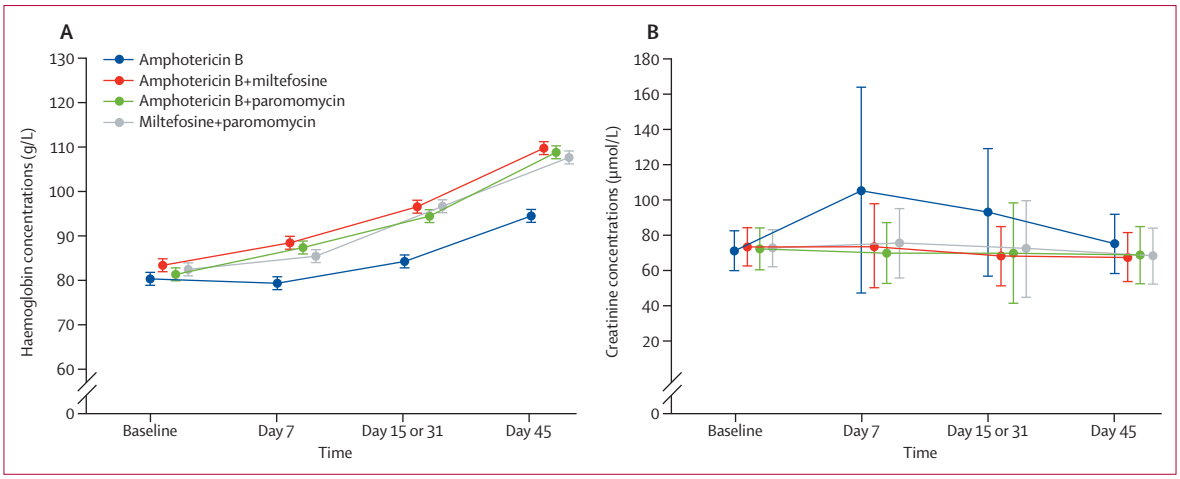

Figure 3: **Improvement of anaemia**
(A) Haemoglobin concentrations (SE). (B) Creatinine concentrations (SE). In the amphotericin B group, 157 patients were included at baseline, 156 at day 7, 149 at day 15, and 150 at day 45. In the amphotericin B plus miltefosine group, 160 patients were included at baseline and 159 for days 7, 15, and 45. In the amphotericin B plus paromomycin group, 158 patients were included for all timepoints. In the miltefosine and paromomycin group, 157 patients were included for all timepoints.

failures in the ITT analysis), the cure rate was 98·6% for the standard treatment. Nephrotoxicity is a well known side-effect of amphotericin B treatment.[17] Therefore, the patients who tolerated amphotericin B treatment had the same outcome as patients in the other groups. Nevertheless, the per-protocol analysis also confirmed the non-inferiority of combination treatment to the standard therapy.

The results of this open-label, non-inferiority trial do not enable discrimination between combination groups, nor can we necessarily detect uncommon safety events. Additionally, this study was done in two established research sites in Bihar on a population of patients whose disease is not severe; therefore, these limitations should be kept in mind when considering the treatments reported here in further implementation projects. However, on the basis of our results, all combinations seem viable treatment options for visceral leishmaniasis. The choice of treatment depends on various factors, ranging from facilities available at the point of care, acceptability, and costs. For example, when intravenous infusion or storage at a temperature below 25°C is not possible, liposomal amphotericin B cannot be used, and miltefosine and paromomycin for 10 days might be the treatment of choice. However, because of the potential teratogenicity of miltefosine,[18] all women of child-bearing age receiving a combination with this regimen should be tested for pregnancy and should agree to long-term contraception.[19] Alternatively, if the local customs do not allow pregnancy testing or use of contraceptives, particularly for unmarried women, liposomal amphotericin B and paromomycin would be the treatment of choice.

Although the study was not powered to stratify for age-groups, we did a post-hoc analysis of efficacy and safety measurements in adults and children (≤18 years), and the results were similar between these age-groups (data not shown).

In addition to these combination regimens, a single dose of 10 mg/kg liposomal amphotericin B can cure more than 95% of patients with visceral leishmaniasis in Bihar,[6] a major advance for the treatment of visceral leishmaniasis. However, the cost of this treatment regimen is higher than that for combination therapy, and the treatment has a higher risk of emergence of drug resistance than does combination treatment.

Consideration of treatments that necessitate no or a short stay in hospital and away from home and workplace is important. At about US$22 per day for treatment in a hospital in Bihar, the standard treatment with amphotericin B and 30 days in hospital is $436 ($368 for the hospital stay and $68 for the drug).[6] Costs of care for these combinations (for the population with visceral leishmaniasis in Bihar and at WHO-negotiated prices; direct costs only) is $71 for miltefosine and paromomycin, $107 for liposomal amphotericin B and paromomycin, $109 for liposomal amphotericin B and miltefosine, and $126 for single-dose liposomal amphotericin B 10 mg/kg for a patient weighing 35 kg.[20] In this study, the cost per year of life and death averted with liposomal amphotericin B and miltefosine (7 days) was $5–6 and $125–160, respectively. In a recent study by Meheus and colleagues,[21] the cost of treatment and cost-effectiveness including drug, other medical, and non-medical or indirect costs of the three combinations studied here compared favourably with those for other conventional monotherapy treatments, including antimonials, amphotericin B, paromomycin, and miltefosine. When other factors are taken into account, including adherence and efficacy, the miltefosine and paromomycin combination was the most cost-effective treatment at $91 per death averted, followed by

www.thelancet.com Published online January 20, 2011 DOI:10.1016/S0140-6736(10)62050-8

Panel: Research in context

Systematic review

In the past 10 years, three drugs (paromomycin, liposomal amphotericin B, and miltefosine) have become available for the treatment of visceral leishmaniasis in south Asia. In particular, single-dose liposomal amphotericin B at 10 mg/kg can cure more than 95% of patients with visceral leishmaniasis. Because of the prolonged duration of the treatment regimens with these drugs, the adherence is likely to be low,[22] and emergence of drug resistance is imminent and resistant parasites have already been isolated. In a phase 2 study, efficacy of a combination of liposomal amphotericin B and miltefosine was reported.[12] These results led to the present study on combinations of all the three effective drugs used for visceral leishmaniasis in India.

There is relatively little research into this disease in south Asia, and knowledge of current or past studies is discussed at meetings organised by WHO and regional authorities. SS, PO, PD, PKS, and FM are members of these expert committees and are aware of current activities in the field; nevertheless, a search of the PubMed database was done on clinical trials for the treatment of visceral leishmaniasis. Randomised controlled trials were the main source of evidence, but data available at the time of design of this trial on combination therapy were scarce.

Interpretation

The results from this study lend support to the development of short, cost-effective, safe, and efficacious treatment regimens with two-drug combinations for visceral leishmaniasis.

liposomal amphotericin B and paromomycin and single-dose 10 mg/kg liposomal amphotericin B.[21] On the basis of safety profiles of combination treatments, our data lend support to the assumptions made previously[20,21] that these combinations can be given on an outpatient basis (panel).

An important outcome of this study is that the definitive cure rates of the three combination treatments reported here and in the previous phase 2 trial[12] were high and similar to those for monotherapies with these drugs.[6,10,18] Therefore, we believe that these combinations can replace monotherapy to extend the effective lives of these drugs. Drug combinations have the advantage of shortening treatment duration from 21–28 days for monotherapy with paromomycin or miltefosine to 8–11 days with combinations. Additionally, continued use of these drugs as monotherapies is associated with a high risk of selecting resistant parasites, as indicated in laboratory experiments.[9,23] Combination drug therapy is expected to reduce the probability of development of resistant parasites, thereby prolonging the useful therapeutic lifespan of these drugs, which are needed for the elimination of visceral leishmaniasis.[24]

Contributors

The authors accept full responsibility for the overall content of this report. SS, FM, PO, PD, PKS, KN, KP, NV, CSL, MR, JC, RA, DKV, SA, PK, and MV designed the trial. SS and PKS were the principal investigators; SS, MR, JC, DKV, SA, PK , JC, PD, KN NV, KP, PKS, CL, and RA enrolled and managed patients, collected clinical data; FM, NSW, SE, PD, SS, MR, JC, RA, DKV, SA, PK, PKS, KN, KP, NV, CL, MB, and MV contributed to writing of the paper. MV analysed the data together with the data management team of the contract research organisation. BS and SE participated in supervision and writing the paper. PO was a clinical adviser.

Conflicts of interest

SS has received grant support for clinical trials and travel funds to attend scientific meetings from Paladin Labs, Institute for One World Health, and GlaxoSmithKline, and his institute has received grants from Bharat Serum and Vaccine Ltd. MR has received travel funds from Paladin Labs to attend a scientific meeting. BS, SE, NSW, MS, MB, and FM are employees of, and MV consultant to, the Drugs for Neglected Diseases initiative (DNDi). All other authors declare that they have no conflicts of interest.

Acknowledgments

We thank B Pecoul, N K Ganguly, S Bhattacharya, M Vray, M Boelaert, S Chang, J Alvar, H Murray, K Weerasuriya, S D Seth, I Ribeiro, and C Royce for their inputs in the concept, protocol design, execution, and review of the manuscript, as well as their support and evaluation of the trial; F Giovannini for assistance in redaction of the manuscript; and P Smith, C P Thakur, R A Pandey, and N K Arora (members of the Data Safety Monitoring Board). This work was jointly supported by DNDi and the Indian Council of Medical Research. We thank Gilead for donating AmBisome used in this study. DNDi would like to thank the following donors for their commitment to the mission and vision of DNDi and for their support of this project, which without their support could not have been accomplished: Médecins Sans Frontières, the Spanish Agency of International Cooperation for Development, the UK Department for International Development, and the Starr International Foundation in Switzerland. The opinions expressed in this paper are those of the authors and might not reflect those of their employing organisations.

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