

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812

JUNE 21, 2007

VOL. 356 NO. 25

Injectable Paromomycin for Visceral Leishmaniasis in India

Shyam Sundar, M.D., T.K. Jha, M.D., Chandreshwar P. Thakur, M.D., Prabhat K. Sinha, M.D.,
and Sujit K. Bhattacharya, M.D.*

ABSTRACT

BACKGROUND

Visceral leishmaniasis (kala-azar) affects large, rural, resource-poor populations in South Asia, Africa, and Brazil. Safe, effective, and affordable new therapies are needed. We conducted a randomized, controlled, phase 3 open-label study comparing paromomycin, an aminoglycoside, with amphotericin B, the present standard of care in Bihar, India.

METHODS

In four treatment centers for visceral leishmaniasis, 667 patients between 5 and 55 years of age who were negative for the human immunodeficiency virus and had parasitologically confirmed visceral leishmaniasis were randomly assigned in a 3:1 ratio to receive paromomycin (502 patients) at a dose of 11 mg per kilogram of body weight intramuscularly daily for 21 days or amphotericin B (165 patients) at a dose of 1 mg per kilogram intravenously every other day for 30 days. Final cure was assessed 6 months after the end of treatment; safety assessments included daily clinical evaluations and weekly laboratory and audiometric evaluations. Noninferiority testing was used to compare 6-month cure rates, with a chosen margin of noninferiority of 10 percentage points.

RESULTS

Paromomycin was shown to be noninferior to amphotericin B (final cure rate, 94.6% vs. 98.8%; difference, 4.2 percentage points; upper bound of the 97.5% confidence interval, 6.9; $P < 0.001$). Mortality rates in the two groups were less than 1%. Adverse events, which were more common among patients receiving paromomycin than among those receiving amphotericin B (6% vs. 2%, $P = 0.02$), included transient elevation of aspartate aminotransferase levels (>3 times the upper limit of the normal range); transient reversible ototoxicity (2% vs. 0, $P = 0.20$); and injection-site pain (55% vs. 0, $P < 0.001$); and in patients receiving amphotericin B, as compared with those receiving paromomycin, nephrotoxicity (4% vs. 0, $P < 0.001$), fevers (57% vs. 3%), rigors (24% vs. 0, $P < 0.001$), and vomiting (10% vs. $<1\%$, $P < 0.001$).

CONCLUSIONS

Paromomycin was shown to be noninferior to amphotericin B for the treatment of visceral leishmaniasis in India. (ClinicalTrials.gov number, NCT00216346.)

From the Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh (S.S.); the Kala-azar Research Centre, Brahmpura, Muzaffarpur, Bihar (T.K.J.); Balaji Utthan Sansthan, Patna, Bihar (C.P.T.); and the Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar (P.K.S., S.K.B.) — all in India. Address reprint requests to Dr. Sundar at the Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India, or at drshyamsundar@hotmail.com.

*Additional members of the Paromomycin for Visceral Leishmaniasis Study Teams are listed in the Appendix.

N Engl J Med 2007;356:2571-81.
Copyright © 2007 Massachusetts Medical Society.

VISCERAL LEISHMANIASIS (KALA-AZAR) IS primarily a fatal vectorborne parasitic disease characterized by fever, hepatosplenomegaly, and pancytopenia. Most of the approximately 500,000 cases of visceral leishmaniasis reported worldwide affect the rural poor in India, Nepal, Bangladesh, Sudan, and Brazil.¹ Treatment options for visceral leishmaniasis are limited. Sodium stibogluconate, a historically effective and affordable pentavalent antimonial compound, is associated with fatal toxic effects,²⁻⁴ and in some regions its use has led to the development of resistant strains of *Leishmania donovani*,⁵ with the result that fewer than 50% of treated patients are cured.^{2,6-8} In regions where antimony resistance is prevalent, intravenous amphotericin B (desoxycholate) (Fungizone, Sarabhai Piramel Pharmaceuticals) is used, but it is expensive and may require weeks of hospitalization with intensive clinical and laboratory monitoring. Liposomal formulations of amphotericin B (AmBisome, Gilead Sciences), which require a shorter treatment course (5 days) and have fewer side effects, remain unaffordable at nearly 30 times the cost of conventional formulations.^{9,10} Miltefosine (Impavido, Aeterna Zentaris), the first effective oral therapy for visceral leishmaniasis,¹¹ is expensive,¹² is potentially teratogenic, and has significant gastrointestinal side effects.¹¹ Safe, effective, and affordable treatments for visceral leishmaniasis in regions where the disease is endemic are urgently needed, particularly in formulations that are compatible with rural settings.

Because humans are the reservoir for visceral leishmaniasis in the Indian subcontinent, the infection could be eliminated with widespread treatment of patients and rigorous vector control¹ (although elimination may not be possible in regions where zoonotic visceral leishmaniasis is prevalent, such as Brazil). Paromomycin, an aminoglycoside antibiotic, has been shown to have a dose-response efficacy in the treatment of visceral leishmaniasis when administered intramuscularly at a dose of 12, 16, or 20 mg of sulfate per kilogram of body weight daily for 21 days.^{13,14} We present the results of a phase 3, multicenter, non-inferiority clinical trial comparing the safety and efficacy of paromomycin and of amphotericin B for the treatment of visceral leishmaniasis in Bihar, India.

METHODS

STUDY DESIGN

This study was an open-label, prospective, randomized trial comparing paromomycin with amphotericin B (Sarabhai Piramel Pharmaceuticals) (hereafter referred to as amphotericin) in which the primary end point was safety and the secondary end point was efficacy. The study was conducted between June 2003 and November 2004 in Bihar, India; all patients provided written informed consent. The protocol was approved by the independent ethics committee at each of the four participating centers, the Drug Controller General of India, and the Steering Committee on Research Involving Human Subjects of the World Health Organization. AmBisome was donated by Gilead Sciences for use as rescue medication in the study, but the company had no role in the design of the study, the accrual or analysis of the data, or the preparation of the manuscript.

STUDY MEDICATIONS

Paromomycin solution, 375 mg per milliliter (500 mg per milliliter as paromomycin sulfate) (Pharmamed Parenterals), was administered by deep gluteal intramuscular injection at a dose of 11 mg per kilogram (15 mg per kilogram as the sulfate) daily for 21 days. Amphotericin was diluted in water and 5% dextrose and, after an initial dose (to test for an allergic response), was infused intravenously for 6 hours at a dose of 1 mg per kilogram every other day for 30 days (a total of 15 infusions). Liposomal amphotericin, infused intravenously at a dose of 3 mg per kilogram daily for 5 days, was used as rescue medication in patients in whom the study treatment failed or relapse occurred.

STUDY PATIENTS

Eligible subjects were between 5 and 55 years of age and had clinically suspected visceral leishmaniasis. Inclusion criteria were parasitologically positive splenic or bone marrow smear; negative serologic testing for the human immunodeficiency virus (HIV); hemoglobin level of at least 5.0 g per deciliter; white blood count greater than or equal to 1×10^9 per liter; platelet count greater than or equal to 50×10^9 per liter; levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase less than or equal to three times the

upper limit of the normal range; prothrombin time less than or equal to 5 seconds greater than that among control subjects; and serum creatinine and potassium levels within the normal limits. Exclusion criteria were treatment for visceral leishmaniasis during the 2 weeks before enrollment, a hearing loss of 75 dB in frequencies 1 through 8 kHz, a history of vestibular or auditory dysfunction, prior treatment with amphotericin without response, allergy or hypersensitivity to aminoglycosides, significant proteinuria ($\geq 2+$ on strip testing), significant coexisting diseases possibly affecting the response to the study treatment response, and pregnancy or lactation.

STUDY PROCEDURES

Enrolled patients were randomly assigned to treatment with paromomycin or amphotericin in a 3:1 ratio in permuted blocks of four. A fraction of the patients in the paromomycin group were also randomly assigned to a substudy in which pharmacokinetic sampling was performed. All patients were hospitalized for the duration of the study treatment; vital signs were assessed daily and adverse events were reported according to the Common Toxicity Criteria (CTC) of the National Cancer Institute.¹⁵ Patients were monitored for hematologic variables, serum chemistry, body weight, and the size of the spleen and liver every week during treatment, at the end of treatment, and at 6 months after the treatment ended. Audiometric testing was performed every week during treatment in all patients and repeated every 2 weeks and then monthly for up to 6 months after treatment ended in patients with ototoxicity. A sparse sampling design was used for the collection of pharmacokinetic samples. Splenic or bone marrow aspiration was performed at the end of treatment, at the 4-week follow-up in patients with few residual parasites at end of treatment, and in those who had a relapse of visceral leishmaniasis, during the 6-month follow-up period. Patients who were not cured or who had a parasitologically confirmed relapse received rescue medication.

END POINTS

Safety

The safety end points were reported adverse events, protocol-defined nephrotoxicity (defined as an increase in serum creatinine that was either double the baseline levels and more than 2.0 mg per deciliter [$177 \mu\text{mol per liter}$], or more than 2.5 mg per

deciliter^{16,17}) and ototoxicity (defined as a confirmed shift from baseline in audiometric thresholds by either 25+ dB at one or more of the tested frequencies [1 to 12 kHz], or 20+ dB at two or more adjacent frequencies), laboratory evaluations, and vital signs. Patients with potential ototoxicity were reviewed by an audiology expert who was unaware of the treatment assignments.

Efficacy

Parasite density was graded on a log scale by pathologists who were also unaware of the treatment assignments. Final cure was defined as an initial cure (clinical improvement with no parasites at the end of treatment or parasite density of 1 at the end of treatment with no parasites on repeated smear 1 month after the end of treatment) and no relapse during follow-up. Relapse was defined as suspected visceral leishmaniasis after an initial cure, followed by a positive result on analysis of a specimen obtained by splenic or bone marrow aspiration. Treatment failure was defined as lack of an initial cure or occurrence of relapse. For pharmacokinetic sampling, plasma paromomycin levels were measured with the use of an assay validated by liquid chromatography–tandem mass spectrometry.

STATISTICAL ANALYSIS

Statistical analyses were performed with the use of SAS software, version 8.2, unless otherwise noted. Statistical tests included Student's t-test, Fisher's exact test, the chi-square test or the Cochran–Mantel–Haenszel test, and general linear equations, generalized estimation equations, or linear mixed-effect models. All tests were two-sided. Efficacy calculations were performed with the use of StatXact, version 7.0 (Cytel). Assuming a 99% cure rate for amphotericin, 666 patients were needed in a 3:1 ratio to support a one-sided, non-inferiority analysis without stratification and with 80% power to detect a type I error rate of 5%. Primary efficacy was calculated as the proportion of patients achieving a final cure; an exact confidence interval for that proportion was computed; the exact, one-sided, upper bound of the 97.5% confidence interval for the difference in success probabilities was compared with the use of $\delta=0.10$ (the chosen margin for noninferiority). All P values except the value for noninferiority were two-tailed. Pharmacokinetic analyses were performed with NONMEM software, version V, level 1.1 (GloboMax).

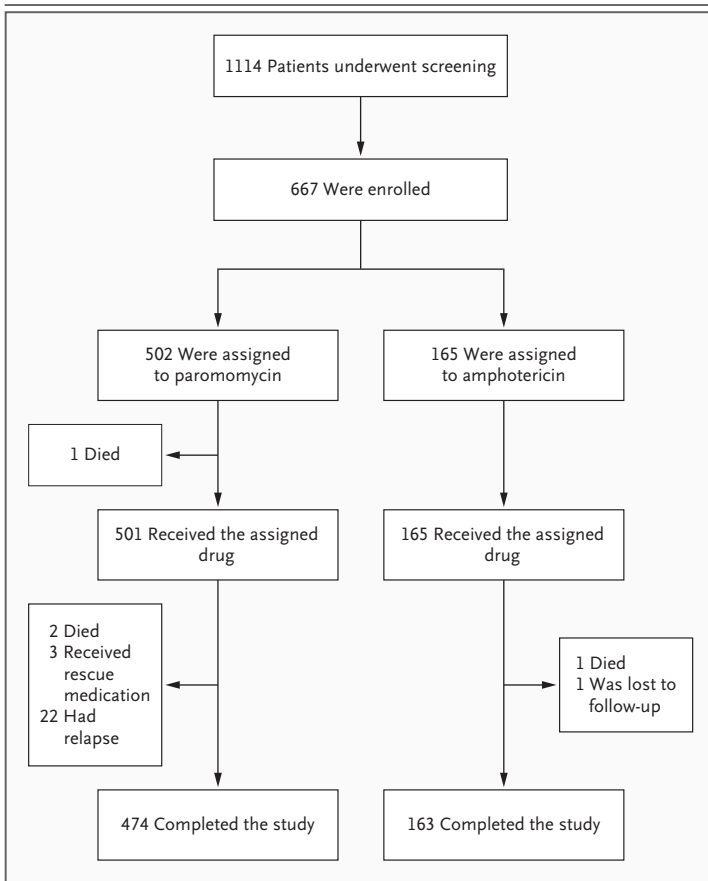


Figure 1. Disposition of the Patients.

One patient in the paromomycin group received a full course of amphotericin. Three patients in the paromomycin group who were not cured at the end of treatment received rescue medication. Relapse was defined as parasitologically confirmed visceral leishmaniasis at any time after an initial cure; the 22 patients who had a relapse were treated with rescue medication. The one patient lost to follow-up could not be located for the 6-month visit. One patient in the amphotericin group discontinued the study drug after receiving eight doses because pulmonary tuberculosis developed, but this patient was followed for the full 6 months.

RESULTS

Of 1114 patients who underwent screening, 667 (60%) were enrolled (Fig. 1). One patient assigned to paromomycin died before administration of the study drug. Ninety-six percent of the patients received the first dose of a study drug within 1 day after randomization. One patient assigned to paromomycin received a full course of amphotericin. Of the enrolled patients, 252 (38%) were pediatric patients, defined as 5 to 14 years of age, and 415 were adult patients, defined as 15 to 55 years of age.

In 10% of the patients, either visceral leishmaniasis did not respond to previous treatment or relapses of visceral leishmaniasis had occurred (Table 1). The two study groups were balanced with respect to baseline characteristics.

SAFETY

Serious Adverse Events

Seven serious adverse events occurred, including four deaths (0.6%) and three events (0.4%) requiring discontinuation of the study drug (Table 2). Of the four deaths, one occurred in a patient before the administration of paromomycin; a second death, deemed by the investigator as possibly related to paromomycin, occurred in a patient with suspected alcoholism who received only two doses of the drug, after which aspartate aminotransferase levels increased to more than six times the upper limit of the normal range; a third death, deemed by the investigator to be unrelated to paromomycin, resulted from septicemia secondary to a thigh abscess after the patient had received 11 doses of the drug (no obvious connection between the injection site and the abscess was found); and a fourth death resulted from gastroenteritis and diarrhea and was considered to be probably related to amphotericin. The three nonfatal serious adverse events included two in patients who had elevated levels of hepatic enzymes after 8 doses of paromomycin (both patients required rescue medication), and one patient with bacterial pneumonia was treated with gatifloxacin after 10 doses of amphotericin.

Five patients (1%) in the paromomycin group had other adverse events requiring drug discontinuation: one patient had reversible ototoxicity and one had elevated levels of hepatic enzymes, and both required rescue therapy; three patients (one with elevated levels of hepatic enzymes, one with transient reversible ototoxicity, and one with injection-site pain) received eight or more doses of paromomycin and did not require rescue therapy. One patient (0.6%) in the amphotericin group discontinued the study drug after receiving eight doses because pulmonary tuberculosis developed.

Audiometric Testing

Audiometry data were available for a total of 589 patients (442 in the paromomycin group and 147 in the amphotericin group). After review by an expert audiologist who was unaware of the treatment as-

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Paromomycin Group (N=501)	Amphotericin Group (N=165)
Age — yr	22.1±12.3	20.8±11.7
Age category — no. (%)†		
Pediatric	188 (38)	64 (39)
Adult	313 (63)	101 (61)
Sex — no. (%)		
Male	321 (64)	95 (58)
Female	180 (36)	70 (42)
Weight — kg	35.5±11.8	33.6±11.0
Height — cm	146.9±18.5	145.9±16.7
Body-mass index‡	15.9±2.9	15.3±2.8
Pediatric	13.8±2.0	13.1±2.2
Adult	17.2±2.5	16.7±2.2
Visceral leishmaniasis — no. (%)		
Newly diagnosed	449 (90)	150 (91)
Previously diagnosed without response to treatment§	52 (10)	15 (9)
Parasite density¶		
Median	2	2
Interquartile range	1–3	1–3
Palpable spleen size — cm below costal margin	6.6±3.8	6.8±4.0
Palpable liver size — cm below costal margin	2.1±1.2	2.1±1.3
Vital signs		
Body temperature — °F	99.9±1.3	100.0±1.4
Heart rate — beats/min	98.1±9.9	98.1±11.3
Respiratory rate — breaths/min	21.9±2.6	22.0±2.9
Systolic blood pressure — mm Hg	105.4±9.8	104.5±10.8
Laboratory values		
White-cell count — ×10 ⁻³ /mm ³	3.1±1.4	3.2±1.5
Platelet count — ×10 ⁻³ /mm ³	120.4±60.8	117.1±54.6
Hemoglobin — g/dl	7.8±1.7	7.7±1.6
Serum alanine aminotransferase — U/liter	40.5±25.6	41.1±26.2
Serum aspartate aminotransferase — U/liter	44.1±22.0	45.1±22.5
Alkaline phosphatase — U/liter	216.4±114	228.2±130
Total bilirubin — mg/dl	0.6±0.2	0.6±0.2
Serum creatinine — mg/dl	0.8±0.2	0.8±0.2
Blood urea nitrogen — mg/dl	11.7±4.2	11.9±3.8
Albumin — g/dl	3.1±0.5	3.1±0.7

* Plus–minus values are means ±SD. To convert the values for bilirubin to micromoles per liter, multiply by 17.1. To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for urea nitrogen to millimoles per liter, multiply by 0.357.

† The pediatric age group was defined as patients 5 to 14 years of age, and the adult age group was defined as those 15 to 55 years of age.

‡ Body-mass index is the weight in kilograms divided by the square of the height in meters. The two groups were similar for all variables other than body-mass index ($P=0.02$); the difference was not statistically significant after adjustment for age and study center ($P=0.06$ for the comparison between the two groups among pediatric patients; $P=0.09$ for the comparison between the two groups among adult patients). Values are adjusted for study center.

§ Patients in this category had received prior treatment for visceral leishmaniasis but remained symptomatic or had been considered cured but had become symptomatic. Among the patients in the paromomycin group who had received previous treatment, 47 received sodium stibogluconate, 4 received miltefosine, and 1 patient received both these drugs. Among the patients in the amphotericin group who had received previous treatment, 14 received sodium stibogluconate and 1 received miltefosine.

¶ Density ranges from 0 to 6, with higher values indicating greater splenic parasite load.

|| The safety substudy included 500 patients in the paromomycin group and 166 in the amphotericin group.

Table 2. Summary of Adverse Events Occurring during the Study.*

Variable	Paromomycin Group (N=500)	Amphotericin Group (N=166)	P Value†
	<i>no. of patients (%)</i>		
Deaths‡	2 (<1)	1 (1)	1.00
Nonfatal serious events§	2 (<1)	1 (1)	1.00
Reported events			
Any	299 (60)	111 (67)	0.12
CTC grade 3 or 4	11 (2)	11 (7)	0.01
Specific events¶			
Injection-site pain	276 (55)	0	<0.001
Pyrexia	13 (3)	94 (57)	<0.001
Rigors	0	39 (24)	<0.001
Vomiting	3 (1)	16 (10)	<0.001
Nephrotoxicity			
Protocol-defined	0	7 (4)	<0.001
Defined post hoc	4 (1)	42 (25)	<0.001
Ototoxicity**	7 (1)	0	0.20
Liver-function values††			
Aspartate aminotransferase >3× ULN	31 (6)	3 (2)	0.02
Aspartate aminotransferase >5× ULN	9 (2)	0	0.12
Alanine aminotransferase >3× ULN	10 (2)	1 (1)	0.31
Alanine aminotransferase >5× ULN‡‡	4 (1)	0	0.58

* Plus–minus values are means ±SD. Patients may have had more than one adverse event. CTC denotes Common Toxicity Criteria, and ULN upper limit of the normal range.

† P values for the comparison between the two groups were calculated with the use of Fisher's exact test.

‡ Of the deaths reported during the study, one occurred before the patient received a study drug, one was considered to be possibly related to paromomycin, one was considered to be probably related to amphotericin, and one was deemed by the investigator to be unrelated to paromomycin and resulted from septicemia secondary to a thigh abscess (no obvious connection between the injection site and the abscess was found).

§ Of the patients who had elevated levels of hepatic enzymes, two were in the paromomycin group and one with bacterial pneumonia was in the amphotericin group.

¶ Specific events were those with an incidence ≥5%.

|| Protocol-defined nephrotoxicity included events for which the value was double the baseline value and greater than 2.0 mg per deciliter, or greater than 2.5 mg per deciliter; nephrotoxic events defined post hoc were those with values greater than or equal to 1.4 mg per deciliter and represented an increase during the treatment period of 50% or more above the baseline serum creatinine level.

** Ototoxicity was defined as a confirmed shift from baseline audiometric thresholds ≥25 dB at one or more of the tested frequencies (1 to 12 kHz), or as a confirmed threshold shift ≥20 dB at two or more adjacent frequencies.

†† Events with a CTC grade of 3 or 4 in the paromomycin group included increased aspartate aminotransferase levels (seven patients), increased alanine aminotransferase levels (three patients), diarrhea (one patient), abnormal audiogram (one patient), neutropenia (one patient), and decreased white-cell count (one patient). Events with a CTC grade of 3 or 4 in the amphotericin group included pyrexia (five patients), diarrhea (one patient), rigors (two patients), increased levels of alkaline phosphatase (one patient), jaundice (one patient), and toxic neuropathy (one patient).

‡‡ All four patients also had aspartate aminotransferase levels greater than five times the upper limit of normal.

signments, seven patients (2%) in the paromomycin group and none in the amphotericin group were found to have confirmed threshold shifts for protocol-specified ototoxicity (P=0.20) (Table 2). In six patients, the threshold shifts were at high frequency above the hearing range, and in all seven patients the shift was transient, with levels returning to near

baseline values during follow-up. No patient reported hearing loss or vestibular dysfunction.

Renal Evaluation

None of the patients in the paromomycin group and seven patients (4%) in the amphotericin group had protocol-defined nephrotoxicity (P<0.001) (Ta-

ble 2). To ascertain potential renal dysfunction, we performed a post hoc analysis of creatinine elevation greater than or equal to 50% of the baseline levels and greater than or equal to 1.4 mg per deciliter during treatment. According to this definition, some renal dysfunction developed in 4 patients (1%) in the paromomycin group and in 42 patients (25%) in the amphotericin group ($P < 0.001$). Mean changes from baseline to the end of treatment in levels of blood urea nitrogen and serum creatinine were significantly higher in the amphotericin group than in the paromomycin group ($P < 0.001$ for both comparisons) (Table 3).

Other Adverse Events

Injection-site pain was the most frequently reported adverse event among patients receiving paromomycin (55%); adverse events that were most frequently reported among patients receiving amphotericin were infusion reactions of fever, rigors, and vomiting (57%, 24%, and 10%, respectively) ($P < 0.001$ for all comparisons) (Table 2). Injection-site pain in those receiving paromomycin was rarely associated with swelling (0.4%) and was generally reported as CTC grade 1 (grade 2, <2%); only one patient (<1%) treated with paromomycin discontinued the study drug because of injection-site pain and swelling. CTC grades 3 and 4 events occurred less frequently in the paromomycin group than in the amphotericin group. In addition, use of concomitant medication was less common in the paromomycin group than in the amphotericin group (14% vs. 77%, $P < 0.001$).

Other Laboratory Tests

Almost half the patients enrolled had elevated levels of alanine aminotransferase or aspartate aminotransferase, consistent with hepatic involvement in 25 to 40% of patients with visceral leishmaniasis.¹⁸⁻²¹ Aspartate aminotransferase levels higher than three times the upper limit of the normal range developed in 31 patients (6%) in the paromomycin group, as compared with 3 patients (2%) in the amphotericin group ($P = 0.02$). In nine (2%) of these patients in the paromomycin group, as compared with none in the amphotericin group, the increases in aspartate aminotransferase levels were greater than five times the upper limit of the normal range ($P = 0.12$) (Table 2); and five patients in the paromomycin group discontinued the study drug because of adverse events (reversible ototoxicity, elevated levels of hepatic enzymes, and injection-site pain). Liver-function testing showed that

levels returned to near baseline for all surviving patients.

EFFICACY

During the treatment period, 12 patients discontinued the study treatment. Of these, five remained cured at the 6-month follow-up visit. A total of 493 patients in the paromomycin group and 164 in the amphotericin group had an initial cure at the end of treatment. One patient in the amphotericin group was lost to follow-up; all 22 patients who had relapses were in the paromomycin group (Fig. 1).

Final cure rates 6 months after the end of treatment were 95% (474 of 501) in the paromomycin group and 99% (163 of 165) in the amphotericin group; the difference in rates was 4.2 percentage points with an upper bound of the 97.5% confidence interval of 6.9 percentage points, demonstrating the noninferiority of paromomycin (Table 4). This finding was consistent across all tested subgroups. Of 449 patients with newly diagnosed visceral leishmaniasis in the paromomycin group, 423 (94%) were cured, and of the 52 patients with prior visceral leishmaniasis, 51 (98%) were cured.

PHARMACOKINETIC ANALYSES

Paromomycin was absorbed quickly after intramuscular injection, reaching peak plasma levels within 1 hour. During the 21 days of treatment, the mean (\pm SD) peak plasma levels of paromomycin at 1 hour after injection ranged from 18.3 μ g per milliliter (± 8.86) to 20.5 μ g per milliliter (± 7.01) and the trough plasma levels at 24 hours after injection ranged from 1.31 μ g per milliliter (± 4.16) to 4.53 μ g per milliliter (± 6.71). The plasma levels on days 1, 8, 15, 21, and 22 were similar, and there was no evidence of drug accumulation or the induction of metabolism. No significant differences in the mean peak or trough plasma levels at day 21 were observed between pediatric and adult patients.

DISCUSSION

Paromomycin (administered intramuscularly at a dose of 11 mg per kilogram daily for 21 days) was shown to be noninferior to and to have an adverse-event profile similar to that of amphotericin (administered intravenously at a dose of 1 mg per kilogram every other day for 30 days) in the treatment of visceral leishmaniasis. On the basis of this study, paromomycin was approved by the Indian government in August 2006 for the treatment of patients

Table 3. Mean (\pm SD) End-of-Treatment Values and Changes in Clinical Measures and in Liver-Function and Renal-Function Values.*

Variable	At End of Treatment		Change from Baseline to End of Treatment [†]		P Value [‡]
	Paromomycin Group	Amphotericin Group	Paromomycin Group	Amphotericin Group	
Safety analysis[§]					
Heart rate (beats/min)	84.4 \pm 5.5	84.8 \pm 7.7	-13.7 \pm 12.2	-13.2 \pm 14.7	0.48 [¶]
Respiratory rate (breaths/min)	20.4 \pm 2.8	20.6 \pm 4.3	-1.4 \pm 3.6	-1.4 \pm 5.1	0.55 [¶]
Systolic blood pressure (mm Hg)	109.4 \pm 9.1	108.5 \pm 9.6	4.0 \pm 8.6	4.0 \pm 8.9	0.55 [¶]
Alanine aminotransferase (U/liter)	48.3 \pm 30.7	32.2 \pm 18.5	7.8 \pm 35.6	-8.9 \pm 30.1	<0.001
Aspartate aminotransferase (U/liter)	52.2 \pm 35.9	33.0 \pm 16.8	8.2 \pm 39.2	-12.1 \pm 26.4	<0.001
Alkaline phosphatase (U/liter)	227.5 \pm 110	249.9 \pm 191	12.5 \pm 123	21.8 \pm 158	0.15
Total bilirubin (mg/dl)	0.6 \pm 0.3	0.6 \pm 0.2	-0.0 \pm 0.4	0.0 \pm 0.3	0.58
Serum creatinine (mg/dl)	0.7 \pm 0.2	1.0 \pm 0.4	-0.1 \pm 0.3	0.2 \pm 0.4	<0.001
Blood urea nitrogen (U/liter)	12.0 \pm 3.4	17.0 \pm 7.4	0.3 \pm 4.8	5.1 \pm 8.0	<0.001
Efficacy analysis					
Temperature ($^{\circ}$ F)	97.5 \pm 0.8	97.6 \pm 1.0	-2.4 \pm 1.5	-2.4 \pm 1.7	0.10
Spleen size (cm below costal margin)	1.7 \pm 2.2	1.3 \pm 1.8	-4.8 \pm 2.6	-5.5 \pm 3.2	<0.001
Weight (kg)	37.0 \pm 12.1	35.1 \pm 11.1	1.5 \pm 2.6	1.5 \pm 1.5	0.94
Hemoglobin (g/dl)	9.6 \pm 1.5	8.4 \pm 1.3	1.7 \pm 1.3	0.7 \pm 1.5	<0.001
Platelet count ($\times 10^{-3}/\text{mm}^3$)	254.5 \pm 122	253.9 \pm 120	133.9 \pm 111	136.8 \pm 111	0.81
White-cell count ($\times 10^{-3}/\text{mm}^3$)	6.7 \pm 2.9	6.9 \pm 2.2	3.6 \pm 2.7	3.7 \pm 2.2	0.65
Albumin (g/dl)	3.5 \pm 0.6	3.5 \pm 0.7	0.4 \pm 0.7	0.4 \pm 0.8	0.94

*To convert the values for bilirubin to micromoles per liter, multiply by 17.1. To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for urea nitrogen to millimoles per liter, multiply by 0.357.

[†]For baseline values, see Table 1.

[‡]Analysis of variance was used to correlate laboratory values at the end of treatment with the baseline value in the model.

[§]Patients in the safety analysis included 500 in the paromomycin group and 166 in the amphotericin group.

[¶]P values were calculated with the use of a mixed model in which heterogeneous variance and a first-order autocorrelation structure are assumed.

^{||}Patients in the efficacy analysis included 501 in the paromomycin group and 165 in the amphotericin group.

with visceral leishmaniasis and is now available as a public health tool in a nationwide program to eliminate visceral leishmaniasis.

The overall cure rate of 95% with the use of paromomycin was similar in pediatric patients (96%), female patients (95%), and male patients (94%), and the difference in cure rates between patients treated with paromomycin or amphotericin was consistently less than 10 percentage points. The cure rate among those whose disease had not responded to previous treatment with sodium stibogluconate or miltefosine or who had had a relapse was high (98%). This finding is important in Bihar, where the failure of sodium stibogluconate therapy is due primarily to drug resistance.^{2,6,7} Although experience with the use of paromomycin during pregnancy is limited, this

drug may be used, when clinically indicated, in women of childbearing potential.²² Furthermore, paromomycin can be administered intramuscularly according to body weight (milligrams per kilogram) to patients with visceral leishmaniasis who have normal renal function, including children, without the need for therapeutic monitoring or dose adjustment.

The duration of treatment with paromomycin (daily for 21 days) is shorter than with amphotericin (every other day for 30 days), sodium stibogluconate (daily for 30 days), or miltefosine (daily for 28 days), though the visit burden may be higher. In this study, protocol-defined nephrotoxicity did not develop in any of the patients treated with paromomycin. When more stringent definitions of potential renal dysfunction were ap-

Table 4. Efficacy (Cure Rate at 6 Months) of Paromomycin versus Amphotericin.

Patients	Paromomycin Group	Amphotericin Group	Difference in Rate	Upper Bound of 97.5% CI for Difference*
	<i>no. cured/total no. (%)</i>		%	%
Overall†	474/501 (94.6)	163/165 (98.8)	4.2	6.9
Age category — no. (%)‡				
Pediatric	181/188 (96.3)	63/64 (98.4)	2.1	6.5
Adult	293/313 (93.6)	100/101 (99.0)	5.4	9.1
Sex				
Male	303/321 (94.4)	95/95 (100)	5.6	8.9
Female	171/180 (95.0)	68/70 (97.1)	2.1	7.2

* The value was determined by a test of noninferiority (margin of noninferiority, 0.10). CI denotes confidence interval.

† This category includes all patients who underwent randomization and received at least one dose of a study drug.

‡ The pediatric age group was defined as patients 5 to 14 years of age, and the adult age group was defined as those 15 to 55 years of age.

plied, only 1% of the patients in the paromomycin group had renal dysfunction, as compared with 25% of those in the amphotericin group. This relative absence of nephrotoxicity among patients with visceral leishmaniasis who were treated with paromomycin at a dose of 11 mg per kilogram is not surprising, since the patients included in the study were generally young and had normal renal function and since leishmania parasites do not typically invade the kidneys. Audiometric data showed transient reversible ototoxicity during treatment in seven patients in the paromomycin group (2%); no long-term clinical hearing loss or vestibular effects were reported. These findings may help to guide the safety monitoring required for large-scale medication use in India.

All the medications used to treat visceral leishmaniasis (pentavalent antimonial compounds, pentamidine, amphotericin, liposomal amphotericin, sodium stibogluconate, and miltefosine) may be associated with a significant increase in levels of liver enzymes during treatment,^{11,19,23,24} which some think may be due to the killing of the parasites in the liver, rather than to direct medication-induced hepatic toxic effects. Aminoglycosides used as parenteral antibiotics are rarely associated with increased levels of liver enzymes.²⁵ Although the exact cause of the transient significant increase in levels of hepatic enzymes, which affected patients in the paromomycin group but not those in the amphotericin group in this study, is difficult to ascertain, the possibilities include faster destruction of the parasites in liver tissue in patients

treated with paromomycin or an emerging toxicity of paromomycin treatment in this setting. Monitoring of aspartate aminotransferase or alanine aminotransferase levels, or both, in a program to control visceral leishmaniasis will be an important consideration, especially in patients with preexisting liver disease.

Limitations of the study include high variation at the study sites in reporting injection-site pain, ranging from 2% to 97%. The data collection was not standardized for this specific outcome. Moreover, the patients were followed for only 6 months, which is the standard for visceral leishmaniasis trials, because most relapses occur during this period.²⁶ Although rare, post-kala-azar dermal leishmaniasis (PKDL) can occur years after treatment²⁷; long-term follow up for PKDL was beyond the scope of this study. Though the overall relapse rate among patients treated with paromomycin was acceptable (4%), for a program to eliminate visceral leishmaniasis to succeed, early detection and prompt treatment of relapses, as well as mechanisms to identify and treat patients with PKDL, are imperative. Combination chemotherapy must also be explored to reduce the risk of drug resistance over time.

It is currently critical to address the elimination of visceral leishmaniasis in the Indian subcontinent, where the rates of HIV infection and HIV-visceral leishmaniasis coinfection are rising.²⁸ Since patients with HIV who are coinfecting with visceral leishmaniasis will probably have relapse without lifelong antiretroviral therapy, they

may remain an infectious reservoir until proper HIV therapy can be deployed in a sustainable manner. Furthermore, a significant proportion of the at-risk population may have subclinical leishmania infection, contributing to transmission of visceral leishmaniasis.²⁹ For widespread public health use, the intramuscular administration of paromomycin is challenging; however, primary health-center personnel have experience with intramuscular administration of sodium stibogluconate, and supervised dosing can limit the resistance due to noncompliance with the regimen.

In conclusion, paromomycin was shown to be noninferior to amphotericin, and, with the exception of mild injection-site pain and a transient increase in values on liver-function testing, it has a reasonable safety profile. Paromomycin may be advantageous because of the shorter duration of its administration and its demonstrated safety and efficacy in pediatric patients and in patients in whom visceral leishmaniasis did not respond to previous treatment. The health care delivery system in India is well suited to the intramuscular administration of paromomycin under directly

observed therapy, and the local manufacture of paromomycin in India, potentially at a very low cost, makes this an approachable therapy in the setting of limited resources.

Supported by grants from the Bill and Melinda Gates Foundation to the Institute for OneWorld Health; and by the Institute for OneWorld Health and the Special Program for Research and Training in Tropical Diseases (TDR) of the United Nations Development Program, the World Bank, and the World Health Organization (WHO).

Dr. Sundar reports receiving travel support from Asta Medica, Zentaris, Liposome, GlaxoSmithKline, DiaMed, Institute for OneWorld Health and grant support from GlaxoSmithKline, Asta Medica, Nexstar, Gilead Sciences, and the WHO; and Dr. Jha, grant support from GlaxoSmithKline. No other potential conflict of interest relevant to this article was reported.

We thank our patients and the staff and administration of the four Kala-Azar Centers of Excellence: Drs. I. Singh, D. Verma, M. Kumar Singh, A. Kumar, K. Pandey, N. Kumar, S.M. Hassan, C.P.N. Thakur, N. Verma, C.S. Lal, S.K. Jaiswal, S.K. Verma, and S. Sharma; many colleagues at the WHO TDR for developing paromomycin for the treatment of visceral leishmaniasis in prior clinical trials in Bihar; Médecins sans Frontières and the International Dispensary Association for providing access to paromomycin; Dr. T. Brewer, of the Bill and Melinda Gates Foundation, and Dr. C. Ley, for reviewing an earlier draft of the manuscript; and A. Herskowitz, V. Hale, C. Rask, G. Crean, A. Llosa, K. Oliver, E. Cooper, D. Tranowski, J. Mordenti, and M. McGuffey, of the Institute for OneWorld Health, and J. Berman, W. Gutteridge, and A. Bryceson, of the Product Development Team, WHO TDR, Geneva.

APPENDIX

Other members of the Paromomycin for VL Study Teams are as follows: **Data Coordinating Centers:** *Institute for OneWorld Health, San Francisco* (lead center): B. Nguyen, E. Kwan, A. Oudin, K. Valcke, S. Mathie, C. Ley; *Majaro InfoSystems, Santa Clara, CA:* M. Rosenberg; *DIEM Computing Services, Newark, CA:* E.L. Gaithersburg, L. Muenz, D. He; **Data Safety Monitoring Board:** *Harvard School of Public Health, Boston:* L.J. Wei (chair); *University of New Mexico, Albuquerque:* B. Ballanchanda; *Diablo Nephrology Middle Group, Walnut Creek, CA:* E. Wronce; *Touro University College of Osteopathic Medicine, Vallejo, CA:* E. Mahmoud; *Northwick Park Hospital, Middlesex, United Kingdom:* R. Davidson; **Audiology Monitoring Committee:** *University of California San Francisco Medical Center, San Francisco:* R. Sweetow (chair); *University of New Mexico, Albuquerque:* B. Ballanchanda; *Washington University Medical Center, St. Louis:* M. Valente; **Pharmacokinetic Analysis Group:** *University of California San Francisco Medical Center, San Francisco:* L. Sheiner (deceased), S. Beal (deceased); *University of California San Francisco Drug Studies Unit, San Francisco:* E. Lin, W. Gee, Y. Huang, H. Chang, X. Li.

REFERENCES

- Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 2004;27:305-18.
- Sundar S, More DK, Singh MK, et al. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. *Clin Infect Dis* 2000;31:1104-7.
- Thakur CP, Sinha GP, Pandey AK, et al. Do the diminishing efficacy and increasing toxicity of sodium stibogluconate in the treatment of visceral leishmaniasis in Bihar, India, justify its continued use as a first-line drug? An observational study of 80 cases. *Ann Trop Med Parasitol* 1998;92:561-9.
- Ahasan HA, Chowdhury MA, Azhar MA, Rafiqueuddin AK, Azad KA. Deaths in visceral leishmaniasis (Kala-azar) during treatment. *Med J Malaysia* 1996;51:29-32.
- Lira R, Sundar S, Makharia A, et al. Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of *Leishmania donovani*. *J Infect Dis* 1999;180:564-7.
- Sundar S, Singh VP, Sharma S, Makharia MK, Murray HW. Response to interferon-gamma plus pentavalent antimony in Indian visceral leishmaniasis. *J Infect Dis* 1997;176:1117-9.
- Thakur CP, Narayan S, Ranjan A. Epidemiological, clinical and pharmacological study of antimony-resistant visceral leishmaniasis in Bihar, India. *Indian J Med Res* 2004;120:166-72.
- Das VN, Ranjan A, Bimal S, et al. Magnitude of unresponsiveness to sodium stibogluconate in the treatment of visceral leishmaniasis in Bihar. *Natl Med J India* 2005;18:131-3.
- Sundar S, Rai M. Advances in the treatment of leishmaniasis. *Curr Opin Infect Dis* 2002;15:593-8.
- Rosenthal E, Marty P. Recent understanding in the treatment of visceral leishmaniasis. *J Postgrad Med* 2003;49:61-8.
- Sundar S, Jha TK, Thakur CP, et al. Oral miltefosine for Indian visceral leishmaniasis. *N Engl J Med* 2002;347:1739-46.
- Sundar S, Murray HW. Availability of miltefosine for the treatment of kala-azar in India. *Bull World Health Organ* 2005;83:394-5.
- Thakur CP, Kanyok TP, Pandey AK, Sinha GP, Messick C, Olliaro P. Treatment of visceral leishmaniasis with injectable paromomycin (aminosidine): an open-label randomized phase-II clinical study. *Trans R Soc Trop Med Hyg* 2000;94:432-3.
- Jha TK, Olliaro P, Thakur CP, et al. Randomised controlled trial of aminosidine (paromomycin) v sodium stibogluconate for treating visceral leishmaniasis in North Bihar, India. *BMJ* 1998;316:1200-5.
- Cancer Therapy Evaluation Program.

- Common toxicity criteria. Bethesda, MD: National Cancer Institute, 1998. (Accessed May 25, 2007, at http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf)
16. Wingard JR, Kublis P, Lee L, et al. Clinical significance of nephrotoxicity in patients treated with amphotericin B for suspected or proven aspergillosis. *Clin Infect Dis* 1999;29:1402-7.
17. Walsh TJ, Pappas P, Winston DJ, et al. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. *N Engl J Med* 2002;346:225-34. [Erratum, *N Engl J Med* 2007;356:760.]
18. Singh UK, Sinha RK, Sharma VK. Fulminant hepatitis in Kala-azar. *Indian J Pediatr* 1995;62:571-4.
19. Jha TK, Sundar S, Thakur CP, et al. Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. *N Engl J Med* 1999;341:1795-800.
20. el Hag IA, Hashim FA, el Toum IA, Homeida M, el Kalifa M, el Hassan AM. Liver morphology and function in visceral leishmaniasis (Kala-azar). *J Clin Pathol* 1994;47:547-51.
21. Aggarwal P, Wali JP, Chopra P. Liver in kala-azar. *Indian J Gastroenterol* 1990;9:135-6.
22. Czeizel AE, Rockenbauer M, Olsen J, Sorensen HT. A teratological study of aminoglycoside antibiotic treatment during pregnancy. *Scand J Infect Dis* 2000;32:309-13.
23. Thakur CP, Kanyok TP, Pandey AK, et al. A prospective randomized, comparative, open-label trial of the safety and efficacy of paromomycin (aminosidine) plus sodium stibogluconate versus sodium stibogluconate alone for the treatment of visceral leishmaniasis. *Trans R Soc Trop Med Hyg* 2000;94:429-31.
24. Chunge CN, Owate J, Pamba HO, Donno L. Treatment of visceral leishmaniasis in Kenya by aminosidine alone or combined with sodium stibogluconate. *Trans R Soc Trop Med Hyg* 1990;84:221-5.
25. Chambers HF. The aminoglycosides. In: Brunton LB, Lazo JS, Parker KL, eds. Goodman & Gilman's The pharmacological basis of therapeutics. 11th ed. New York: McGraw-Hill, 2006:1155-71.
26. Collin S, Davidson R, Ritmeijer K, et al. Conflict and kala-azar: determinants of adverse outcomes of kala-azar among patients in southern Sudan. *Clin Infect Dis* 2004;38:612-9.
27. Zijlstra EE, Musa AM, Khalil EA, el-Hassan IM, el-Hassan AM. Post-kala-azar dermal leishmaniasis. *Lancet Infect Dis* 2003;3:87-98.
28. Sinha PK, Bimal S, Singh SK, Pandey K, Gangopadhyay DN, Bhattacharya SK. Pre- and post-treatment evaluation of immunological features in Indian visceral leishmaniasis (VL) patients with HIV co-infection. *Indian J Med Res* 2006;123:197-202.
29. Sundar S, Maurya R, Singh RK, et al. Rapid, noninvasive diagnosis of visceral leishmaniasis in India: comparison of two immunochromatographic strip tests for detection of anti-K39 antibody. *J Clin Microbiol* 2006;44:251-3.

Copyright © 2007 Massachusetts Medical Society.

CLINICAL TRIAL REGISTRATION

The *Journal* requires investigators to register their clinical trials in a public trials registry. The members of the International Committee of Medical Journal Editors (ICMJE) will consider most clinical trials for publication only if they have been registered (see *N Engl J Med* 2004;351:1250-1). Current information on requirements and appropriate registries is available at www.icmje.org/faq.pdf.