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Failure of Pentavalent Antimony in Visceral Leishmaniasis in India: Report from the Center of the Indian Epidemic

In India, 320 patients with visceral leishmaniasis (209 in the state of Bihar and 11 in the neighboring state of Uttar Pradesh) received identical pentavalent antimony (Sb) treatment. Sb induced long-term cure in 35% (95% confidence interval [CI], 28%–42%) of those in Bihar versus 86% (95% CI, 79%–93%) of those in Uttar Pradesh. In Bihar, the center of the Indian epidemic, traditional Sb treatment should be abandoned.

Of the estimated 500,000 new cases of visceral leishmaniasis (kala-azar) that occur annually throughout the world, as many as one-half are thought to occur in India, and up to 90% of these cases are found in the northern region of the state of Bihar [1, 2]. In India, kala-azar is also endemic in neighboring states, including Uttar Pradesh and West Bengal; sporadic cases are found elsewhere as well [1, 2]. In addition to representing the center of what is now the decades-old kala-azar epidemic in India, north Bihar has also been watched closely, since it appears to be the first region in the world where large-scale therapeutic failure of conventional treatment, pentavalent antimony (Sb), has emerged [3, 4].

Despite the associated adverse reactions and the need for prolonged parenteral treatment, Sb has remained the traditional, first-line therapy for kala-azar throughout the world, primarily because it is affordable and effective and is surely a time-tested therapy [5]. Although low-level unresponsiveness to Sb was expressed in India as early as the 1970s [4], incremental increases in both the recommended dosage (to 20 mg/kg/day) and the duration of treatment (to ⩾28 days) satisfactorily compensated for the growing resistance to Sb in India until approximately 1990 [3–5]. Since that time, however, there has been a steady erosion in the capacity of Sb to induce long-term cure in patients with kala-azar who live in Bihar [4–7].

In 1994, we set out to formally document the level of efficacy of Sb treatment in patients with kalaz-azar who live in Bihar and to determine whether unresponsiveness to Sb had spread to an adjacent region, eastern Uttar Pradash. From May 1994 through June 1997, we enrolled a total of 320 subjects in a 2-site study in which conventional Sb therapy, 20 mg/kg/day for 30 days, was given to previously untreated patients in a similar and closely supervised fashion.

The Kala-Azar Medical Research Center consists of 2 treatment units, one of which is located in Muzaffarpur in north Bihar and the other of which is located 200 miles to the northwest at Banaras Hindu University Hospital in Varanasi, Uttar Pradesh [6]. From May 1994 through June 1997, consecutive patients were enrolled at both sites to receive conventional Sb therapy.

Male and female patients of any age were eligible for the study if they had typical symptoms and signs of kala-azar (e.g., fever, weight loss, hepatosplenomegaly, and pancytopenia), if they had characteristic amastigotes observed microscopically on splenic or bone marrow aspirate smears, and if they had not received prior antileishmanial treatment [6]. Patients were excluded from this study if they had a serious underlying illness (e.g., congestive heart failure, renal dysfunction, or diabetes) or an associated infection (e.g., tuberculosis or malaria), if they were receiving immunosuppressive therapy, or if one of the following laboratory abnormalities was present: a serum glutamic oxaloacetic transaminase value >5 times the normal value, a serum creatinine level >2.0 mg/dL, or a prothrombin time >5 seconds above the control value. Eleven eligible patients (9 at the Bihar site and 2 at the Uttar Pradesh site) either were excluded because of the preceding criteria (9 patients) or chose not to participate (2).

In addition to routine biochemical and hematologic tests, evaluation of history, complete physical examination, and assessment of the Karnofsky performance status, other standard

This study was approved by the Ethics Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, and informed consent was obtained either from the subjects or from their parents or guardians.

S.S. participated in designing the study, collecting the data, and writing the paper and served as the principal investigator. D.K.M., M.K.S., V.P.S., S.S., A.M., and P.C.K.K. were involved in the care and clinical assessment of patients and in the collection of data; H.W.M. collaborated on the design of the study, was responsible for data interpretation, and helped with the writing of the paper.

Reprints or correspondence: Dr. Shyam Sundar, 6, S.K. Gupta Nagar, Lanka, Varanasi 211 005, India (shyams@nde.vsnl.net.in).

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Table 1. Clinical and laboratory features, at study entry, of 320 patients with visceral leishmaniasis (kala-azar).

Feature	Patients from Bihar (Muzaffarpur) ($N = 209$)	Patients from Uttar Pradesh (Varanasi) ($N = 111$)	P
Age, years (range)	19 ± 1 (1–70)	26 ± 2 (3–65)	<.0006
Male sex, %	66	70	NS
Duration of illness, months	3.0 ± 0.2	3.1 ± 0.2	NS
Weight, kg	29 ± 1	38 ± 1	<.0001
Karnofsky performance score	73 ± 1	80 ± 1	<.0001
Spleen size, cm	7.0 ± 0.3	6.7 ± 0.4	NS
WBC count, $\times 10^3$ cells/mm^3 (n)	3.9 ± 0.1 (206)	5.1 ± 0.2 (104)	<.0001
Hemoglobin, g/dL (n)	8.4 ± 0.1 (206)	8.9 ± 0.3 (103)	<.05
Platelet count, $\times 10^3$ cells/mm^3 (n)	99 ± 3 (204)	137 ± 6 (103)	<.0001
Splenic aspirate parasite score (n)[a]	2.6 ± 0.1 (188)	2.8 ± 0.1 (105)	NS

NOTE. Data are means ± SE, unless otherwise indicated. The 4 laboratory tests were not performed for every patient. NS, not significant ($P > .05$).

[a] A total of 21 patients from Bihar and 6 from Uttar Pradesh did not undergo splenic aspiration; these 27 patients had positive bone marrow aspirate smears at study entry.

pretreatment tests for subjects enrolled at both sites included urinalysis, electrocardiography, and chest radiography. Spleen size was measured in the anterior axillary line. Parasite density score was determined microscopically in Giemsa-stained splenic aspirate smears, by use of a logarithmic scale ranging from 0 (no parasites per 1000 oil-immersion fields) to +6 (>100 parasites per field) [6]. Stained bone marrow aspirate smears were designated as either "positive" (if amastigotes were present) or "parasite-free" (if no amastigotes were seen in 1000 oil-immersion fields).

Sodium antimony gluconate (Albert David Limited, Calcutta), 20 mg/kg (without any upper dose limit), was administered once daily by means of iv injection. The same methods of administration and batches of the drug were used at both treatment sites. Treatment was given for 30 days, at which time the parasitologic response was measured by repeat splenic or bone marrow aspirate smear examination. Hematological and biochemical testing and electrocardiography were repeated weekly during treatment. The treatment drug was discontinued if evidence suggesting cardiac toxicity, including appearance of the signs and symptoms of heart failure, prolongation of the QT interval corrected for heart rate (known as the "QTc interval"; >0.50 s), or marked ST-T wave changes [5, 8] developed. Sb was also stopped if clinically significant renal or hepatic dysfunction developed.

Designation of apparent cure at the end of treatment on day 30 required the absence of fever, general clinical improvement, a decrease in spleen size, and a parasite-free splenic or bone marrow aspirate smear [6]. Definitive cure, measured 6 months after treatment, required that the patient be healthy with no symptoms or signs of relapse [6]. Patients with primary treatment failures and patients who experienced relapse were offered alternative therapy, usually either amphotericin B or one of its lipid formulations [9].

Data were expressed as means ± SE. The 2-sample t test was used to detect differences between the clinical and laboratory results for the 2 patient groups, with the exception of differences in sex and response rates (χ^2 test). Standard binomial 95% CIs for the individual proportions of patients responding to each treatment regimen were computed. A P value of <.05 was considered significant.

During the study period, 320 patients were enrolled; 209 were enrolled at the Bihar site (Muzaffarpur) and 111 at the Uttar Pradesh (Varanasi) site. Table 1 summarizes the clinical and laboratory features at study entry. There were significant differences between the 2 patient groups at baseline, with regard to age, weight, Karnofsky performance score, and the 3 hematologic test values. However, with the possible exception of the Karnofsky score, we believe that it is unlikely that the other differences noted would have meaningfully influenced the response to treatment. In addition, the groups were similar with respect to duration of illness, spleen size, and splenic aspirate parasite density score, which are variables of equal or perhaps greater clinical relevance.

Although it was not routinely performed, ELISA testing of serum for anti-HIV antibody showed negative results for the 219 patients tested (176 patients from Bihar and 43 from Uttar Pradesh). In a parallel study, species identification of parasites isolated from 99 cultures of pretreatment splenic or bone marrow aspirates showed that 100% of the isolates from patients from both Bihar (77 patients) and Uttar Pradesh (22) were *Leishmania donovani* [10].

Table 2 summarizes the results of the study, in which 286 of

Table 2. Response to 30 days of pentavalent antimony (Sb) treatment, for 320 patients with visceral leishmaniasis (kala-azar).

Response	Patients from Bihar ($n = 209$)	Patients from Uttar Pradesh ($n = 111$)
Removed from treatment		
before day 30	25	9
Self-withdrawal	2	0
Cardiotoxicity		
Nonfatal	8	4
Fatal	15	5
At day 30		
Completed treatment	184	102
Treatment failure	95[a]	2[b]
Apparent cure	89	100
At 6 months		
Relapse	14	1
Lost to follow-up	2	4
Definitive cure, no. (%)	73 (35)[c]	95 (86)[d]

[a] Of the 95 patients who had treatment failure, 12 had clinical failure despite apparent parasitologic response (negative tissue aspirate but presence of persistent fever and/or no decrease in spleen size; repeat aspirate in subsequent weeks showed parasites in all 12 patients), 18 had parasitologic failure despite apparent clinical response (either an increase or no change in aspirate parasite density or failure to achieve parasite-free aspirate on day 30), and 65 had both clinical and parasitologic failure.

[b] Of the 2 patients who had treatment failure, one had clinical failure, and the other had clinical and parasitologic failure.

[c] 95% CI, 28%–42%.

[d] 95% CI, 79%–93%.

the 320 patients completed 30 days of treatment. Six of the patients designated as having apparent cure on day 30 did not return for final evaluation and were therefore considered to have treatment failure at 6 months. Overall, in an intention-to-treat analysis, 73 (35%; 95% CI, 28%–42%) of 209 patients from Bihar and 95 (86%; 95% CI, 79%–93%) of 111 subjects from Uttar Pradesh ($P < .0001$) were healthy and relapse free 6 months after treatment and were designated as having definitive cure. On-treatment analysis of subjects who completed 30 days of therapy and who were not lost to follow-up indicated definitive cure in 73 (40%; 95% CI, 33%–47%) of 182 patients from Bihar and in 95 (97%; 95% CI, 94%–99%) of 98 patients from Uttar Pradesh.

Consistent with Sb-induced effects [5, 8], cardiotoxicity developed in 32 (10%) of the 320 subjects (table 2). Nineteen patients (5.9%) died, and all deaths were attributed to Sb-associated cardiotoxicity; autopsies were not performed, as per local custom. At the 2 treatment sites, Bihar and Uttar Pradesh, the frequency of cardiotoxicity (11% vs. 8%, respectively) or death (7.2% vs. 4.5%, respectively) was not statistically different.

The 182 subjects at the Bihar site who completed the 30-day treatment protocol and who were not lost to follow-up were separately analyzed to determine whether clinical or laboratory findings recorded at baseline (table 1) could distinguish those who had treatment failures. We compared (a) patients with initial apparent cure (87 patients) and nonresponders (95) on day 30, (b) patients with apparent cure who had relapse (14) and those with apparent cure who did not have relapse (73) within 6 months, and (c) patients with a definitive cure at 6 months (73) and all patients who failed treatment at day 30 or at 6 months (109). These analyses (data not shown) did not identify a pretreatment feature that distinguished between these subsets of patients in Bihar.

These results clearly indicate that traditional pentavalent Sb therapy is no longer effective among patients from north Bihar. On the basis of the alarmingly high failure rate (65%) identified in this study, we abandoned the use of Sb at our treatment unit in Muzaffarpur after June 1997, and we have since turned primarily to the use of either amphotericin B or one of its lipid formulations [9, 11] and to the testing of a new oral agent [12]. Results from 2 other less extensive studies from Bihar, which were performed at approximately the same time, indicate somewhat lower failure rates among patients treated with Sb (37% [7] and 42% [4]). Nevertheless, the clinical conclusion of these studies and our own study is essentially the same: after being used effectively in India for 50 years, the usefulness of Sb in the treatment of kala-azar among patients in the high-prevalence, high-transmission epidemic region of Bihar has come to an end.

At the same time, it is also clear that, in other areas of India [13] (and in all other countries around the world where kala-azar is found [5, 14]), Sb has maintained a high level of efficacy when it is used daily at a proper dose (20 mg/kg) and for prolonged periods of ⩾28 days [5]. For example, in Varanasi, just 200 miles from Muzaffarpur, nearly 90% of patients were cured by the same regimen that failed in two-thirds of the subjects in Bihar.

A number of factors, primarily thought to be related to years of providing suboptimal treatment that led to drug resistance among parasites [3, 4, 15], have likely contributed to the failure of Sb for the treatment of kala-azar in patients from Bihar. One factor that can directly undermine the response to Sb is coinfection with HIV [16]. However, 68% of our patients were tested and were determined to be seronegative for HIV, and coinfection has not been detected in prior studies of patients from Bihar who have kala-azar [9, 12, 17]. Thus, coinfection with HIV is currently uncommon in this part of India, and it does not explain unresponsiveness to Sb therapy.

It is possible that the magnitude of Sb treatment failure may remain regional in and unique to India. However, resistance may also now be increasing in Sudan [18], the other major epidemic area for kala-azar; thus, clinicians in other endemic regions should be on the alert for the future possibility of the declining efficacy of Sb. The results reported in the present study also reemphasize the pressing need to support the development of new antileishmanial drugs [12, 19].

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Shyam Sundar,[1] Deepak K. More,[1] Manoj K. Singh,[1] Vijay P. Singh,[1] Sashi Sharma,[1] Anand Makharia,[1] Prasanna C. K. Kumar,[1] and Henry W. Murray[2]

[1]The Kala-Azar Medical Research Center, Banaras Hindu University Institute of Medical Sciences, Varanasi, India; and [2]Department of Medicine, Weill Medical College of Cornell University, New York, New York

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Death from Inappropriate Therapy for Lyme Disease

A 30-year-old woman died as a result of a large *Candida parapsilosis* septic thrombus located on the tip of a Groshong catheter. The catheter had been in place for 28 months for administration of a 27 month course of intravenous cefotaxime for an unsubstantiated diagnosis of chronic Lyme disease.

A 30-year-old woman was admitted to the Mayo Clinic (Rochester, MN) in May 1999 following a grand mal seizure. She reported a several-week history of anorexia that was accompanied by a 23-kg weight loss over an 8-month period; 4 days before admission, she noticed twitching of her upper extremities. She appeared ill and had a blood pressure of 124/82 mm Hg, a pulse rate of 85, a temperature of 37°C, and a respiratory rate of 20. The patient was confused and unable to provide a coherent history. She was icteric and had diffuse myoclonus. Cardiac auscultation revealed a prominent pulmonic second sound. A Groshong catheter was in place. Hepatosplenomegaly was noted.

Her family provided a pertinent medical history. She had had a history of bilateral knee pain since childhood. She resided in Iowa; however, she had lived in Westchester County, New York, until the age of 16 years and in northern California for a short period thereafter. In 1994, she underwent cholecystectomy and since that time she had had chronic abdominal pain, whole body pain, an episode of Bell's palsy, occasional headaches, and periods of what were described as "mental fogginess" and "transient numbness." She also reportedly had a periodic rash that was thought to be a possible "Lyme rash." In 1996, she was evaluated by an infectious diseases physician in New York who specializes in chronic Lyme disease and was diagnosed with chronic Lyme disease. This diagnosis was made despite 6 EIAs negative for *Borrelia burgdorferi,* 7 Western blot assays negative or indeterminate for *B. burgdorferi,* and 4 PCR assays of blood, 5 PCR assays of urine, and 1 PCR assay of CSF, all negative for *B. burgdorferi.* MRI of the brain, as well as CSF examination, had been unremarkable in 1996. One PCR assay of blood for the *ospA* gene (Boston Biomedica Inc., New Britain, CT) was reportedly positive in January 1997.

She was initially treated with oral doxycycline, and then, for an 8-month period (1995–1996), she was treated with iv ceftriaxone; this treatment was followed by courses of oral clarithromycin and minocycline as well as parenteral penicillin G benzathine. A Groshong catheter was placed in January 1997, and a prolonged course of therapy with iv cefotaxime (up to 4 g every 8 h) was started. Intravenous doxycycline (300 mg every 12 h) was added to this therapeutic regimen in 1998. The patient reported only partial relief of her chronic symptoms during administration of this antibiotic regimen. Therapy with iv antibiotics was discontinued 1 month before evaluation at our institution, when a family physician noted abnormal results of liver function tests and thrombocytopenia. Another infectious diseases physician was consulted; this physician thought that the patient did not have chronic Lyme disease.

The patient was also being treated for chronic diffuse body pain, with several pain medications, including sustained release morphine sulfate (300 mg t.i.d.) and immediate release morphine sulfate (~45 mg/d), according to the recommendations of a fourth physician in Illinois.

At our institution, laboratory tests revealed the following abnormal results: hemoglobin level, 6.3 g/dL; WBC count, 2.2×10^9 cells/L; platelet count, 16×10^9 cells/L; rare schistocytes and helmet cells on a peripheral blood smear; prothrombin time, 19.9 s; alkaline phosphatase level, 435 U/L; aspartate

Reprints or correspondence: Dr. Robin Patel, Divisions of Infectious Diseases and Clinical Microbiology, Mayo Clinic, 200 First St. SW, Rochester, MN 55905 (patel.robin@mayo.edu).

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