Improving Outcome of Treatment of Kala-Azar by Supplementation of Amphotericin B with Physiologic Saline and Potassium Chloride

Chandeshwar P. Thakur,* A. Kumar, Dipendra K. Mitra, Ambak Roy, Arun Kumar Sinha, and Alok Ranjan

Balaji Utthan Sansthan, Patna, India; All India Institute of Medical Sciences, New Delhi, India; Department of Statistics, Patna University, Patna, India; Rajendra Memorial Research Institute of Medical Sciences, Patna, India

Abstract. Complications of amphotericin B limit its wide application in the treatment of patients with kala-azar. This study was undertaken with an aim to minimize anti-renal complications and severe rigor in course of treatment with this drug. Parasitologically confirmed kala-azar cases ($n = 230$) were randomized equally into two groups: a control group received amphotericin B only at a dose of 1 mg/kg of body weight/day for 20 days and a patient (test) group received 500 mL of physiologic saline and 30 mL (60 meq/L) of KCl with amphotericin B. We observed a significantly lower increase in serum creatinine levels ($P = 0.0001$) and a lower incidence of severe rigor and fever ($P = 0.0165$) in the test group than in the control group. However, the ultimate cure rate was not significantly different ($P = 0.5637$) between two groups after 12 months of follow-up. Relapses occurred after even after six months in both groups. Persons with relapses were treated with 25 infusions of amphotericin B and cured. Supplementation of amphotericin B with 500 mL of physiologic saline and 30 mL (60 meq/L) of KCl during treatment could help prevent an increase in serum creatinine levels and severe rigor and would make the treatment of kala-azar with amphotericin B easier.

INTRODUCTION

Amphotericin B, a polyene antibiotic and an antifungal agent, was first used in the treatment of kala-azar (visceral leishmaniasis) in 1960s.¹ Patients with kala-azar who were unresponsive to treatment with sodium antimony gluconate and pentamidine were treated with amphotericin B. Use of pentamidine as a second-line drug for treatment of patients with sodium antimony gluconate-resistant kala-azar in Bihar, India was discontinued because of its severe toxicity.^{2,3} The gradual increase in unresponsiveness of kala-azar patients to sodium antimony gluconate led to replacing it with amphotericin B as a first-line drug.^{4,5}

During the 1980s and in 2000, increased use of amphotericin B for treatment of kala-azar may have resulted in a decrease in the incidence of post-kala-azar dermal leishmaniasis.^{6,7} We have not seen any cases of post-kala-azar dermal leishmaniasis in patients treated with a 20-day regimen of amphotericin B. However, this drug causes many serious side effects (increase in serum creatinine level, acute and chronic renal failure, severe rigor, fever, cardiac failure, and death).^{8–10}

We have prevented some of the serious complications of treatment with amphotericin B by adopting simple procedures. Severe cardiac arrhythmia and heart failure in some patients with kala-azar were prevented by correcting serum electrolyte level before starting treatment with amphotericin B.¹¹ Serious cardiac arrhythmia and death among those patients who had myocarditis-like features caused by treatment with sodium antimony gluconate were prevented by providing 10 days of rest before starting treatment with amphotericin B.¹² Our 20-day regimen of amphotericin B (1 mg/kg of body weight/day) was based on a polymerase chain reaction study and was confirmed by a clinical trial.^{13,14} Our 20-day regimen of amphotericin B showed better results than 1 mg/kg alternate day regimen for 15 infusions and 1 mg/kg of body weight for 10 days.^{13,15}

We also showed that death could be prevented by increasing the hemoglobin level to 5 g/dL before using amphotericin B.¹⁶ In trials with miltefosine and paromomycin, amphotericin B was used as a comparator drug but the follow-up period was fixed at six months.^{17,18} We observed that some patients showed relapse even after six months of follow-up. In addition, amphotericin B severely affects sodium and potassium levels in some patients.

Therefore, we conducted a randomized trial to test the hypothesis that use of 500 mL of physiologic saline and 30 mL (60 meq/L) of KCl daily with amphotericin B treatment can prevent complications such as acute renal failure and severe rigor and to assess the utility of 12 months of follow-up of treatment with amphotericin B. We report the outcome of this study.

MATERIALS AND METHODS

Sample size. The aim of this study was to reduce complications such as rigor and acute renal failure caused by treatment with only amphotericin B by simultaneous administration of 500 mL of physiologic saline and 30 mL (60 meq/L) of KCl in adults and a correspondingly lower dose in children. A sample size of 100 patients in each group was required for 90% power to detect a difference of 11% reduction in complications in the test regimen group compared with the control regimen group at 5% significance level.¹⁹ On the basis of our previous experience, the drop-out rate was assumed to be 15% at this center. When we adjusting for the compliance rate, the final sample size was estimated to be 115 patients in each group.²⁰

Methods. This study was conducted as an open-label, randomized trial of 230 patients at Balaji Utthan Sansthan, Patna, India. The first patient was enrolled on February 1, 2006, the study was completed on September 30, 2007, and follow-up was completed on September 30, 2008.

Patient enrollment. Patients with kala-azar 5–55 years of age with signs and symptoms of visceral leishmaniasis (intermittent fever, shivering, enlarged liver and spleen, leukopenia, positive results for rk-39 antigen and diagnosis confirmed by demonstration of Leishman-Donovan bodies in aspirates of spleen/bone marrow) were considered for

*Address correspondence to Chandeshwar P. Thakur, Balaji Utthan Sansthan, Uma Complex, Fraser Road, Patna 800 001, Bihar, India. E-mails: cpthakur1@rediffmail.com or thakurcp@gmail.com

inclusion. Total and differential leukocyte counts count, hemoglobin %, thrombocyte count, prothombine time, serum levels of alanine and aspartate aminotransferases, creatinine, sodium, and potassium were estimated in each case. A chest radiograph and an electrocardiograph were also obtained.

Parasite load was graded on a logarithmic scale in which 1+ = 1–10 amastigotes per 1,000 high-power fields and 6 + $\geq$ 100 amastigotes per high-power field.²¹ Exclusion criteria included patients who had tuberculosis, infection with human immunodeficiency virus, acquired immunodeficiency syndrome, kidney and heart disease, and leukocyte counts < 1,000 cells/ μ L, a hemoglobin concentration < 5 g/dL, serum aspartate aminotransferase and alanine aminotransferase levels > 3 times the upper limit of the reference range, thrombocyte counts < 60,000 cells/ μ L, or serum creatinine levels > 1.5 times the upper limit of the reference range. If patients had hemoglobin levels < 5 g/dL or a thrombocyte count < 65,000 cells/ μ L, whole blood transfusions or platelet transfusions were given, respectively. If these two parameters reached acceptable levels, only then were the patients included in the trial.

Dosage and duration. Patients in control group (group A) received amphotericin B (fungizone; Nicolas Piramal India Ltd., Mumbai, India) at a dose of 1 mg/kg of body weight in an intravenous infusion given over a four-hour period every day for 20 days. Patients in the test group (group B) received 500 mL of physiologic saline, 30 mL (60 meq/L) of KCl, and an infusion of amphotericin B. A 50-mg vial of amphotericin B was dissolved in 10 mL of sterile water, and the required dose was mixed with 500 mL of 5% dextrose and infused slowly over a four-hour period through a scalp vein cannula with a small dose of hydrocortisone to minimize thrombophlebitis. Antipyretic and dexamethasone were used to treat any allergic reactions. The study staff who treated the patients opened consecutively numbered envelopes containing the treatment assignment after eligible patients fulfilled the entry criteria. Clinicians who provided treatment were not blinded to the treatment given.

Study procedure. Patients were evaluated daily during treatment. If patients had severe loss of appetite and serum creatinine levels reached twice the initial level, these patients were withdrawn from the study. During withdrawal period, they were hydrated daily with 5% glucose, electrolyte deficiency, if any, was corrected, and a high protein diet was restricted. After 10 days, patients were re-evaluated and if creatinine levels were within normal ranges, only then were they returned to their original study and treated accordingly. Any adverse events were noted. These events were graded according to common toxicity criteria of the National Cancer Institute (Bethesda, MD).²²

On day 21 and at 12 months, patients in both groups were evaluated clinically and biochemically. Splenic aspirations were performed to assess parasitologic cure. Patients were instructed to report immediately if they had fever or any adverse effect during follow-up. Patients were followed-up monthly for 12 months and evaluated at the end of 12 months for ultimate cure. The primary end point was to assess any complications such as an increase in serum creatinine levels and severe rigor. The secondary end point was to assess clinical cure at the end of treatment and at the end of the 12-month follow-up.

Clinical cure. On day 21, patients were examined, fever was rated, spleen size was measured, splenic aspiration was

conducted, body weight was measured, and biochemical and hematologic investigations were conducted. Patients were asked to come for follow-up monthly for 12 month. If fever occurred, they were asked to report immediately. In that case, patients were re-evaluated for any relapse, and splenic aspiration was conducted for parasitologic evaluation.

Cure. A patient was classified as clinically cured if he or she was afebrile and had no parasites in splenic aspirates on day 21. Ultimate cure was defined as fever during 12 months of follow-up and no parasites in splenic aspirates. If patients had relapses, they were given 25 infusions of amphotericin B.

Ethics. The study and consent forms were reviewed and approved by the ethics committee of Balaji Utthan Sansthan. Written informed consent was obtained from all adult patients and from guardians of patients < 18 years of age.

Statistical analysis. All data were entered into the computer using Epi-Info version 3.2 (Centers for Disease Control and Prevention, Atlanta, GA). Statistical analyses were conducted by using Stata version 10 (StataCorp, College Station, TX). Fisher's *t*-test was used to compare continuous variable between two groups. The paired *t*-test was used to detect significant change in variables before and after treatment within the groups for continuous variable. Fisher's exact test (one-tailed) was used to test proportions between the two groups.

RESULTS

The demographic, baseline hematologic, and biochemical variables of study participants are shown in Table 1. The two groups were comparable for all demographic, hematologic, and biochemical variables at baseline except for leukocyte counts and sodium levels. Hematologic, biochemical, and complication variables in two groups after the end of treatment and follow-up for 12 months are shown in Table 2). Two groups were comparable with response to treatment because we did not observe any significant difference in spleen size, liver size, hemoglobin percent, and sodium and potassium levels between the two groups, except for leukocyte counts and creatinine levels.

The number of patients with parasitologic cure on day 21 were nearly identical in both groups. Thirteen patients in the control group and none in the test series had to be withdrawn from the study because of anorexia and unacceptable increase in creatinine levels ($P = 0.0001$). Also, two patients in group A and one patient in group B had relapses that appeared to

TABLE 1
Demographic, hematologic, and biochemical characteristics of patients in the two study groups at baseline, India*

Characteristic	Group B	Group A	<i>P</i> †
No.	115	115	
Sex	M = 85, F = 30	M = 85, F = 30	
Weight, kg	38.95 (36–41.6)	40 (37–43)	0.754
Age, years	25.6 (22.7–28.5)	24.5 (21.5–27.4)	0.586
Spleen size, cm	3.28 (2.97–3.58)	2.81 (2.6–3.01)	0.09
Liver size, cm	1.42 (1.33–1.53)	1.32 (1.23–1.41)	0.122
Hemoglobin %	7.66 (7.33–7.99)	7.89 (7.51–8.28)	0.437
Leukocyte count/mm ³	4,352 (3,986–4,718)	5,321 (4,952–5,690)	0.0004
Na, mEq/L	130 (129–132)	133 (132–35)	0.0006
K, mEq/L	4.84 (2.57–7.10)	3.87 (3.77–3.97)	0.401
Creatinine, mg/dL	0.93 (0.85–1.01)	0.95 (0.89–1.02)	0.8029

*Values are mean (95% confidence interval) unless otherwise indicated. Group B received amphotericin B, saline, and KCl. Group A received amphotericin B.

† By Fisher's *t*-test for comparison of means between two groups before treatment.

TABLE 2

Comparison of hematologic and biochemical variables, adverse effects, and ultimate cure in the two study groups, India*

Characteristic	Group B	Group A	P†
Spleen size, cm	0.49 (0.36–0.61)	0.41 (0.31–0.51)	0.21
Liver size, cm	0.095 (0.033–0.155)	0.026 (0–0.064)	0.0539
Hemoglobin %	7.95 (7.66–8.51)	8.23 (7.88–8.57)	0.2951
Leukocyte count/mm ³	6,021 (5,849–6,194)	6,806 (6,606–7,005)	0.0001
Na, mEq/mL	137 (136–138)	136 (135–38)	0.5077
K, mEq/mL	3.53 (3.41–3.65)	3.64 (3.52–3.76)	0.2374
Creatinine, mg/dL	0.98 (0.91–1.04)	1.19 (1.10–1.28)	0.0002
Rigor, no.	5	15	0.0165‡
No. withdrawn because of increase in creatinine level	0	13	0.0001‡
No. with ultimate cure	114	113	0.5637‡

*Values are mean (95% confidence interval) unless otherwise indicated. Group B received amphotericin B, saline, and KCl. Group A received amphotericin B.

† By Fisher's *t*-test.

‡ By Fisher's exact test (one-tailed).

be similar. Fifteen patients in control group had severe rigor compared with five patients in test group who had mild shivering; the difference between two groups was significant ($P = 0.0165$). On day 21, all patients in control group were clinically and parasitologically cured. After 12 months, 113 patients in the control group and 114 patients in the test group were cured. The three patients who had relapses were cured after receiving 25 infusions of amphotericin B. The relapses in the two patients in the control group occurred at seven and nine months, and the relapse in one patient in the test group occurred at 12 months. The ultimate cure rates were similar in two groups ($P = 0.5637$).

DISCUSSION

The addition of 500 mL of physiologic saline and 30 mL (60meq/L) of KCl prevented an increase in serum creatinine levels in the test group; a significant increase was observed in the control group. These patients in control group were temporarily withdrawn from the study because of an increase in serum creatinine levels to twice the initial levels. These patients were given 10 days of rest, the electrolyte imbalance was corrected, and they were properly hydrated. During this period, serum creatinine levels returned to reference levels, and treatment with amphotericin B treatment was started. All 13 patients completed a full treatment regimen and were cured. An increase in serum creatinine levels ultimately leading to acute renal failure is a serious complication of treatment with amphotericin B. If treatment is continued despite an acceptable increase in serum creatinine levels, patients could eventually have acute renal failure. This complication of treatment with amphotericin B was prevented. Thus, supplementation with physiologic saline and KCl prevented an increase in serum creatinine levels and further deterioration of renal function to acute renal failure.

Amphotericin B can cause hypokalemia; renal, cardiac, and hepatic toxicities; bone marrow suppression; myocarditis; and sudden death.^{23,24} The nephrotoxicity of amphotericin B is dose dependent.²⁵ It has also been suggested that low salt intake increases the risk of nephrotoxicity.²³ After considering these factors, we did not increase the daily dose of amphotericin B but increased the intake of sodium and potassium. This regi-

men helped reduce renal complications. Timely withdrawal of patient from treatment, correcting the electrolyte imbalance, and dehydration prevented acute renal failure in patients in the control group. Fifteen patients in control group had severe rigor compared with mild shivering in 5 five patients in test group ($P = 0.0165$). Thus, sodium and potassium supplementation prevented some serious complications of treatment with amphotericin B. The difference in cure rate between two groups was not significant ($P = 0.5637$) and was likely the result of the same total dose of amphotericin B being given to both groups (1 mg/kg of body weight for 20 days).

Relapses occurred after six months of follow-up in both groups. All patients with relapses were given five additional infusions of amphotericin B (25 infusions of 25 mg/kg of body weight). Two patients in control group had relapses at seven and nine months, and one patient in the test group had a relapse at 12 months. Therefore, there was no difference between two groups regarding cure and relapses. Thus, when amphotericin B is used as a comparator drug, follow-up should be conducted for 12 months. Patients treated with sodium antimony gluconate usually had relapses within six months. However, in patients treated with amphotericin B, relapses usually occur after 6 months.

Death is a serious complication of treatment with amphotericin B.^{23–26} None of the patients died during treatment with amphotericin in this study and in our medical practice. This result may have been caused by the precautions taken during the trial. Before starting treatment in the two groups, precautions such as correcting serum electrolyte levels, increasing hemoglobin levels to > 5 g/dL, and not using amphotericin B in patients treated with sodium antimony gluconate with myocarditis-like symptoms without first providing 10 days of rest likely prevented deaths. Additional precautions such as withdrawing treatment from patients when severe loss of appetite developed and when serum creatinine levels increased to twice basal levels helped reduce renal complications and death. Addition of physiologic saline and potassium played a useful role in preventing these complications. We have not seen any patient with permanent treatment failure. The patients who had relapses in this trial were also cured with after receiving 25 of amphotericin B.

A voluntary organization working in Bihar, India has reported that poor patients with kala-azar lack access to diagnosis and treatment. This finding could have adverse effects on their quality of treatment and could be one of the factors resulting in increased drug resistance. Increased drug resistance could lead to serious outbreaks in leishmaniasis-endemic communities. Treatment failure in patients with kala-azar patients receiving treatment in the private sector has been observed. The market price of liposomal amphotericin B is 21,855 Rupees (350 Euros) per patient per treatment. This cost is unaffordable for poor patients.²⁷ However, we have eliminated kala-azar from some parts of Bihar by treatment only with amphotericin B treatment.²⁸ We have been working continuously to minimize toxicities of amphotericin B so that this drug could show higher cure rates.

We have demonstrated that increasing hemoglobin levels to 5 g/dL, correcting serum electrolyte deficiencies, and providing 10 days of rest before starting treatment with amphotericin B in patients previously treated with sodium antimony gluconate for myocarditis-like features will prevent occurrence of acute renal failure and cardiac complications. Use of physiologic

saline and KCl will prevent additional complications. Similarly, withdrawing a patient from a trial when creatinine levels increased to twice initial levels and providing 10 days of rest will also prevent acute renal failure. These simple procedures could help prevent serious complications during treatment with amphotericin B. Twelve months of follow-up will enable proper assessment of treatment with amphotericin B compared with other drugs. In this study, all relapses occurred after six months. Use of these precautions will help make amphotericin B more effective in controlling kala-azar.

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Authors' addresses: Chandeshwar P. Thakur and A. Kumar, Balaji Utthan Sansthan, Uma Complex, Fraser Road, Patna 800 001, Bihar, India, E-mails: cpthakur1@rediffmail.com or thakurcp@gmail.com, and dr.amitkumar@yahoo.com. Dipendra K. Mitra and Ambak Roy, All India Institute of Medical Sciences, Sarojani Nagar, New Delhi 110029, India, E-mails: salimiltra@yahoo.com and ambakrai@yahoo.com. Arun Kumar Sinha, Department of Statistics, Patna University, Ashok Raj Path, Patna 800005, Bihar, India, E-mail: arunkrsinha@yahoo.com. Alok Ranjan, Division of Biostatistics, Rajendra Memorial Research Institute of Medical Sciences, Indian Council of Medical Research, Agam Kuan, Patna 800 007, Bihar, India, E-mail: aranjn30@yahoo.com.

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