Daily versus alternate-day regimen of amphotericin B in the treatment of kala-azar: a randomized comparison*


Using a randomized study, we compared a daily and an alternate-day regimen of amphotericin B for the treatment of kala-azar, with respect to efficacy, adverse reactions, cost-effectiveness, and tolerance. The study subjects were 80 kala-azar patients, drawn from the first four decades of life and matched by age, sex, and parasite load. The patients were randomly allocated to treatment groups A and B (40 patients per group). Patients in group A received a daily regimen of amphotericin B, starting with an escalating dose of 0.05 mg/kg body weight per day until a daily dose of 1 mg/kg was reached; the latter dose was then given daily till a total dose of 20 mg/kg body weight had been administered. The patients in group B also started with an escalating dose of 0.05 mg/kg but when 1 mg/kg was reached the drug was given on alternate days. All 80 patients using the two treatment regimens were cured, no patient relapsed in either group in 6 months of follow-up, and their bone-marrow aspirates were free of amastigotes.

Treatment of kala-azar patients with the daily regimen of amphotericin B at a dose of 1 mg/kg body weight was as effective, not more toxic, equally well tolerated, and much more cost-effective than the alternate-day regimen and should be adopted for treatment of this condition.

Introduction

Sodium stibogluconate and pentamidine, two drugs commonly used in the treatment of kala-azar, have become less effective over the years. In 1953 a 10-day course of sodium stibogluconate was sufficient to cure 94% of patients, but by 1988 a 40-day course was required (1, 2). Similarly, 10 injections of pentamidine were required to cure all cases of antimony-unresponsive kala-azar in 1978 but 33 injections were required by 1991 to achieve a 98% cure rate (3, 4). Also, kala-azar patients unresponsive to both sodium stibogluconate and pentamidine have been reported, and this has necessitated the use of a third drug, amphotericin B, to treat the condition. Amphotericin B cures all antimony- and pentamidine-unresponsive patients (5–7) and even with fresh cases it cures all patients, compared with only 80% of cases cured with sodium stibogluconate (8). The high cure rate achieved with amphotericin B has resulted in its use to treat large numbers of multidrug-resistant kala-azar patients. Kala-azar is common in Bihar (ca. 250,000 cases) and the number of multidrug-resistant cases is high (9). Traditionally, amphotericin B has been administered in an escalating dose of 0.05 mg per kg body weight until a dose of 1 mg/kg is reached, whereupon it is given on alternate days. A total dose of 1 g is given to a 50-kg adult on the basis of 20 mg per kg body weight. This approach requires at least 45 days of treatment, that the patient remains close to the treatment centre, and that two relatives look after each patient. This causes great economic hardship to poor patients. Also it places a heavy strain on hospital beds. Most protocols advocate the alternate-day regimen (10–17), although two state that amphotericin B can be given daily (15, 17). We therefore carried out a study to compare the efficacy, safety, tolerability, and cost of daily and alternate-day regimens of amphotericin B for the treatment of kala-azar in order to assess the feasibility of introducing the daily regimen for its treatment. Our findings are reported in this article.

Materials and methods

A total of 80 kala-azar patients, whose diagnosis was confirmed by demonstration of Leishman-Donovan bodies in aspirates of spleen or bone marrow, were entered into the trial. The patients were randomly...
assigned to two treatment groups (A and B). Each group consisted of 40 patients, comprising 10 patients from each decade from the first to the fourth decades of life, selected and matched with respect to age, sex, duration of illness, and parasite load.

**Initial assessments**

All patients received a thorough physical examination. Spleen sizes were measured in centimetres from the costal margin to the tip in the anterior axillary line, while liver sizes were measured in centimetres in the mid-clavicular line from the costal margin to its edge. The body weight of patients was measured in kg. The total white cell count, haemoglobin concentration, serum bilirubin, alanine, aspartate transaminase, creatinine and potassium levels were estimated, electrocardiograms and chest X-rays recorded, and urine analyses carried out. Splenic aspirates were stained with Giemsa for demonstration of parasites. Patients who did not agree to come for follow-up for 6 months were excluded from the study, as were those with pneumonia, jaundice, cardiac and renal complications, and tuberculosis. Patients infected with hookworm, roundworm and *Entamoeba histolytica* were, however, not excluded, since such infections were very common among the study group; they were treated concurrently with appropriate drugs. At the outset the decision was made to withdraw from the study any patient who experienced any serious toxic reaction or hypersensitivity to amphotericin B. Patients whose haemoglobin concentration was <30 g per litre were also excluded from the study.

**Treatment regimen**

The patients in group A received increasing doses of amphotericin B as follows: 0.05 mg per kg body weight on the first day, 0.10 mg/kg on the second day, 0.25 mg/kg on the third day, 0.5 mg/kg on the fourth day, 1 mg/kg on the fifth day and subsequently 1 mg/kg daily until a total dose of 20 mg/kg had been administered. Over the first 5 days patients in group B received the drug in the same way as those in group A and were then given 1 mg/kg on alternate days until a total of 20 mg/kg had been administered. If parasites persisted in splenic aspirates at the end of the respective treatment regimens, more of the drug was given. Parasitological cure was the objective for both the groups.

Amphotericin B is dispensed as a dry powder. It was reconstituted by adding the appropriate amount to 10 ml of sterile water, diluting this solution with 500 ml of 5% (w/v) glucose solution, and then infusing patients intravenously over 6 hours. Sensitivity tests were carried out on the first day by infusing each patient with 30 ml of the drug solution. A low dose of hydrocortisone was given to each patient beforehand to avoid rigor, fever, and thrombophlebitis. Antihistamines and paracetamol were kept ready.

**Post-trial assessment.** After the trial was over, the patients’ body weights were measured, all the initial assessment investigations were repeated, and splenic aspirations were performed and the samples examined for amastigotes.

**Adverse reactions.** In both the groups adverse reactions were monitored closely every day. Patients were asked about the tolerability of the drug.

**Cost-effectiveness.** The cost of stay of a patient and two relatives was calculated on the basis of the minimum market rate.

**Follow-up.** Patients were followed up monthly for 6 months and were asked to report any fever or infection during this period.

**Statistical analysis.** Appropriate statistical analyses were carried out and χ² tests were used to calculate significances.

**Relapse and unresponsiveness.** Patients who did not respond after 20 infusions of amphotericin B were taken to be unresponsive to the drug. Patients who relapsed during follow-up were re-treated with the drug.

**Cure**

**Clinical cure** was taken to have occurred if the general condition of the patient improved, fever subsided, and splenic size regressed.

**Parasitological cure** occurred if no parasites were found in splenic aspirates at the end of treatment. **Ultimate cure** was defined to be no clinical or parasitological relapse during 6 months of follow-up after initial clinical and parasitological cure.

**Results**

Table 1 compares the initial characteristics of the two groups of patients. Intermittent fever with shivering, loss of weight, loss of appetite, splenomegaly, hepatomegaly, anaemia, leukopenia and Leishman-Donovan bodies in splenic aspirates occurred in all the patients in the two groups. Other patient characteristics were also comparable (Table 1).
Thrombophlebitis occurred in one patient in group A and in two patients in group B, and in both instances improved with treatment.

Diminution of appetite occurred in 12 patients in group A and 14 in group B, but 2 weeks after the end of treatment it had normalized.

### Cure

**Clinical cure.** The mean duration of fever was 9.5 days for group A and 10.2 days for group B. There was regression in the size of the spleens of patients in both the groups. In group A, 16 (40%) had palpable spleens, 28 (70%) had spleens that were just palpable, and six (15%) had spleens > 2 cm. In group B, 18 (45%) patients had palpable spleens, 25 (62%) had spleens that were just palpable, and seven (17.5%) had spleens > 2 cm. The mean gain in weight was 0.7 kg for patients in group A and 1.2 kg for those in group B. The greater weight increase in group B was due to the longer duration of treatment. In both groups there was an increase in haemoglobin concentration and white blood cell count at the end of the treatment.

**Parasitological cure.** All patients in both group A and group B achieved parasitological cure at the end of the treatment.

### Table 1: Initial characteristics of the kala-azar patients in the two treatment groups

<table>
<thead>
<tr>
<th>Initial characteristic</th>
<th>Treatment group:</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A; n = 40</td>
<td>B; n = 40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(daily regimen)</td>
<td>(alternate day regimen)</td>
<td></td>
</tr>
<tr>
<td>No. with intermittent fever</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Age range (years)</td>
<td>3.5–46</td>
<td>4–42</td>
<td></td>
</tr>
<tr>
<td>Duration of illness (months)</td>
<td>4.4</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Mean splenic size (cm)</td>
<td>6.8</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Mean haemoglobin concentration (g/dl)</td>
<td>6.8</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>Mean WBC count ((\times 10^9/l))</td>
<td>6.6</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>L-D bodies in splenic aspirates(^b)</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) WBC = white blood cell.
\(^b\) L-D = Leishman-Donovan.

### Table 2: Distribution of adverse reactions in the two groups of patients

<table>
<thead>
<tr>
<th></th>
<th>Daily regimen (group A)</th>
<th>Alternate-day regimen (group B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of episodes</td>
<td>520/920(^a)</td>
<td>545/920(^a)</td>
</tr>
<tr>
<td>No. of patients with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe reactions</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Raised creatinine values</td>
<td>8 (20)(^b)</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>Creatinine level &gt; 3 mg/dl</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fever alone</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Interruption of treatment</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No symptoms</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Fall in serum potassium level</td>
<td>18 (45)</td>
<td>16 (40)</td>
</tr>
<tr>
<td>Diminution of appetite</td>
<td>12 (30)</td>
<td>14 (35)</td>
</tr>
<tr>
<td>Thrombophlebitis</td>
<td>1 (2.5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Mean body weight (kg)</td>
<td>25.5/26.2(^c)</td>
<td>27.4/28.6(^c)</td>
</tr>
<tr>
<td>Mean WBC count after treatment ((\times 10^9/l))</td>
<td>7.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Mean haemoglobin concentration (g/dl)</td>
<td>7.4</td>
<td>8.6</td>
</tr>
</tbody>
</table>

\(^a\) No. of episodes/total number of infusions.
\(^b\) Figures in parentheses are percentages.
\(^c\) Before/after treatment.
**Ultimate cure.** No patients in any group relapsed by the end of 6 months of follow-up and their bone marrow aspirates were free of amastigotes.

Secondary infections (bronchitis (5 patients), otitis media (2), gastrointestinal tract infection (1)) occurred in eight (20%) patients in group A and 11 (27.5%) in group B (bronchitis (8), otitis media (1), gastrointestinal tract infection (2)) during treatment. These infections were treated and cured.

**Cost of treatment**

The cost of drugs and the accessories for their administration by transfusion was the same in both the groups. The minimum expenditure incurred on board and lodging of two relatives of each patient was US$ 92 for group A and US$ 225 for group B. This did not include the loss of income of the relatives and the cost of nursing care for the patients.

**Tolerance**

Rigor and fever on the day of infusion and diminution in appetite were the main complaints of the patients during treatment. Children tolerated the drug better, and no patient had to discontinue treatment because of intolerance.

**Discussion**

The results of the study showed that a daily regimen of amphotericin B was as efficacious as an alternate-day regimen and produced no more toxic reactions. Infusion-related adverse reactions such as shivering, rigor, or fever were not any less frequent with the alternate-day regimen. These reactions are mediated through production of tumour necrosis factor (18), release of prostaglandins (19), or the direct effect of the drug on mammalian cells (20). The incidence of severe reactions, including renal complications, was also the same in both treatment groups. Serum creatinine and potassium levels returned to normal 2 weeks after completion of the treatment.

It has been suggested that there is a significant correlation between the increase in serum creatinine (follow-up level minus pre-treatment level) and the total dose of amphotericin B (21). Also, renal function abnormalities are more frequent in patients who received a total dose of more than 4 g of amphotericin B (22). In our study no patient received a total dose of more than 1 g, an amount that was based on the results of a pilot study (6). A total dose of 971 mg of amphotericin B was used by Pratta to treat visceral leishmaniasis in Brazil (23), and WHO recommends a total dose of 1–3 g of the drug for visceral leishmaniasis. It has also been shown that the toxicity of amphotericin B may not be dose-related (24). If the serum creatinine concentration exceeds 3 mg/dl it has been suggested that treatment with amphotericin B should be interrupted for 24–48 hours to prevent uraemia (24). On this basis, its alternate-day administration has been advocated to reduce renal toxicity; there is, however, little evidence to justify this approach (24). Our study showed that in both treatment groups there were patients whose serum creatinine level was greater than 3 mg/dl; after interruption of treatment these levels remained normal even after receiving further amphotericin B at a dose of 1 mg/kg, daily or on alternate days. These findings suggest that there might be other factors that are responsible for the renal damage.

It has also been proposed that a daily dose of 1–1.5 mg/kg of amphotericin B might cause serum creatinine levels to exceed 3 mg/dl (22). Our findings do not confirm this. Only one patient in each group had a serum creatinine level that exceeded 3 mg/dl, and this normalized within 5 days and remained so even when treatment at the same dose was restarted.

The cellular effects of amphotericin B are complex and depend on a variety of factors such as the growth phase of the cells, dose, and mode of its administration, e.g., single or fractional doses (25). It appears that more work needs to be carried out to identify the exact mechanism of production and prevention of toxicity by amphotericin B.

The cost of the alternate-day treatment was almost twice that of the daily treatment. The patients had to remain twice as long in hospital and at least two relatives had to give up work for 1.5 months to look after each patient. For the daily regimen the duration of treatment was 0.83 months. In Bihar, where there are 250 000 cases of kala-azar and a large number of patients who are unresponsive to sodium stibogluconate, the daily regimen of amphotericin B has much in its favour.

Possibly because of concerns about it toxicity, amphotericin B has been mainly advocated as an alternate-day regimen. Unless the amphotericin B/lipid complex, which is less toxic and can be used as a daily dose (26), is available, amphotericin B could be used daily at a dose of 1 mg/kg body weight. While guidelines for use of amphotericin B were being formulated, it was suggested that it could be used at a daily dose of 1 mg/kg for several months, but that the daily dose should not exceed 1.5 mg/kg (17). The daily dose used in our study was 1 mg/kg over a period that did not exceed 3 weeks.

In conclusion, our data suggest that the daily regimen of 1 mg/kg body weight of amphotericin B was as effective, no more toxic, more cost-effective,
and as well tolerated as the alternate-day regimen of 1 mg/kg, and is therefore the preferred treatment for kala-azar.

Résumé

**Amphotéricine B en administration quotidienne ou un jour sur deux dans le traitement du kala-azar: comparaison randomisée**

Au moyen d'une étude randomisée, nous avons comparé l'efficacité, les effets indésirables, le rapport coût-efficacité et la tolérance de deux posologies d'amphotéricine B pour le traitement du kala-azar, l'une quotidienne et l'autre avec administration du produit un jour sur deux. Au total, 80 sujets atteints de kala-azar, âgés de 0 à 40 ans et appariés selon l'âge, le sexe et la charge parasitaire, ont été répartis par tirage au sort en deux groupes A et B de 40 sujets chacun. Les sujets du groupe A ont reçu l'amphotéricine B en doses quotidiennes, en débutant avec 0,05 mg/kg de poids corporel et en augmentant progressivement la dose jusqu'à 1 mg/kg, dose maintenue ensuite jusqu'à ce que la dose totale reçue soit de 20 mg/kg. Les sujets du groupe B ont reçu le même traitement au départ mais lorsque la dose atteignait 1 mg/kg, l'amphotéricine B était administrée un jour sur deux. Les 80 sujets de l'étude ont été guéris quelle que soit la posologie, aucun cas de rechute n'a été noté au cours des 6 mois de suivi, et les prélèvements de moelle osseuse ne contenaient pas d'amastigotes.

Huit sujets du groupe A et 7 sujets du groupe B ont présenté une élévation de la créatinine sérique, mais avec des taux restant dans les valeurs normales pour 7 sujets du groupe A et 6 sujets du groupe B. Un seul sujet de chaque groupe avait un taux de créatinine dépassant 3 mg/dl. Chez ces deux sujets, le traitement a été interrompu puis repris au bout d'une semaine à la même dose; à la fin du traitement, ni l'un ni l'autre ne présentait d'élévation de la créatinine. Des frissons, une rigidité et de la fièvre ont été observés le jour de la perfusion lors de 520 perforations sur 920 chez les sujets du groupe A et de 545 perforations sur 920 dans le groupe B. Cette différence n'était pas statistiquement significative. Une forte réaction fébrile a été observée chez 6 sujets du groupe A et 5 sujets du groupe B. Une fièvre isolée a été notée après la perfusion chez 12 sujets du groupe A et 14 sujets du groupe B. On a observé une chute de la kaliémie chez 18 sujets du groupe A et 16 sujets du groupe B, mais les taux sont remontés à la normale au bout de 2 semaines. Une surinfection au cours du traitement a eu lieu chez 8 sujets du groupe A et 11 sujets du groupe B. Ces infections ont été traitées avec succès.

En conclusion, le traitement du kala-azar par l'amphotéricine B en administration quotidienne à la dose de 1 mg/kg est aussi efficace, pas plus toxique, aussi bien toléré et d'un bien meilleur rapport coût-efficacité que le traitement un jour sur deux, et devrait donc être adopté pour le traitement de cette affection.

**Références**