Magnitude of unresponsiveness to sodium stilbogluconate in the treatment of visceral leishmaniasis in Bihar


ABSTRACT

**Background.** The Indian government proposes to eliminate kala-azar, which has been a serious public health problem in Bihar. This study aimed to assess the magnitude of unresponsiveness to sodium stilbogluconate in the treatment of new cases of visceral leishmaniasis and to identify the associated factors.

**Methods.** Patients with clinically and parasitologically confirmed visceral leishmaniasis (n = 182) who had received no prior treatment, were enrolled for the study. The patients were treated with sodium stilbogluconate (20 mg/kg body weight; upper limit 850 mg), intramuscularly for 30 days. The vital parameters and side-effects, if any, were monitored. Patients who developed toxicity during treatment were excluded from the study but were given rescue treatment with liposomal amphotericin B. All patients who completed the treatment were followed up for 6 months.

**Results.** Unresponsiveness to sodium stilbogluconate at the end of treatment was 43%. It was higher in women (48%) compared to men (40%). A significant association was observed between unresponsiveness and level of endemicity (p = 0.0002), large spleen size (p = 0.04) and immune response (migration inhibition factor) (p = 0.00002). At the end of 6 months' follow up, 27% of patients relapsed, giving a total unresponsiveness rate of 58%.

**Conclusion.** Unresponsiveness to sodium stilbogluconate is a serious problem in the management of patients with visceral leishmaniasis. In patients with factors associated with non-response to sodium stilbogluconate, alternative drugs such as miltefosine or amphotericin B should be considered as first-line drugs.


INTRODUCTION

Visceral leishmaniasis (VL) has been a serious public health problem in Bihar for many decades. The increasing trend of patients not responding to sodium stilbogluconate (SSG) therapy has aggravated the problem and raised doubts about the use of SSG as the drug of choice in the treatment of kala-azar. The recent availability of an oral drug (miltefosine) for the treatment of VL and the government’s proposed efforts to eliminate kala-azar from India make it important to assess the magnitude of unresponsiveness to SSG.

We assessed the extent of unresponsiveness to SSG treatment among new cases of VL and determined the associated factors.

**METHODS.** All patients attending the outpatient department of our institution with fever, splenomegaly, Leishman–Donovan (LD) bodies in the bone marrow and/or splenic aspirate and without any history of treatment for kala-azar were included in the study. All patients were admitted to hospital and prior to starting treatment, detailed clinical parameters such as duration of illness, body weight, fever, spleen size, liver size as well as the place of residence of the patient were recorded. The total and differential white blood cell count, haemoglobin, chest X-ray and electrocardiogram were done. Patients with a clinical suspicion of VL were subjected to bone marrow and/or splenic aspiration for confirmation of the disease. Briefly, the number of amastigotes were counted on a Giemsa-stained slide of the bone marrow (taken from the posterior superior iliac spine) or splenic aspirate and the parasite load was assessed as follows:

- 1–10 amastigotes/1000 oil immersion fields
- 1–10 amastigotes/100 oil immersion fields
- 1–10 amastigotes/1 oil immersion fields

Parasitological examination was performed on 59 of 67 patients; 8 patients had already had a bone marrow and/or splenic aspiration done recently (<7–15 days). After confirming the presence of LD bodies in the bone marrow and/or splenic aspirate, the patients were included in the study.

The patients were given SSG (20 mg/kg body weight intramuscularly daily for 30 days; maximal daily dose 850 mg). All vital signs were monitored daily. The patients were reviewed mid way, and at the end of treatment. Clinical as well as immune response evaluation was done every seventh day till the end of treatment.

Migration inhibition factor (MIF) was evaluated in all the patients as an index of cell-mediated immunity. In brief, lymphocytes were separated from heparinized blood and cells were washed three times with RPMI-1640. Finally, the aliquots were distributed in 4 wells each in a preparation of agaroase in a petri dish (15x90 mm). Two wells were filled with soluble or LD antigen while the rest were filled with the medium (control wells). The petri dish was incubated overnight at 37 °C in a humified chamber with 5% CO₂. The next day, the cells that had migrated under the agaroase were fixed and stained. The diameter of the migration areas was measured to calculate the MIF.

Patients who were free of clinical symptoms, i.e. they showed an overall improvement in their general condition and were parasitologically negative were termed as having achieved ‘apparent cure’. After discharge, the patients were followed up at 1, 2, 3 and 6 months and those with no relapse were categorized as ‘cured’. Patients were categorized as ‘unresponsive’ if they had
of the patients before starting treatment were found to be significantly associated with unresponsiveness while factors such as duration of illness, haemoglobin and total leucocyte count showed no association (p>0.05; Table III).

DISCUSSION
The treatment of kala-azar with SSG has posed a problem in Bihar. In the 1950s, SSG therapy cured more than 95% of patients. In the 1970s, 30% of patients were found to be unresponsive to treatment. When the duration of treatment was extended to 20 days, the cure rate increased and unresponsiveness declined. Over the years, the diminishing effect of SSG therapy has been reported and recently many authors have reported a 50%–55% cure rate with SSG.15,16,12 Besides, several studies have also shown regional variations in the cure rate.13,14 The final cure rate with SSG therapy was about 42% in the present study, the lowest ever reported in Bihar. In 1998, the reported cure rate was 58% in Bihar.15 There is also a clear difference in the drug response pattern of patients from other parts of India compared to Bihar.15 We observed that the cure rate among patients coming from low-endemic areas was high (100%) as compared to those from high- (48%) and meso-endemic areas (64%). Similar observations have been reported earlier.15 In Pakistan, Nepal and Sudan, cure rates in the range of 98%–100% have been reported.17–19 The diminishing therapeutic responsiveness to SSG in areas with different endemicity in the state of Bihar poses great therapeutic challenge.15,18 A wide range of doses of SSG have been recommended for the treatment of kala-azar.15,20–23 In a recent meeting of experts of the Government of India, WHO and the Government of Bihar,21,22 a 30-day regimen was recommended.

We found that factors such as the level of endemicity, MIF and spleen size were significantly associated with the cure rate. The ongoing studies do not incorporate in their approach the evaluation of cellular immunity, the preferential downregulation of which has been reported to be a conspicuous feature of kala-azar.24 We evaluated MIF in our patients, since earlier reports suggested that its inadequate release caused depression of cell-mediated immunity permitting the parasite to survive in the host.25,26 Our

Table I. Distribution of patients unresponsive to sodium stibogluconate

<table>
<thead>
<tr>
<th>Endemic area</th>
<th>Treated</th>
<th>Unresponsive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>105</td>
<td>55 (52)</td>
</tr>
<tr>
<td>Meso</td>
<td>35</td>
<td>12 (34)</td>
</tr>
<tr>
<td>Low</td>
<td>16</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>67 (43)</td>
</tr>
</tbody>
</table>

persistence of clinical signs and symptoms and/or were parasitologically positive. Relapse was defined as the reappearance of fever and presence of parasites in the bone marrow and/or splenic aspirate during follow up of 6 months. The criteria for exclusion from the study included any side-effects, namely, fall of blood pressure, cardiac, liver or renal dysfunction, severe anaemia or any toxic features. Such patients were managed according to standard treatment guidelines.

RESULTS
Of the 182 patients enrolled in the study, 156 patients (102 men, 54 women) were given a full course of 30 days of SSG. Twenty-six patients were excluded from the study due to adverse events or toxic effects of the drug. Two patients developed cardiac toxicity. Sixty-seven patients (43%) were unresponsive at the end of therapy, while 89 responded to the treatment. Of these 89 patients, 24 relapsed during the follow up period. Therefore, a total of 91 patients (58%) did not respond to SSG or had relapsed; these were referred to as the ‘unresponsive’ group.

Most patients (n=105) belonged to areas highly endemic for kala-azar and 52% of them were unresponsive (Table I). All the patients, who came from low endemic areas were cured by SSG therapy.

The clinical and haematological variables of the responsive and unresponsive groups before the start of treatment were comparable (Table II). At the end of treatment, variables such as fever, body weight, spleen size, haemoglobin and total leucocyte count were found to have significantly improved in those who responded to treatment as compared to those in the unresponsive group (Table II). Factors such as endemicity, MIF value and spleen size

Table II. Clinical and haematological characteristics of responsive and unresponsive groups of patients before and after treatment

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Responsive group (n=89)</th>
<th>Unresponsive group (n=67)</th>
<th>p value (after treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever(°F)</td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>101.34 (1.57)</td>
<td>99.99 (1.68)</td>
<td>101.91 (1.81)</td>
<td>99 (1.99)</td>
</tr>
<tr>
<td>101–101.66</td>
<td>98.74–99.44</td>
<td>101.21–102.08</td>
<td>100.08–101.03</td>
</tr>
<tr>
<td>30.51 (12.10)</td>
<td>31.64 (12.22)</td>
<td>29.96 (13.04)</td>
<td>31.52 (13.62)</td>
</tr>
<tr>
<td>28–33</td>
<td>31–37</td>
<td>27–33</td>
<td>27–34</td>
</tr>
<tr>
<td>5.83 (2.70)</td>
<td>2.78 (3.08)</td>
<td>5.33 (3.28)</td>
<td>3.97 (3.45)</td>
</tr>
<tr>
<td>5.27–6.39</td>
<td>2.14–3.42</td>
<td>5.54–6.11</td>
<td>3.14–4.80</td>
</tr>
<tr>
<td>7.80 (1.70)</td>
<td>8.50 (1.75)</td>
<td>7.70 (1.65)</td>
<td>8.85 (1.73)</td>
</tr>
<tr>
<td>7.45–8.15</td>
<td>8.13–8.86</td>
<td>7.30–8.09</td>
<td>8.43–9.26</td>
</tr>
<tr>
<td>Total count (x/mm³)</td>
<td>4657.78 (3350.82)</td>
<td>3505.62 (2756.52)</td>
<td>3468.81 (1427.19)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>43.09 (13.25)</td>
<td>44.80 (13.30)</td>
<td>40.16 (13.75)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>40.34–45.84</td>
<td>42.03–47.56</td>
<td>36.10–42.08</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>49.82 (13.98)</td>
<td>44.53 (14.39)</td>
<td>52.51 (14.13)</td>
</tr>
<tr>
<td>Parasite load</td>
<td>2.45 (1.41)</td>
<td>0</td>
<td>2.68 (1.30)</td>
</tr>
</tbody>
</table>

* The first row of each characteristic is the mean (SD) and the second row provides the 95% confidence intervals
unpublished data have also shown that sequential increase in MIF production by T-cells in a patient with kala-azar can be an important criterion to monitor the response of patients to therapeutic agents. Low immunity and large spleen size of patients at the initial screening may play some role in achieving a final cure. Assessment of MIF and spleen size of patients from a particular area of disease prevalence can help the clinician to use SSG as the first-line drug, or opt for other treatment regimens. However, these relationships need further investigation.

The Government of India proposes to start a programme for the elimination of kala-azar. This programme requires passive and active kala-azar case finding followed by effective treatment. It is, therefore, important to assess the magnitude of unresponsiveness to the existing drugs so as to develop a rational therapeutic approach to these patients. Our study has important implications for policy-makers and the proposed kala-azar elimination programme.

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References