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Unresponsiveness to AmBisome in some Sudanese patients with kala-azar

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Summary In Sudan, two treatments are currently registered for visceral leishmaniasis: sodium stibogluconate (SSG) as first line and liposomal amphotericin B (AmBisome) as second line. We present 64 patients (52 relapse cases to SSG, 12 new but complicated cases) treated with AmBisome in eastern Sudan. AmBisome was administered at 2.5—8.2 mg/kg (15—49 mg/kg in total) per dose six times (days 1, 2, 3, 5, 10, 15) as an intravenous infusion. We measured outcome according to clinical response and parasitological clearance (lymph node aspiration). Patient outcomes fell into three groups: group 1, clinical responders (cured) with a negative test of cure ($n = 35$); group 2, clinical responders with a positive test of cure ($n = 19$); group 3, clinical non-responders (failures) with a positive test of cure ($n = 10$). Of the 10 failures, six were already relapse cases. All of group 3, and 15 from group 2, were also treated with additional SSG (20 mg/kg intramuscularly daily for 30—50 d) with resulting clinical and parasitological improvement. Parasite persistence and clinical failure were associated with a higher parasite density on admission ($P < 0.002$) and underlying immunosuppressive disease: tuberculosis (three cases) or HIV (two cases). Because AmBisome monotherapy may fail in Sudan, a combination of AmBisome and SSG is recommended for relapse cases.

1. Introduction

In Sudan, kala-azar (visceral leishmaniasis, VL) is endemic and caused by *Leishmania donovani*. Two drugs are currently registered for the treatment of this disease. Sodium stibogluconate (SSG) is recommended as first-line treatment, while liposomal amphotericin B (AmBisome) is used as second-line treatment by Médecins Sans Frontières for VL patients who have relapsed after SSG. AmBisome is usually given to a total dose of 20—30 mg/kg for patients who have presented as relapse cases. Exceptionally, AmBisome is also used as first-line treatment for those patients at high risk of death who may be too unwell to tolerate SSG. We retrospectively evaluated the safety and efficacy of AmBisome in patients treated in the Médecins Sans Frontières

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kala-azar treatment centre in Um el Kher, Gedaref State, Sudan, between January 2004 and February 2005.

2. Materials and methods

2.1. Context

The Um el Kher treatment centre was established by Médecins Sans Frontières in 1996. It is located on the Rahad River in Gedaref State in eastern Sudan. This area is endemic for kala-azar, with outbreaks occurring on an intermittent basis. The centre is located in a remote area, with poor road links and communications. All patients were treated as in-patients. Drugs for kala-azar, as well as supportive treatment, nursing and food were given free of charge. Daily food rations and supplementary or therapeutic feeding were given as indicated.

Most patients came from Gedaref or neighbouring Sennar State. Transport in the area was scarce and most patients journeyed for several hours or days to reach the centre. On admission we routinely recorded demographic data (age, sex, residence), previous medical history and presenting signs and symptoms for all patients.

2.2. Case definition

Visceral leishmaniasis patients were diagnosed by laboratory confirmation of those fitting a clinical case definition of fever for >2 weeks plus weight loss and either splenomegaly or lymphadenopathy. Other typical features of VL are hepatomegaly, recurrent epistaxis and anaemia.

2.3. Laboratory investigations

Laboratory confirmation (in a patient meeting the clinical case definition) was by: the presence of visible amastigotes of *Leishmania* in Giemsa-stained aspirates of lymph nodes; a high titre ($\geq$6400) of anti-*Leishmania* antibodies in the direct agglutination test (DAT); or the rK39 (DiaMed-IT-Leish®) rapid diagnostic dipstick test for primary kala-azar patients. Relapsed patients were all diagnosed by microscopy of lymph node aspirations. Aspirates were graded using a logarithmic grading from 0 (0 parasites/1000 fields) to 6 (>100 parasites/field) (Chulay and Bryceson, 1983). Splenic aspiration is currently not permitted by the Sudanese Ministry of Health in rural health centres such as Um el Kehr.

The laboratory in Um el Kehr was able to perform microscopy (urine, stool, blood, sputum, lymph node and bone marrow), measurement of haemoglobin concentration (by haemoglobinometer) and rapid diagnostic tests for urinalysis, malaria, HIV and meningitis. Voluntary counselling and HIV testing (VCT) were offered from May 2004 to all patients >15 years and for younger patients when indicated. HIV status was not always recorded in the patient's case records, for reasons of confidentiality. Tuberculosis (TB) diagnosis was established with sputum smears.

2.4. AmBisome treatment

AmBisome was given in six doses of 2.5—8.2 mg/kg over a period of 15 d (days 1, 2, 3, 5, 10, 15) by intravenous infusion over 60—120 min. The wide variation of dose was due to the fact that doses were rounded up or down by staff to the nearest 50 mg vial in order to avoid wastage. As well as VL treatment, all VL patients received a standard supportive treatment of vitamin A, folic acid, ferrous sulfate, multivitamins and a high kilocalorie nutritional regimen. Antibiotics, antimalarials, and anti-TB drugs were added when indicated. Antiretroviral drugs were not available. During treatment and at discharge patients were followed-up clinically, to document primarily clearance of fever and weight gain. Spleen and liver regression was also measured. For every patient on AmBisome a lymph node aspiration as test of cure (TOC) was undertaken at days 25 and 30 of treatment.

2.5. Measurement of outcome

Patients were considered cured when symptoms (especially fever) resolved and they did not re-present with kala-azar to the centre in the 3 months after the end of treatment. Patients with persisting fever at the end of treatment, with a negative test for malaria, were considered likely to be treatment failures. To be confirmed as treatment failures for the purpose of this study, patients had: (1) to have been treated with at least one antibiotic to reduce the likelihood of an additional bacterial infection such as typhoid; and (2) to show clearance of fever after starting re-treatment with SSG. Due to the harsh field conditions and especially poor communication and road links, discharged patients could not be actively followed-up.

2.6. Data analysis

We documented the clinical outcome and the result of the lymph node aspirate of all patients treated with AmBisome between January 2004 and February 2005. Data entry and statistical analysis was done in Excel (Microsoft Corp., Seattle, WA, USA) and EpiInfo (CDC, Atlanta, GA, USA). Proportions were compared using the χ^2 test with Yates' correction, or Fisher's exact test, where appropriate, and medians were compared using the Mann—Whitney *U* test.

3. Results

3.1. Overall outcomes

Between January 2004 and February 2005, 2835 patients were treated for VL in the Um el Kher centre. The majority of these patients had received SSG treatment, except 64 patients (2.3%) who received six doses (days 1, 2, 3, 5, 10, 15) of AmBisome at a dose ranging from 2.5 to 8.2 mg/kg (a total of 15—49 mg/kg). Of these, 52 (81%) were relapse cases after SSG treatment. After treatment two lymph node aspirations were routinely performed as a TOC on each patient. For the analysis, the patients were divided into three groups (Table 1).

Group 1 comprised patients who showed no signs and symptoms of VL at the end of treatment, accompanied with a negative TOC. Group 2 was composed of patients with a positive TOC, but without any signs of the disease. Group 3 consisted of clinical and parasitological treatment failures: patients with a positive TOC and persistent symptoms

Table 1 Baseline characteristics of patients treated with AmBisome

	Group 1 TOC negative/clinically cured	Group 2 TOC positive/clinically cured	Group 3 TOC positive/clinical treatment failures
Total	35	19	10
Male (%)	20 (57)	10 (53)	8 (80)
Female (%)	15 (43)	9 (47)	2 (20)
Relapse (%)	29 (83)	17 (89)	6 (60)
Primary kala-azar (%)	6 (17)	2 (11)	4 (40)
Age (years) [median (range)]	11 (1—35)	7 (2—30)	6.5 (1—35)
Nutritional status[a]			
Not malnourished[b] (%)	21 (60)	12 (63)	5 (50)
Moderately malnourished[c] (%)	10 (29)	7 (37)	4 (40)
Severely malnourished[d] (%)	3 (9)	0	1 (10)
Not known (%)	1 (3)	0	0
Spleen size (cm)	$n = 34$	$n = 17$	$n = 10$
[median (range)]	4 (0—21)	6 (0—16)	5 (0—14)
Liver size (cm)	$n = 34$	$n = 17$	$n = 10$
[median (range)]	1.7 (0—5)	0 (0—6)	1.25 (0—8)
Haemoglobin (g/dl)	$n = 34$	$n = 19$	$n = 9$
[median (range)]	9.5 (4.5—13.3)	8 (5—11)	9 (7—11)
Malaria co-infection	13	4	3
HIV/tuberculosis co-infection	1	0	4
Parasitologically confirmed (%)	31 (88)	17 (89)	8 (80)
Grade >4 (%)	1 (3)	3 (18)	4 (50)
Grade 2—4 (%)	7 (23)	9 (53)	4 (50)
Grade 1 (%)	21 (68)	4 (23)	0
Grade not specified (%)	2 (6)	1 (6)	0
Serologically confirmed (%)	4 (11)	2 (11)	2 (20)

Where there are missing data, n = number of subjects for whom data are available. TOC: test of cure.
[a] Weight for height (W/H) for patients aged 1—14 years; body mass index (BMI) for patients >14 years.
[b] BMI >18 kg/m^2 or W/H >80%.
[c] BMI 16—18 kg/m^2 or W/H 70—80%.
[d] BMI < 16 kg/m^2 or W/H < 70%.

of kala-azar. There were no significant differences among the patients from the three groups on admission in: age and sex distribution, reason for treatment with AmBisome (52 relapsed, five jaundiced, five pregnant, one with cardiac arrhythmia, one with SSG toxicity), nutritional status, haemoglobin, liver size, co-infection with malaria, or dose of AmBisome.

In group 1, 35 patients (54%) were clinically cured, with a negative TOC. In group 2, 19 patients (30%) were TOC-positive but clinically cured. In group 3, 10 patients (16%) were clinical treatment failures, of whom nine had a positive TOC. Six of these cases were relapse patients who had not previously responded to SSG. Thus 6/52 (11.5%) of relapse patients showed no clinical and parasitological response to AmBisome.

3.2. Co-infection with either HIV or TB

Four patients from group 3 (40%), and one from group 1 (2%), were known to be co-infected with HIV (two cases, both in group 3) or TB (three cases, one in group 1 and two in group 3). There were no patients co-infected with both HIV and TB. However, HIV status was known for only seven patients.

Three patients co-infected with HIV or TB had VL diagnosed by lymph node aspirate. These co-infected patients had higher parasite density (median = 6) on admission than other patients (median = 2). Furthermore, the median TOC aspirate grade for HIV or TB co-infected patients was 5. This was 1.5 among other patients without known HIV or TB.

3.3. Other factors associated with treatment failure

Fifty-six patients (88%) were initially diagnosed by lymph node aspiration; the remaining 8 (12%) were diagnosed serologically by either DAT or rK39 dipstick. The parasite grade on admission was higher (median = 4) in those from group 3 than in those who clinically responded (groups 1 and 2; median = 1; $P = 0.002$). Similarly, the parasite grade among those who had parasite persistence (groups 2 and 3) was higher (median = 3) than among those who cleared parasites (group 1, median = 1, $P < 0.0001$).

There was a significantly lower weight gain and regression of the spleen in group 3 after treatment compared with patients in groups 1 and 2 combined ($P < 0.05$). However, data for splenic regression were limited, as spleen size, even if measured, was frequently only recorded before discharge when a further course of SSG had already been given (Table 2).

Table 2 Outcome of patients

	Group 1 TOC negative/clinically cured[a]	Group 2 TOC positive/clinically cured[a]	Group 3 TOC positive/clinical treatment failures
Total	35	19	10
Total AmBisome dose (mg/kg) [median (range)]	3.6 (2.5—7.4)	3.8 (2.8—8.1)	3.4 (2.6—8.2)
Clearance of fever [median (range)]	1.5 (1—14)	2 (1—17)	All still febrile
Weight gain (kg) [median (range)]	*n* = 32 1.2 (−5 to 8)	*n* = 19 1.5 (−3 to 11)	*n* = 8 0.15 (−2 to 1.5)
Spleen regression (cm)[b] [median (range)]	*n* = 33 2 (−8 to 8)	*n* = 4 3.5 (2—4)	*n* = 4 −1 (−5 to 1)
Liver regression (cm)[b] [median (range)]	*n* = 34 0 (−8 to 5)	*n* = 4 0 (−2 to 0)	*n* = 4 −3.5 (−5.5 to −2)
Test of cure aspirate			
Grade >4	0	1	3
Grade 2—4	0	9	5
Grade 1	0	9	1
Grade 0	35	0	1

Where there are missing data, *n* = number of subjects for whom data are available.
TOC: test of cure.
[a] Cure was primarily defined as clearance of fever and weight gain with resolution of other symptoms at presentation.
[b] Spleen and liver regression were used only as secondary measures of cure. Patients generally had spleen and liver recorded in their notes at admission and before discharge. Hence many patients in groups 2 and 3 who received further sodium stibogluconate treatment only had spleen/liver recorded after this point and not after initial AmBisome treatment.

3.4. Significant adverse events

Only one patient from group 1 had a significant adverse event (an allergic reaction), which occurred during the fifth dose of AmBisome. The reaction disappeared shortly after stopping the drug infusion. The patient was discharged well with a negative TOC.

3.5. Management of non-responding cases

Following AmBisome treatment, all 10 patients in group 3 received treatment with SSG 20 mg/kg/d intramuscularly. Eight patients received a course for 30—37 d. Two patients received longer (50-d) courses of SSG. One was due to development of a post-kala-azar dermal leishmaniasis with mucosal involvement, while another was an HIV-positive patient. These 10 patients were all clinically and parasitologically cured at the end of SSG treatment. Clearance of fever was seen at a median of 4 d (range 3—15 d) after start of SSG. The 10 patients also gained weight (median 1.3 kg) and their spleens regressed (by median 2.8 cm), in marked contrast to their poor response to AmBisome (Table 3).

Fifteen patients from group 2 were also treated with 30—37 injections of SSG 20 mg/kg/d intramuscularly until they had two negative TOCs. Of all 25 patients treated with additional SSG, two experienced adverse events (one pancreatitis, one nephritis). All patients were finally discharged clinically cured.

4. Discussion

Our experience of AmBisome treatment of complicated and relapsed cases of VL was in a small proportion (2.3%) of the 2835 VL cases we treated. Ten of these patients (16% of those treated with AmBisome and 0.35% of the VL cases treated) showed a poor response to AmBisome. We were unable to

Table 3 Outcomes of clinical treatment failure group

	After AmBisome	After additional sodium stibogluconate
Total number	10	10
Fever clearance (days) [median (range)]	All still febrile	4 (3—15)
Weight gain (kg) [median (range)]	*n* = 8 0.15 (−2 to 1.5)	*n* = 10 1.3 (0.4—8)
Spleen regression (cm) [median (range)]	*n* = 4 −1 (−5 to 1)	*n* = 10 2.8 (0—6)
Liver regression (cm) [median (range)]	*n* = 4 −3.5 (−5.5 to −2)	*n* = 8 0 (−5.5 to 6)

Where there are missing data, *n* = number of subjects for whom data are available.

follow-up our patients at 6 months to ensure definitive cure, but we expect that most patients would have been able to return for re-treatment had their VL relapsed. In our experience, AmBisome is safe, with minimal adverse events. This finding is consistent with other studies and animal models (Adler-Moore and Proffitt, 2002; Berman et al., 1998; Thakur et al., 1996).

The lack of clinical or parasitological response in 10 patients could be due to poor-quality drugs, parasite resistance or host factors. We consider the first of these explanations to be unlikely, because AmBisome was not used after its expiry date, nor stored at temperatures outside of 4—25 °C. Secondary parasite resistance is unlikely, because none of these patients had previously received amphotericin B in any form. Primary parasite resistance is also unlikely, because very few VL patients have been exposed to amphotericin B in this region. We are thus left with the likeliest possibility, namely that patients were unable to respond to AmBisome because of some host factor. Much recent literature has emphasized the rapid and complete response that AmBisome produces in VL, yet treatment with AmBisome failed to produce clinical improvement in 10 patients (16%). All had persistent signs of VL: fever and poor weight gain or weight loss. In some instances, inadequate spleen and liver regression was also noted at the end of treatment. Six of these patients were already relapse patients whom had been previously treated with a 30-d course of SSG — yet all 10 patients responded well to a further course of SSG, contrary to our expectations.

High parasite density in the lymph node aspirations before treatment was linked to both parasite persistence and clinical failure. It might therefore be that patients with a higher parasite density need a higher dose of AmBisome; it should be noted that, in India, total doses of AmBisome as low as 5 mg/kg produce high cure rates in VL. However, in two patients even doses of 37 and 49 mg/kg produced no clinical improvement. These findings support our previous findings (Seaman et al., 1995), in which we found that relatively high total doses of AmBisome (25—30 mg/kg) are needed to cure Sudanese patients with VL, and that patients with a higher parasite density at admission are likely to relapse and require additional kala-azar treatment.

In addition, a severe underlying disease, such as HIV or TB, was found in four of the 10 (40%) clinical treatment failures, and in a further six of these patients HIV testing was not performed. HIV co-infection, with or without extrapulmonary or smear-negative TB, could have been a major host factor determining unresponsiveness to AmBisome treatment. The parasite density in patients with underlying TB or HIV was higher at the end of AmBisome treatment compared with other patients. These patients therefore require special VL regimens as first line.

Until now, initial treatment failures with AmBisome have been described in only a small cohort of patients in south Sudan (Seaman et al., 1995). Cases of relapses following AmBisome treatment were found in immunocompromised patients, in AIDS patients in Europe with VL caused by *L. infantum* (Russo et al., 1996), in children under 5 years old with Brazilian VL (due to *L. chagasi*), and, more exceptionally, in Indian kala-azar (Berman, 2003, 2005; Davidson and Russo, 1994; Freire et al., 1997; Montana et al., 2004). AmBisome-resistant *L. donovani* parasites can also be generated in vitro (Espuelas et al., 2000).

4.1. Recommendations

Since April 2004, the Sudanese Ministry of Health has drawn up recommendations for the use of AmBisome. The protocol suggests 3 mg/kg on alternate days for 14 d to make a total dose of 21 mg/kg. This is not dissimilar to the Médecins Sans Frontières protocol of 18—30 mg/kg (3—5 mg/kg over six doses in 15 d). The Sudanese Ministry of Health also recommends its use as an alternative treatment for cases that have either not responded to SSG or in which SSG is contraindicated. They currently recommend combination therapy in cases of multiple relapse (third relapse) that have already received both SSG and AmBisome (National Malaria Schistosomiasis Leishmaniasis Administration, Sudan, 2004).

However, in light of our experience, in which we have seen a high proportion of treatment failures to the first course of AmBisome (even at relatively high doses), we would recommend a combination of 30 mg/kg AmBisome (e.g. six doses over 2 weeks) followed or overlapped by a 30-d course of 20 mg/kg SSG in Sudanese VL patients who have relapsed. The combination of the two drugs may also reduce the possibility of development of parasite resistance. For a typical VL patient weighing 30 kg, the cost of SSG therapy (US$15) is low compared with AmBisome monotherapy ($135—810 per patient in developing countries). We need also to consider the 19 patients (30%) from group 2 who had a good clinical outcome despite a positive TOC. One explanation for this discrepancy might be the tissue distribution of AmBisome. AmBisome is distributed mainly in the reticulo-endothelial tissue of organs (especially the liver and spleen), where *Leishmania* parasites are principally found during VL, rather than the skin (Adler-Moore and Proffitt, 2002). This may result in successful clearance of the parasite from the viscera but persistence (of living and dead parasites) in the superficial lymph nodes. Our data seem to substantiate this. We strongly recommend that patients after treatment should be judged clinically, rather than by lymph node aspirate. Criteria of clinical improvement should focus on clearance of fever, weight gain and splenic regression. In doubtful cases, a spleen or bone marrow aspiration should be performed to aid assessment of cure. Research in reliable diagnostic tests based on antigen detection would also be useful.

Conflicts of interest
The authors have no conflicts of interest concerning the work reported in this paper. No specific funding was obtained for this study. The authors undertook the work described in the course of their roles in patients' care while working for Médecins sans Frontières.

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