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Safety and effectiveness of amphotericin B deoxycholate for the treatment of visceral leishmaniasis in Uganda

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Amphotericin B for visceral leishmaniasis in Uganda

ABSTRACT

Supply of antimonials for the treatment of visceral leishmaniasis (VL) to Amudat Hospital, Uganda, was interrupted between September 2003 and April 2004. Therefore, all VL patients admitted during this period were treated with amphotericin B deoxycholate (AmB). We analysed the safety and effectiveness of AmB in comparison with a historical cohort of patients treated with meglumine antimoniate (SbV) in the same hospital. Demographic and clinical data were collected before and after treatment. Adverse effects were recorded passively in the whole cohort, and actively in a sub-group of patients with a pre-designed questionnaire. The in-hospital case-fatality rate was 4.8% [95CI: 2.4 – 8.8] in the 210 patients treated with AmB compared to 3.7% [95CI: 1.4 – 7.9] in the 160 patients treated with SbV ($p > 0.20$). Adverse effects requiring treatment interruption were rare in both cohorts. Treatment failures (non-responses or relapses) were found in 2.9% [95CI: 1.2 – 6.4] of patients treated with AmB compared with 1.2% [95CI: 0.1 – 4.4] in patients treated with SbV ($p > 0.20$). AmB had a similar effectiveness and safety profile as antimonials for the treatment of VL in Uganda.

INTRODUCTION

Visceral leishmaniasis (VL), or kala-azar, is a life-threatening haemo-protozoal disease affecting an estimated 500 000 new cases every year worldwide (WHO 2000). Treatment of VL remains a major problem, especially in resource-poor areas (Guerin et al. 2002). Patients' access to branded pentavalent antimony such as meglumine antimoniate (Glucantime™; Aventis, France) or sodium stibogluconate (Pentostam™; GlaxoSmithKline, United Kingdom) is limited, mainly because of their high cost. The generic form of sodium stibogluconate (SSG) manufactured by Albert David Ltd, Calcutta, India, has substantially reduced the cost of treatment and both its safety and efficacy were shown to be similar to the branded drugs (Moore et al. 2001;Ritmeijer et al. 2001;Veeken et al. 2000). Unfortunately, this generic SSG is still not authorized for importation in many countries. Amphotericin B, either in its conventional (amphotericin B deoxycholoate) or liposomal form, is a well-known drug for VL (Sundar et al. 2004). Amphotericin B deoxycholate (AmB) is now used as a first-line drug in the Bihar State of India, where resistance to antimonials has become a major problem (Mishra et al. 1994;Thakur & Narayan 2004), and as a second-line drug in some Latin-American (Santos et al. 2002) and Asian countries such as Nepal (Rijal et al. 2003). In India, its efficacy and safety have been demonstrated even in rural settings (Thakur & Ahmed 2001) . Interestingly, AmB is not licensed for the treatment of VL in any country in the world but, in contrast with generic SSG, the drug is available as it is licensed for other indications (fungal infections). Data on the use of AmB for VL in Africa are lacking and cannot be extrapolated as such from Indian studies, as the drug sensitivity profile of the parasite and the characteristics of the hosts may differ (Berman et al. 1998). Liposomal Amphotericin B (AmBisome™; Gilead, USA) is better tolerated and more practical to use than conventional

AmB (Sundar et al. 2004). It is used as first-line treatment for VL in developed countries, but its high price restricts its access for the developing world (Bern et al. 2006).

In Uganda, VL is only known to be endemic in Pokot County, Nakapiripirit District. This county forms a single endemic focus with the neighboring West Pokot District in Kenya, inhabited by the same semi-nomadic pastoralists, the Pokot. VL in this area is caused by *L. donovani* and is transmitted by *Phlebotomus martini* (Wykoff et al. 1969). Epidemiological data on VL in the Pokot area are lacking. The proportion of VL patients co-infected with HIV is believed to be currently low in this endemic focus; an unlinked anonymous survey conducted by MSF at Amudat Hospital in 2002 and 2003 showed that only 3 out of 203 VL patients (1.4%) were co-infected with HIV (Chappuis, unpublished data). Médecins Sans Frontières (MSF) opened a VL program in Amudat in 2000, establishing the only treatment centre for VL in the area. SbV was used as the first-line drug and AmB as a second-line drug. In 2003, the production of Glucantime™ by Aventis was interrupted due to technical problems in the production line. Large quantities of Pentostam™ were not available in the short term, and the generic form of SSG was not authorized for importation in Uganda. Facing this shortage of antimonials, first-line treatment was temporarily switched to AmB. In this article, we present a descriptive analysis of our experience with AmB as first-line treatment of VL, focusing on its safety and effectiveness. This is, to our knowledge, the first report on the use of AmB in Africa.

PATIENTS AND METHODS

Amudat Hospital is a 120-bed rural hospital with neither surgical nor radiological facilities. The laboratory performs basic tests such as malaria thick blood smears, colorimetric haemoglobin estimation (using the Lovibond method), white blood cell count, stool examination, urine analysis, Gram stain, sputum-examination, serology for HIV, HbsAg, VDRL, and brucellosis. No chemistry tests are performed except dipsticks for blood glucose. Since 2001, the diagnosis of VL is based on an algorithm that combines results of the Direct Agglutination Test (DAT) and microscopic examination of Giemsa-stained spleen aspirates for borderline DAT titers (1:1600 -1:12800). DAT diagnostic titers were locally validated against spleen aspirates in 2000 and 2001 (Chappuis, unpublished data). Demographical and clinical data such as age, sex, origin, symptoms at admission, duration of symptoms, weight and height, were routinely collected for every VL suspect patient as part of the project monitoring.

Between September 2003 and April 2004, patients were treated with AmB in its branded (Fungizone^{TM}; Bristol-Myers Squibb, USA) or its generic (Photericin B; Cipla, India) form, depending on which drug was available in the country. The decision to use AmB was an operational decision, as no antimonials were available in the country. The drug was administered as a daily dose of 1 mg/kg body weight given in slow infusion over 8 to 12 hours to improve tolerance. The total dose was 15 mg/kg, given on alternate days during a 30-day period in order to reduce the risk of nephrotoxicity. Renal function tests were not available. In case of edema, the body weight used for dose calculations was was reduced by 10%. Patients were stimulated to drink abundantly. Access to safe water was secured and ORS salts were distributed. In case of clinical dehydration, IV rehydration using 0.9% NaCl or Ringer lactate solutions was used. Patients were given daily supplements of oral K+ and Mg2+ tablets, in addition to multivitamin, vitamin C, folic acid and ferrous sulfate tablets. All

patients received supplementary feeding. Temperature was monitored daily and weight weekly. Antibiotic treatment for suspicion of bacterial infections and blood transfusions in case of severe anemia were given if necessary. Concomitant nephrotoxic medications were avoided.

Initial outcome was assessed at day 25 of treatment, based on the patient's general condition, spleen size, and hemoglobin count. Initial cure was defined as clearance of fever plus an improvement in general condition, decrease in spleen size, and increase in hemoglobin count. In the case of persistent symptoms and/or no reduction in spleen size, a spleen aspiration was performed (test of cure). If positive, the treatment was prolonged at the same dose until 2 successive negative spleen aspirates were obtained. Such cases were defined as slow-responders. Treatment failures included both non-responders (defined as neither clinical nor parasitological response to treatment) and relapses (defined as VL diagnosed within 6 months after initial cure). Definite cure was defined as the absence of relapse 6 months after hospital discharge. Amudat Hospital was the only center offering treatment for VL in the whole endemic area during the study period. Therefore, we assume that most relapse cases would have presented to our center. Active follow-up after discharge was not possible due to logistic constraints. The initial cure, in-hospital case-fatality rate and treatment failure rate were chosen as primary outcomes, whereas variations in hemoglobin count and spleen size between admission and end of treatment were secondary outcomes.

The occurrence of adverse effects of AmB was assessed daily during the clinician's rounds. In order to minimize non-reporting bias due to the language barrier, a weekly questionnaire targeting 19 symptoms was administered by a translator to the subgroup of patients admitted between December 2003 and February 2004. Adverse effects were graded as mild/moderate

or, if treatment interruption was necessary, severe. Association of the adverse effects with the drug was classified as possible, probable or certain.

As an indicator of comparison, we selected a historical cohort consisting of all patients diagnosed with first-time VL admitted in Amudat Hospital between September 2002 and April 2003 and treated with SbV, which was administered by intramuscular injections of 20 mg/kg without upper limit dose for 30 days. We compared baseline characteristics, outcomes and occurrence of severe adverse effects between the cohorts of patients treated with AmB and SbV.

Data were entered in a Microsoft Excel spreadsheet by the clinician in charge. The database was cleaned by identifying aberrant data. Categorization of nutritional status was based on weight-for-height for children under 137cm (females) or 145cm (males) as defined by the WHO, and on Body Mass Index for adults (BMI <16: severe malnutrition, BMI 16-17: moderate malnutrition, BMI 17-18: mild malnutrition, >18: no malnutrition). Statistical analysis was performed using the STATA^{TM} software. Comparison between cohorts were performed using one-way analysis of variance for continuous variables, Kruskal-Wallis test for discrete variables (hemoglobin count), and chi-square or Fisher's exact test for proportions. Results are presented with 95% confidence intervals where appropriate.

RESULTS

Demographic and clinical characteristics of the 210 patients treated with AmB are shown in Table 1. The presenting symptoms most frequently reported by the patients were abdominal

mass (96.2%), fever (92.8%), cough (68.9%), headache (39.7%), weight loss (31.6%), anorexia (21.5%), epistaxis (14.3%), weakness (9.1%), edema (5.3%) and vomiting (2.9%). The diagnosis of VL was based on the DAT (titre $\geq$ 1:25600) in 181 cases (86.2%), and on spleen aspirates in 29 patients (13.8%). Branded AmB was administered in 114 (54.3%) patients whereas 74 patients (35.2 %) were treated with generic AmB and 22 patients (10.5%) received the two products. Moreover, specific treatment of accompanying infections was given to 25 (11.9%) patients with malaria, 22 (10.5%) with respiratory tract infection and 21 (10.0%) with other bacterial infections such as typhoid fever, otitis media, pharyngitis, brucellosis or dysentery.

Outcome

Among the 210 patients treated with AmB, 194 were initially cured, including one slow-responder, resulting in an initial cure rate of 92.4% [95CI: 87.9 – 95.6]. Ten patients died during their stay in the hospital, resulting in a case-fatality rate of 4.8% [95CI: 2.4 – 8.8]. Causes of death consisted of bleeding (3 patients), infectious complications (2), severe anemia (1), cardiac failure (1), ileus (1), and unknown (2). Six patients relapsed, resulting in a treatment failure rate of 2.9% [95CI: 1.2 – 6.4]. The median hemoglobin count increased by 1.4 g/dl and spleen size showed a mean reduction of 5.3cm at the end of treatment compared to admission values. We observed no difference in case-fatality rates between patients treated with either branded AmB, generic AmB, or a combination of the two products (5.3%, 5.4% and 0.0%, respectively; $p>0.20$). We found a trend towards a higher treatment failure rate among patients who received the branded drug compared to those receiving only the generic drug (4.4 % vs. 0.0 %; $p=0.08$).

Tolerance

The mean total dose of AmB administered was 13.6 mg/kg, corresponding to a mean daily dose of 0.93 mg/kg. Within the whole cohort, adverse effects requiring interruption of treatment occurred in 4 cases (1.9%, 95CI: 0.5 – 4.8): vomiting (2 patients), itching (1 patient) and anaphylactic reaction (1 patient). All patients recovered. Recording of adverse effects on the pre-designed questionnaire was performed in 55 consecutive patients (26% of the cohort). The main reported adverse effects in this sub-group were fever (52.7%), sweating (40%), abdominal pain (38.9%), headache (37.0%), diarrhea (22.2%), itching (14.8%), and shivering (14.5%). All these adverse effects were considered as mild to moderate, and none required an interruption of treatment. The association of these adverse effects with the treatment was considered as probable in 54%, possible in 43%, and unknown in 3%. The intensity of adverse effects decreased during the course of the treatment. Only 29% of patients did not mention any of the listed adverse effects.

Comparison of patients treated with AmB or SbV

The demographic characteristics of the cohort of 210 patients treated with AmB were similar to the historical cohort of 161 patients treated with SbV (see Table 1). The clinical characteristics of the two cohorts were similar, with the exception that a higher proportion of patients in the SbV group presented with moderate to severe anemia upon admission (Hb count below 7 g/dl in 49.1% vs. 30.1% in the SbV and AmB group, respectively; $p<0.001$). We found no differences in either initial cure rates (92.4% vs. 95.0%; $p>0.20$), case-fatality rates (4.8% vs. 3.7%; $p>0.20$) or treatment failure rates (2.9% vs. 1.2%; $p>0.20$) between the two cohorts (Table 2). One patient did not respond to SbV and was switched to AmB with a good clinical response. We found a greater spleen size reduction in the SbV cohort (6.8 cm vs. 5.3 cm; $p<0.001$). The median increase of Hb count was also more significant in the SbV

cohort (2.4 g/dl vs. 1.4 g/dl; p=0.032) but the absolute Hb counts at the end of treatment were similar (9.7 g/dl in both cohorts; p=0.13). Adverse effects requiring interruption of treatment occurred in 2 patients treated with SbV (1.2%, 95CI: 0.1 – 4.4), both due to clinical pancreatitis, compared to 4 patients (1.9%, 95CI: 0.5 – 4.8) treated with AmB (p>0.20).

DISCUSSION

The effectiveness and safety profiles of amphotericin B deoxycholate were comparable to antimonials for the treatment of VL in Amudat, Uganda. The case-fatality rate found in our study (4.8%) was slightly higher than previously reported figures from India. However, the Indian trials were conducted using strict exclusion criteria such as any serious concurrent infection, neutropenia or severe anaemia, which led to the exclusion of the most severe cases, whereas our field evaluation included all treated patients (Sundar et al. 2004; Thakur & Narayan 2004). In Africa, similar or higher case-fatality rates were reported with other treatments (Moore et al. 2001;Ritmeijer et al. 2001;Ritmeijer et al. 2006;Veeken et al. 2000). The number of severe adverse effects requiring treatment interruption was low (1.9%). When searched actively, mild or moderate adverse-effect(s) were reported by most patients. Interestingly, the occurrence of adverse effects decreased after the first week of treatment.

The clinical outcome and the incidence of severe adverse effects were comparable between the cohort treated with AmB and the historical cohort of patients treated with SbV. The statistical comparison of these two cohorts has some limitations. Firstly, the two cohorts are consecutive and not concurrent. In the absence of randomization, discrepancies in the demographic or clinical characteristics of the patients might have remained unnoticed.

Secondly, the physicians who assessed the patients changed during the study period, leading to possible differences in the general management of the patients, the manual evaluation of the spleen size, or the reporting of adverse effects.

The low rate of treatment failure (2.9%) found in patients treated with AmB indicates that it can be considered as an effective drug for treatment of VL in Uganda. Treatment failures are unlikely to be due to acquired resistance of *Leishmania* parasites, as secondary resistance to AmB is believed to be an unlikely phenomenon in the absence of HIV co-infection (Bryceson 2001). There are some indications that AmBisome^{TM} needs to be given in higher dose regimen in Africa as compared to India (Berman et al. 1998). This might also be true with AmB although the low treatment failure rate found in our study does not support this hypothesis. We cannot exclude an underestimation of the treatment failure rate as patients were not actively followed-up after hospital discharge. We nevertheless believe that the number of missed relapses was small considering that Amudat Hospital was the only functional VL treatment center in the area during the study period. Although no difference in treatment failure rates were found between the two groups, the reduction of spleen size and the increase of hemoglobin count during treatment were more marked in the SbV group. The latter finding should be interpreted with caution considering that the two cohorts differed in Hb admission values.

The relatively good tolerance of AmB in our cohort is likely to be related to the long infusion time, the maintenance of adequate hydration and the supplementation of electrolytes. There is some evidence that a slower infusion flow (4 hours vs. 45 minutes) reduces the occurrence of infusion-related adverse effects (Ellis et al. 1992). The administration of AmB by continuous infusion over 24 hours appears to reduce nephrotoxicity (Eriksson et al. 2001), but this

approach was not feasible in our hospital. As it was not possible to monitor renal function, we administered the drug on alternate days, although there is no clear evidence that this reduces the risk of nephrotoxicity (Thakur et al. 1994). The toxicity of AmB appears to vary with the patient population, the underlying clinical condition, the use of concomitant nephrotoxic drugs, and the mode of administration (Girmenia et al. 2001;Mayer et al. 1999). Among VL patients, acute renal failure appears to be rare (Thakur et al. 1999), although transient increases in creatinine levels are observed in up to 20% of the patients (Thakur et al. 1994). AmB should be considered as a relatively safe drug in VL patients, provided that proper attention is given to the practical aspects of drug administration, including a permanent presence of nursing staff to monitor infusion flow. This implies an increased workload for the medical and nursing team compared to antimonials that require only one intramuscular injection daily. Moreover, the use of AmB is logistically more demanding, as it requires a cold chain and significant capacities for the transport and storage of intravenous fluids.

In conclusion, conventional AmB is an effective and reasonably safe drug for the treatment of VL in Uganda. Liposomal AmB would be a better tolerated and more practical option that would also shorten the duration of hospital stay (Bern et al. 2006). A recent study conducted in Ethiopia showed that miltefosine could be an interesting therapeutic alternative for VL in East Africa (Ritmeijer et al. 2006). Moreover, clinical trials coordinated by the Drugs for Neglected Diseases initiative are currently evaluating paromomycin in several East African countries. The number of treatment options for VL, preferably using drugs in combination (Bryceson 2001), is thus likely to increase in the coming years. In the mean time, the main priority in sub-Saharan Africa, where resistance to antimonials is still a negligible problem, is to ensure an affordable and sustainable access to antimonials for the neglected populations suffering from VL (Berman et al. 1998;Yamey & Torreele 2002).

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Table 1: Demographic and clinical characteristics of patients with visceral leishmaniasis treated with amphotericin B deoxycholate (AmB) or meglumine antimoniate (SbV) in Amudat Hospital, Uganda

	AmB (N=210)		SbV (N=161)		p-value
	n	(%)	n	(%)	
Age (in years)					>0.20[b]
- 0 to 5	42	(20.0)	32	(19.9)	
- 5 to 15	101	(48.1)	71	(44.1)	
- over 15	67	(31.9)	58	(36.0)	
Gender					>0.20[b]
- Males	154	(73.3)	116	(72.0)	
- Females	56	(26.7)	45	(28.0)	
Origin					>0.20[b]
- Uganda	59	(28.1)	44	(27.3)	
- Kenya	151	(71.9)	117	(72.7)	
Median duration of illness, in weeks (IQR)	4 (4;12)		7.7 (4;8)		>0.20[c]
Nutritional status[a]					>0.20[b]
- normal or mild malnutrition	92	(44.7)	69	(43.7)	

- moderate malnutrition	61	(29.6)	38	(24.0)	
- severe malnutrition	53	(25.7)	51	(32.3)	
Mean spleen size upon admission, in cm (SD)	13.7 (4.2)		13.3 (4.1)		>0.20[c]
Anemia upon admission					<0.001[d]
- Severe (Hb <5g/dl)	3	(1.4)	4	(2.5)	
- Moderate (Hb 5-7 g/dl)	60	(28.7)	75	(46.6)	
- Mild (Hb 7-11 g/dl)	139	(66.5)	82	(50.9)	
- No anemia (Hb >11 g/dl)	7	(3.3)	0	(0.0)	

NOTE: IQR: Interquartile range, SD: Standard deviation

[a] malnutrition defined for children under 137 cm (females) or 145 cm (males) as weight-for-height: 80-89%: mild; 70-79%: moderate; <70%: severe malnutrition. Otherwise BMI 17-18: mild; BMI 16-17: moderate; BMI <16: severe malnutrition.

p-value from chi-square test[b], one-way analysis of variance[c], or Fisher's exact test[d]

Table 2: Clinical outcomes of patients with visceral leishmaniasis treated with amphotericin B deoxycholate or meglumine antimoniate in Amudat Hospital, Uganda

	Amphotericin B (N=210)			Meglumine antimoniate (N=161)			
	n	%	[95% CI]	n	%	[95 % CI]	p-value
Initial cure	194	92.4	[87.9 – 95.6]	153	95.0	[90.4 – 97.8]	> 0.20 [a]
Deaths	10	4.8	[2.4 – 8.8]	6	3.7	[1.5 – 8.3]	> 0.20 [a]
Treatment failures	6	2.9	[1.2 – 6.4]	2	1.2	[0.2 – 4.9]	> 0.20 [b]

NOTE: p-value from chi-square[a] or 2-sided Fisher's exact test[b]

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