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A 6 day course of liposomal amphotericin B in the treatment of infantile visceral leishmaniasis: the Italian experience

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***Objectives*: To evaluate in a retrospective analysis the efficacy and safety of a 6 day course of liposomal amphotericin B (L-AmB) in infantile cases of Mediterranean visceral leishmaniasis (VL) diagnosed over a 10 year period in Italy.**

***Patients and methods*: Patients included were diagnosed as having VL consecutively admitted from December 1992 to December 2001 at four main referral children's hospitals in Italy and treated with six intravenous doses of 3 mg/kg L-AmB given on days 1–5 and 10 (a total dose of 18 mg/kg). Demographic data, nutritional status, underlying diseases, clinical and laboratory findings, and therapy outcome were considered.**

***Results*: A total of 164 HIV-negative children (median age 1.6 years; range 4 months to 14 years) were enrolled. All patients were initially cured by the given treatment, and did not present adverse events related to drug infusion. Seven patients (4.3%) had a clinical and parasitological relapse 3–15 months after therapy. All relapses were successfully retreated with 3 mg/kg L-AmB for 10 consecutive days (a total dose of 30 mg/kg).**

***Conclusions*: This study highlights the efficacy (>95%) and safety of the six dose L-AmB regimen and validates it as a first-line treatment for Mediterranean VL in children.**

Keywords: *Leishmania infantum*, Italy, therapy

Introduction

Visceral leishmaniasis (VL) is endemic in all the countries bordering the Mediterranean basin, where it is caused by the protozoan *Leishmania infantum* and is transmitted by the bite of phlebotomine sandflies. Dogs are the proven reservoir of infection.[1]

After their introduction in the therapy of VL in the early part of the 20th century,[2] pentavalent antimonial drugs have been considered the standard treatment for VL for more than 60 years.[3] They have been used extensively in children and have been demonstrated to be generally safe and effective.[4,5] However, during the last decade, the emergence in certain geographical areas of *Leishmania* strains resistant to pentavalent antimonials, coupled with some drug toxicity and prolonged administration, has prompted the evaluation of alternative drugs, including lipid formulations of amphotericin B.[3]

Liposomal amphotericin B (L-AmB) is the only drug approved by the US Food and Drug Administration for the treatment of any VL (i.e. caused by *Leishmania donovani*,

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L. infantum or *Leishmania chagasi*) in immunocompetent patients, with a regimen of seven infusions of 3 mg/kg given on days 1–5, 14 and 21 (total dose 21 mg/kg).[6] Results from a dose-decreasing study have a shown that a short course of L-AmB (six infusions of 3 mg/kg given on days 1–5 and 10; total dose 18 mg/kg) could be safe and effective in treating children with Mediterranean VL.[7]

We report here our 10 year experience with the use of this short course of L-AmB for the treatment of paediatric VL in Italy.

Patients and methods

Four paediatric centres participated to the study. (i) The G. Di Cristina Hospital in Palermo, which is the largest children's hospital in Sicily. It is a tertiary-care university hospital with 350 beds, and acts as referral paediatric centre for western Sicily. (ii) The Santobono-Pausilipon Hospital for Children's Diseases is a high speciality structure, and represents an outstanding centre for paediatric emergencies and many branches of paediatric pathology for the southern Italian population. (iii) The Giannina Gaslini Institute for the Cure, Protection and Care of Infants and Children is classified as public Research Institute and Children's Hospital, and acts as referral paediatric centre for northern Italy. (iv) Policlinico Umberto I, University 'La Sapienza' of Rome, acts as referral paediatric centre for several areas of central Italy.

Children aged 0–14 years consecutively diagnosed as having VL and treated with six infusions of 3 mg/kg L-AmB given on days 1–5 and 10, during the period December 1992 to December 2001 were evaluated. The patient's clinical histories were stored in a database that included demographic characteristics, clinical and laboratory findings recorded on admission and during hospitalizations, therapeutic interventions and clinical outcome.

Diagnosis of VL was established if patients had at least one of the following criteria: (i) presence of clinical signs and symptoms compatible with VL (fever, splenomegaly, pancytopenia, hypergammaglobulinaemia) and detection of serum *Leishmania* antibodies by immunofluorescent antibody test (IFAT) at a titre ≥1:40; and/or (ii) demonstration of *Leishmania* parasites in bone marrow or spleen aspirates by microscopy and/or culture.

Fever was defined as a temperature ≥38.0°C; anaemia was defined as a haemoglobin value of <9.0 mg/dL; leucopenia was defined as <4000 white blood cells/μL; and thrombocytopenia as <150 000 platelets/μL.

IFAT was performed using an in-house manufactured antigen preparation (from the WHO reference strain of *L. infantum*) or a commercial kit (*Leishmania*-Spot IF; bioMérieux, Marcy l'Étoile, France). Cultures were performed by seeding bone marrow aspirates into blood agar media (Evans' modified Tobie's medium and/or 'sloppy' Evans' medium). Established positive cultures were characterized by the electrophoretic analysis of 15 enzymatic loci, using the techniques and zymodeme nomenclature of the WHO Collaborating Centre of Montpellier, France.[8]

Clinical response was assessed at the completion of treatment on day 10, and was either initial cure (defined as defervescence, restoration of laboratory parameters and reduction of spleen size), or failure, which was defined as persistent or worsening clinical and laboratory findings. A VL relapse was defined as the recurrence of signs and symptoms of disease associated with demonstration of *Leishmania* in bone marrow after initial successful treatment. All the patients were followed at the hospital's outpatient clinic for at least 6 months after treatment was completed.

The *t*-test for dependent samples was computed to evaluate the differences in clinical and laboratory data before (day 0) and at the completion of (day 10) treatment. A two-tailed level of P value ≤0.05 was used for all analyses.

Results

In the 10 year period considered, 164 children met the inclusion criteria. Thirty-seven (22.6%) had been enrolled in previous clinical trials on L-AmB and have already been described.[7,9,10] Seven patients (4.2%) had been previously treated for VL with meglumine antimoniate, and had a relapse. Three patients (1.8%) who had started on antimonial treatment were shifted onto L-AmB because of poor response (two cases) or toxicity (one case). In the remaining 117 patients (71.4%), L-AmB was used as first-line treatment on the basis of the ongoing good results obtained in the aforementioned studies on this drug.

The median age of patients was 1.6 years (range 4 months to 14 years); 42 patients (25.6%) were aged <12 months. Ninety-one (54.1%) were males. HIV serology was negative in all cases.

At admission, analysis of the nutritional status of the patients showed that 27 (16.5%) were below the tenth percentile on the weight-for-age growth chart. Twelve (7.3%) had underlying conditions at hospital admission: one was affected by tetraparesis, one by pseudomembranous conjunctivitis and 10 by thalassaemic trait. Concurrent bacterial or viral infections were diagnosed in 10 patients (6.1%): seven had respiratory infections (two bronchitis and five pneumonia), two rotavirus gastroenteritis and one chickenpox.

The median time from the onset of symptoms to hospital admission was 15 days (range 4–90 days). At admission, fever and splenomegaly were present in all patients. Anaemia and thrombocytopenia were frequently observed (77.5% and 67% of patients, respectively); leucopenia was present in 27.8% of cases, and a neutrophils count of <500/μL was found in 10.3% of the patients. Pancytopenia was present in 19.6%, and an association of leucopenia and thrombocytopenia without anaemia in 4.8% of cases. Albumin value was <3 g/dL in 24.2% of cases, and 49.4% of patients showed inversion of A/G ratio. A value for aspartate aminotransferase and alanine aminotransferase >80 IU/L was reported in 8.4% and 7.2% of cases, respectively. An erythrocyte sedimentation rate >40 mm/h was found in 93.5% of patients (Table 1).

A bone marrow aspirate was obtained in all patients, and *Leishmania* amastigotes were detected in 160 cases (97.6%). Serology was performed and found to be positive in all of them, with a geometric mean titre of anti-*Leishmania* antibodies of 661 (range 80–10 240). Hence, laboratory diagnosis of VL was established by bone marrow microscopy in association with positive serology in 160 patients and by means of serology only in the remaining four patients (2.4%).

Culture from bone marrow aspirate was performed in 112 patients and found to be positive in 96 (85.7%). Zymodeme analysis was carried out on 87 established isolates; 56 were characterized as *L. infantum* zymodeme MON 1 (the most common genotype in the Mediterranean area), 30 as *L. infantum* MON 72

Table 1. Clinical, haematological and biochemical features of 164 children with VL before (day 0) and at the completion of (day 10) treatment with L-AmB

Variables	Day 0[a]	Day 10[a]	% Difference[b]
Weight (kg)	14.1 ± 8.4 (5.9–71.5)	14.5 ± 8.7 (6–76)	↑ 2 ± 4.6
Spleen size (cm from the left costal margin)	6 ± 2.4 (2–14)	3.5 ± 2 (0–10)	↓ 40 ± 24.5
Red blood cells ($\times 10^6$ cells/μL)	3.7 ± 0.5 (1.9–4.9)	4.2 ± 0.4 (2.2–5.4)	↑ 13.8 ± 1.1
Haemoglobin (g/dL)	7.9 ± 1.4 (3.8–12)	9.5 ± 1.1 (5.7–14)	↑ 16 ± 0.9
White blood cells ($\times 10^3$ cells/μL)	5.3 ± 2.3 (1.8–14.7)	8.1 ± 2.7 (2.4–18.7)	↑ 30.6 ± 1.9
Neutrophils ($\times 10^3$ cells/μL)	1.2 ± 6.8 (0.2–5.4)	2 ± 1.4 (0.2–7.8)	↑ 45 ± 55
Platelets ($\times 10^3$ cells/μL)	119.8 ± 50 (18–311)	277.8 ± 101 (38–565)	↑ 53.2 ± 23.6
Erythrocyte sedimentation rate (mm/h)	82.1 ± 27.4 (24–138)	44.2 ± 24.1 (7–138)	↓ 43.2 ± 32
C-reactive protein (mg/100 mL)	12.9 ± 15.8 (0.7–76)	3.2 ± 9.6 (0–44)	↓ 89.9 ± 12
Albumin (g/dL)	3.1 ± 0.5 (1.4–5.5)	3.5 ± 4.2 (2.4–4.9)	↑ 11.5 ± 1.9

[a]Values are presented as means ± S.D. (range).
[b]The direction of the arrow indicates whether the day 10 values were increased or decreased compared with the day 0 values. Values are the means ± S.D. of the percentage differences between day 10 and day 0 values (computed using the paired data for each patient).

(the typical genotype of the Naples area) and one as *L. infantum* MON 80.[11]

All 164 patients had initial cure with L-AmB treatment, with no relevant adverse effects. Anaemic condition required blood transfusion in 18 (11.0%). Defervescence occurred after a mean of 43.8 ± 13 h (range 12–76 h) from the first L-AmB infusion.

Table 1 shows changes from baseline clinical and laboratory results to those obtained at the end of treatment. Increase of red blood cells, haemoglobin, neutrophils, platelets and albumin, and reduction of spleen size, erythrocyte sedimentation rate and C-reactive protein, were statistically significant on day 10 ($P<0.0001$). The duration of hospitalization was 8 ± 3 days (range 5–27 days), with most of the cases being treated on day 10 as outpatients.

Patients were followed at the outpatient clinic for a median of 1 year (range 6 months to 3 years) after treatment was completed. Seven patients (4.3%) had a VL relapse at 3, 4, 5, 5.5, 6, 13 and 15 months, respectively; hence, the definitive cure rate was 95.7%. All the relapsing patients were successfully re-treated with L-AmB administered intravenously at a dosage of 3 mg/kg for 10 consecutive days (total dose 30 mg/kg).

Discussion

Pentavalent antimony compounds (sodium stibogluconate and meglumine antimoniate) have been considered the standard antileishmanial treatment for >60 years, and, notwithstanding the emergence of a high level of resistance in some parts of India (especially in North Bihar), they are still the first-line drugs in the other VL foci in the Indian subcontinent;[12,13] however, antimonials need a long period of hospitalization (3–4 weeks) and are characterized by non-negligible adverse effects. In 1997, the US Food and Drug Administration approved L-AmB for the treatment of VL, with a regimen consisting of seven doses of 3 mg/kg (total dose 21 mg/kg).[6] In the same year, di Martino *et al.*[7] showed that in immunocompetent children with VL caused by *L. infantum*, a lower total dose of L-AmB (i.e. 18 mg/kg given in six doses over 10 days) had a high rate of response (95.8%), and proposed this as the treatment schedule of choice.

In this uncontrolled, retrospective, multicentre study, we confirm and extend previous results regarding the efficacy and safety of that 6 day schedule of L-AmB in a much larger cohort of paediatric patients recruited in Palermo, Naples, Genoa and Rome. Our findings indicate that a total dose of 18 mg/kg of L-AmB induces a cure rate of 95.7% for Mediterranean VL in children, and is at least as effective as conventional antimonial therapy. In fact, as reported in a recent large retrospective study, the antimonial cure rate in Italy was found to range from 90% to 95%.[14] Moreover, several studies have shown that time of defervescence, reduction in spleen size and correction of haematological parameters occurs more quickly among patients (adults and children) treated with L-AmB in comparison with those treated with meglumine antimoniate.[15,16] Increase of red blood cells and haemoglobin are an earlier index of response to treatment, and reinforce the attitude of waiting before carrying out transfusion.

The main objection to the use of L-AmB for the treatment of VL in countries where resistance to antimonials has been rarely reported is the high cost of the drug. In Italy, the wholesale price of a 50 mg vial of L-AmB is €151, whereas that of a vial of meglumine antimoniate containing 425 mg of antimonium is almost negligible (about €0.7). In a 15 kg patient (the median weight of our patients), the cost of the L-AmB regimen is about €900; however, the high cost of the treatment is counterbalanced by the reduction of the hospitalization period: assuming an average cost of €250 per day of hospital stay in Italy, the treatment with L-AmB is more affordable than the 21 day hospitalization period needed using antimonial therapy (€3031 versus €5265).

Recently, in Greece, a 2 day regimen of L-AmB (10 mg/kg/day) was compared with a 5 day regimen of L-AmB (4 mg/kg/day) and with a 30 day regimen of meglumine antimoniate, in an open, prospective study on infantile Mediterranean VL. No differences in the response rates among the comparison groups were observed, but the pharmacoeconomic analysis showed a lower cost for patients treated with two doses of L-AmB (€1664) compared with those treated with five doses of L-AmB (€2284) or 30 doses of meglumine (20 mg/kg/day) (€2600).[16] However, it should be noted that this high dose given in a 2 days regimen is not currently approved for this condition,

and needs further demonstration of its good tolerability in children. In India, a study conducted on 91 patients affected by VL and employing a total dose of 5 mg/kg of L-AmB (administered as a single infusion or as a 5 day infusion of 1 mg/kg daily) showed a 92% cure rate, with a cost of US$519;[17] however, for developing countries this cost, despite being more affordable, is still considered too high. We are aware of only a single case-report of Mediterranean VL treated with a single dose of L-AmB.[18] It should be remembered that regional differences in response to treatment have been observed, probably related to the infecting *Leishmania* strain; in fact, Brazilian infection caused by *L. chagasi* seems to be less responsive to L-AmB than Indian Kala-azar (caused by *L. donovani*).[19]

In conclusion, our study confirms the safety and efficacy of a total dose of 18 mg/kg for the treatment of Mediterranean VL in children, but the same efficacy for VL acquired outside the Mediterranean basin should not be inferred. Furthermore, in developed countries, for the paediatric population, the reduction of length of hospitalization seems to be cost-effective.

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