Early efficacy of liposomal amphotericin B in the treatment of visceral leishmaniasis

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Abstract

The rapidity and efficacy of a short course of liposomal amphotericin B was evaluated in 29 children affected by visceral leishmaniasis (Leishmania infantum). Their overall health status was assessed using the prognostic inflammatory and nutritional index (PINI), and their haematological status by the reticuloocyte count and haemoglobin blood levels. All these quantities were measured on day 0, and 3 and 10 d after starting therapy. A significant decrease of inflammatory signs, associated with an improved reticuloocyte count, was recorded after 3 d of therapy. A significant increase of haemoglobin levels was also observed 10 d after the start of treatment. The early reduction of inflammatory signs and the improvement of bone marrow function in most patients confirmed the validity of amphotericin B therapy. The PINI score is helpful in assessing the severity of visceral leishmaniasis and the follow-up of its treatment.

Keywords: leishmaniasis, Leishmania infantum, chemotherapy, liposomal amphotericin B, PINI score

Introduction

Visceral leishmaniasis (VL), caused by Leishmania infantum, is endemic in Italy; it is frequently seen in immunocompetent children, especially those below 4 years of age (Lemna & Kent, 1989). The clinical and laboratory signs are those of systemic inflammation and bone marrow suppression, with detection of Leishmania in bone marrow, spleen or lymph node aspirate. A total dose of 18 mg/kg of liposomal amphotericin B administered over 6 d has been recently shown to be effective in the treatment of VL (Davidson et al., in press), and this drug is now included by the World Health Organization (WHO) among the recommended therapies for VL (Gradoni et al., 1995). However, one of the major questions about this treatment concerns the efficacy of a short course, even with a drug that is effective and with such favourable pharmacokinetics, as liposomal amphotericin B (Davidson et al., 1994; Gradoni et al., 1995). During a multicentre, international, phase 2 trial evaluating the efficacy of this drug for treatment of VL (Davidson et al., in press), we conducted an ancillary study in a small subgroup of children admitted to Italian hospitals. The aim of the study was to verify whether it is possible to cure the signs of bone marrow depression and the laboratory signs of inflammation after minimal dosage of liposomal amphotericin B, i.e. to assess the efficiency and rapidity of response to this therapy.

To the best of our knowledge, this is the first study dealing with this particular aspect of therapy, since until recently antimony derivatives were the sole therapeutic agents for VL and there is no report of the speed of response normally expected in VL treated conventionally.

Patients and Methods

Twenty-nine immunocompetent children (15 males and 14 females), aged 10-177 months (median 25 months), weighing 9.5-47.2 kg (median 12 kg), and affected by VL, were enrolled in a phase 2 trial to evaluate the efficacy of liposomal amphotericin B in the treatment of this parasitic disease. The study lasted from 1 May 1993 to 31 May 1994. A total dosage of 15 mg/kg of liposomal amphotericin B was administered to the first 10 patients (group 1). Among them 3 relapses were observed within the first 3 months following treatment, necessitating resumption of therapy, which was successful using a higher dosage of 3 mg/kg daily for 10 d. The next 10 patients, newly diagnosed as VL, were treated according to another protocol with a total dose of 18 mg/kg of liposomal amphotericin B (group 2).

Haematology and inflammatory signs were evaluated before treatment (day 0), at day 4, after 3 doses of liposomal amphotericin B, and at day 10, after 4 doses in group 1 and 5 doses in group 2. The haematological status was determined by the haemoglobin (Hb) levels and reticuloocyte count (RC). For evaluation of the inflammatory response, we used the prognostic inflammatory and nutritional index (PINI) score, calculated from a combination of sensitive indicators of inflammation (C-reactive protein and α1-acid glycoprotein) and of protein nutritional status (albumin and thyroxine-binding prealbumin) according to the following equation:

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\text{PINI score} = \frac{\text{C-reactive protein (mg/L)} \times \text{α1-acid glycoprotein (mg/L)}}{\text{albumin (g/L)} \times \text{thyroxine-binding prealbumin (mg/L)}}
\]

The PINI formula has a discriminatory power greater than that of its 4 components considered individually (Ingbreenleek & Carpenter, 1985; Pressac et al., 1990). The PINI score allows stratification of patients with inflammation, including those with infections and cancer, into different prognostic groups: PINI scores > 30 indicate a prognosis of high risk; 11–30 indicate medium risk; 1–10 indicate low risk; and values < 1 indicate 'not infected' (Ingbreenleek & Carpenter, 1985; Kickrakona et al., 1990; Pressac et al., 1990; Veer et al., 1991). Changes of the PINI score with time, rather than a single value, are considered the best indicators of the clinical course of the patient (Ingbreenleek & Carpenter, 1985; Pressac et al., 1990). Because of the possible influence on the PINI score of impaired nutritional status, this was assessed in all patients before the start of treatment by means of the computer program for anthropometric calculations Anthrol (Centers for Disease Control, Atlanta, Georgia, USA), to detect acute or chronic malnutrition, defined according to international standard criteria (Rickett et al., 1986; Gibson, 1990). Serum samples were taken on the prescribed day, frozen and sent to the First Laboratory of Clinical Analysis, G. Gaslini Children's Hospital, Genoa, Italy, where the analyses were performed in a single session, using the Array 360 System® (Beckman, Galway, Ireland) with 300 µL of plasma.

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Statistical analysis used the paired or unpaired *t* test, as appropriate. Statistical significance was defined as *P* ≤ 0.05.

**Results**

*Hematology*

All 29 patients presented anaemia at the time of diagnosis with a mean Hb value of 7.8 g/dL (95% confidence interval [CI] 7.3–8.3). Hb and RC on days 0, 4 and 10 were available for only 21 children. In these patients, the mean Hb value at day 0 was 7.9 g/dL (95% CI 7.3–8.5). At day 4 the mean Hb value was 8.5 g/dL (95% CI 8.0–9.0) (*P* = 0.046), and at day 10 it was 9.7 g/dL (95% CI 9.1–10.4) (*P* < 0.001). The mean RC was 18/10^3 at day 0 (95% CI 12–24), 24/10^3 at day 4 (95% CI 16–32) (*P* = 0.026), and 18/10^3 (95% CI 14–23) at day 10 (*P* = 0.86).

**PIN1 score**

The PIN1 score was calculated for all 29 patients at the time of diagnosis, with a mean value of 67 (median 24, range 1–633). Data for days 0, 4 and 10 were available for 21 children; their mean PIN1 score was 48 (95% CI 10–86) on day 0, 10 (95% CI 2–20) on day 4 (*P* = 0.035) and 0 (95% CI 0–1) on day 10 (*P* = 0.043). At the time of diagnosis of VL, malnutrition was detected in 2 patients only (1 acute and 1 chronic). The abnormalities of PIN1 score in our patients were therefore mainly attributable to the inflammatory process induced by leishmaniasis.

In patients who had a relapse of VL after treatment with the lower dose of liposomal amphotericin B, the time course of the PIN1 score was similar in the first and in the second episodes, with high values at time of diagnosis and a return to normal after treatment.

There was no statistically significant difference in the mean values of inflammatory signs and haematological values between the 2 patient groups (total doses of 15 mg/kg or 18 mg/kg of liposomal amphotericin B) at days 0, 4, or 10 (data not shown).

The PIN1 score and RC were both available for 14 patients. The time course of their changes was similar to those observed in the whole group (data not shown).

**Discussion**

VL is characterized by the presence of fever and signs of inflammation, hypergamma globulinemia, and anaemia secondary to bone marrow infiltration by Leishmania. Liposomal amphotericin B is now included by the WHO among the recommended therapies for this disease (Gradoni et al., 1995). We examined the speed of response in children receiving liposomal amphotericin B for treatment of VL, assessing bone marrow function and inflammatory response within a few days from the start of treatment. At day 4 (after 3 doses of therapy) we observed recovery of bone marrow function, indicated by a significant increase in mean RC. This was associated with a significant decrease in the inflammatory response. The mean baseline PIN1 score indicated moderate to severe inflammatory status (mean = 48) but it rapidly decreased to low risk status (mean = 10).

At day 10 (after 4 doses of therapy in group 1 and 5 doses in group 2) there was a significant increase in the mean Hb value, associated with a return to normal of the inflammatory signs. These changes were similar in patients receiving either 15 or 18 mg/kg of liposomal amphotericin B. Unfortunately, this fast early response did not preclude the possibility of relapse, suggesting that other, probably cellular, mechanisms control relapse (De Rosset et al., 1992).

In conclusion, we have demonstrated that bone marrow normally recovers and inflammatory markers subside very early after the beginning of treatment with liposomal amphotericin B in immunocompetent children affected by VL, supporting the usefulness of this drug for its treatment. The PIN1 scoring system appeared to be an appropriate laboratory tool for assessing the severity of VL and the follow-up of treatment.

**References**


