SUCCESSFUL TREATMENT WITH AMPHOTERICIN B OF PATIENTS WITH VISCERAL LEISHMANIASIS RESISTANT TO MEGLUMINE ANTIMONATE

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Abstract- Visceral leishmaniasis (VL) is usually treated with pentavalent antimonial drugs given alone or in combination with other drugs. The efficacy of these treatments is diminishing, however, and development of alternative treatments has been recommended.We studied 13 patients with leishmaniasis who were unresponsive to meglumine antimonate, at a dose of 20 mg/kg body weight/per day given for 30 days. These patients were treated with daily regimen of amphotericin B, starting with an escalating dose of 0.1mg/kg body weight per day until a day dose of 1.5 mg/kg was reached for a period of 4 weeks. All 13 patients using the treatment regimen were cured; no patient relapsed in one year of follow-up. Treatment of visceral leishmaniasis (kala-azar) with the daily regimen of amphotericin B at a dose of 1.5 mg/kg body weight was effective.

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Key words: Visceral leishmaniasis, meglumine antimonate, amphotericin B

INTRODUCTION

Leishmanial infections are caused by a number of species of the protozoan *leishmania* and include three major clinical syndromes: cutaneous, mucosal, and visceral leishmaniasis (VL). In VL, the infection is widespread through the reticuloendothelial system and is manifested as a chronic febrile illness, with marked hepatosplenomegaly, pancytopenia, and hyperglobulinemia. VL is estimated to affect 400000 people yearly and children are considered to be at greater risk of infection (1). Although leishmanial infection is generally considered as a disease typical of tropical and subtropical developing countries, it is also endemic in areas of many developed countries such as France, Italy, and the United States (1).

VL is usually treated with pentavalent antimonial agents given alone or in combination with interferon-γ or allopurinol (2). The efficacy of these treatments is diminishing, however, because of increasing drug resistance. Disease relapse after these conventional treatments have been documented (1). Also problematic are toxic effects of pentavalent antimonials, which frequently include hepatotoxicity, cardiotoxcity and rash. Available alternatives to antimonials include pentamidine, lipid associated amphotericin B, and amphotericin B (3). Pentamidine has substantial toxic effects and must be given in prolonged courses.Lipid associated amphotericin B is an effective drug with low toxicity but unfortunately it is not avaliable in Iran and is expensive. Amphotericin B has been used successfully in the treatment of antimony-unresponsive patients. Compared with only 88% of cases cured with meglumine antimonite, the high cure rate achieved with amphotericin B resulted in its use to treat large numbers of multidrug-resistant VL (kala-azar) patients (4,5). Our experience with this drug is reported in this article.

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MATERIALS AND METHODS

During a 15 year period, March 1986 to February 2000, 123 patients with VL were treated at the Children's Medical Center. The criterion for admission to this study was that patient should have persistent clinical and parasitological disease at the end of a course of treatment with meglumine antimonate given in a dose of at least 20 mg elemental meglumine antimonite per kg body weight daily for

30 days. Seven patients met this criterion fully on admission. The other six had relapsed for the fourth, third or second time after receiving 20 mg per kg/body weight meglumine antimonate.These patients (8 boys, 5 girls) were unresponsive to antimony confirmed by the demonstration of Leishman bodies in aspirates of bone marrow or IFA (indirect immunofluoresene antibody test). Titres more than 1/160 was considered positive.

Certain terms used in this paper are defined as follows: Primary unresponsiveness was defined as no clinical or parasitological improvement at the end of the first defined course of treatment. Relapse means reappearance of parasitologically confirmed disease after initial cure; relapse is not distinguished from reinfection (6).

Initial assessments

All patients underwent a thorough physical examination. Spleen size was measured in centimeters from the costal margin to the tip in the anterior axillary line, while liver size was measured in centimeters in the mid-clavicular line from the costal margin to its edge. Body weight, total white cell count, haemoglobin concentration, serum bilirubin, alanine and aspartate transaminase, creatinine and potassium levels were measured, electrocardiograms and chest X-rays recorded, and urine analysis were carried out.

Treatment regimen

The patients received increasing doses of amphotericin B as follows: 0.1mg per kg body weight on the first day, 0.2mg/kg on the second, 0.3 mg/kg on the third, 0.5 mg/kg on the fourth, 0.75 mg/kg on the fifth and 1 mg/kg on the sixth day. Subsequently 1.5 mg/kg daily was administered, for a period 4 weeks. Amphotericin B is dispensed as a dry powder. It was reconstituted by adding the appropriate amount to 10 ml of sterile water, diluting this solution with 500 ml of 5% glucose solution, and then infusing patients intravenously over 6 hours.

Adverse reactions were monitored closely every day. Patients were asked about the tolerability of the drug.

Post-trial assessment

After the trial was over, the patients body weights were measured and all the initial assessments were repeated. Patients were followed up monthly for one year and were asked to report any fever or infection during this period.

Outcome

Patients who did not respond after 20 infusions of amphotericin B were taken to be unresponsive to the drug.Clinical cure was taken to have occurred if the general condition of the patient improved, fever subsided, and splenic size regressed. Ultimate cure was defined as no clinical or parasitological relapse during one year of follow-up.

RESULTS

Age, weight, duration of illness, splenomegaly, hepatomegaly, anemia, leukopenia and other characteristics of patients are shown in Table 1. Severe reactions that necessitated the use of acetoaminophen occurred in one patient (Table 2). Fever alone anytime after infusion occurred in two patients. The reactions occurred mostly during the first week of the study. A total of three patients had elevated creatinine levels, the level did not exceed 3 mg/dl in these patients. The treatment was stopped for four days, their creatinine values returned to the normal range, and the treatment was restarted with the respective regimens.

Table 1. Initial characteristics of the visceral leishmaniasis patients

Initial characteristic (n=13)	Mean±SD
Age (years)	2.10±2
Weight (kg)	9±2
Duration of illness (months)	3.5±1.5
Spleen size (cm)	9±0.51
Liver size (cm)	3.9±1.9
Haemoglobin (g/dl)	7±0.25
Leucocyte count ($\times 10^6$ /L)	5.4±0.20
Platelet count ($\times 10^9$ /L)	108±8.30

Table 2. Treatment outcomes(n=13)

Characteristic	n (%)
Severe reactions	1 (8%)
Fever alone	2 (15%)
Raised creatinine values	3 (23%)
Fall in serum potassium level	6 (46%)
Diminution of appetite	3 (23%)
Thrombophlebitis	1 (8%)
Weight (kg)	10.5±1*
WBC count after Treatment ($\times 10^6$ /L)	8.2±0.37*
Haemoglobin Concentration (g/dl)	9.6±1.2*

*Mean ± Standard deviation

These patients exhibited normal creatinine levels, as did all the other patients, 2 weeks after completion of the treatment. Serum potassium fell below normal levels in six patients. No patients exhibited any evidence of abnormal renal function at the end of one year of follow up. Thrombophlebitis occurred in one patient that got improved with treatment. Diminution of appetite occurred in two patients but one week after the end of treatment it became normal. The mean duration of fever was nine days. There was regression in the size of the spleen of patients. Six had impalpable spleens, six had spleens that were just palpable, and one had spleen >2cm.The mean gain in weight was 1.5kg.

No patients relapsed by the end of one year of follow-up.Children tolerated the drug well, and no patient had to discontinue treatment because of intolerance.

DISCUSSION

The development of effective treatment strategies in VL has been recommended, following the observation of increasing leishmanial infection world wide. Increasing cases of leishmaniasis, are either imported or acquired in many developed countries (1). For instance, in France and in Italy new endemic areas were identified, and in the United States several foci of endemic leishmaniasis have been reported (1). Several concurrent factors have been proposed to explain the increased number of leishmanial infection in these countries, including the increasing frequency of international travel by the local citizens as well as the influx of immigrants. An additional risk factor to be considered in the spread of VL in developed countries is the typical mode of transmission of this infection. It is important to note that VL is zoonotic in most areas, and dogs and other carnivores are the commonest reservoirs. Therefore defective epidemiologic strategies and lack of appropriate treatments for animals in many developed countries or in old world (1) may contribute as an additional risk for the population exposed, in particular for those groups most susceptible to the infection such as young children.

This study demonstrated amphotericin B to be effective for treatment of VL. Amphotericin B (AMB) was approved for clinical use in the mid-1950s and quickly became the mainstay of antifungal therapy (7). It remains today the gold standard in the eyes of many clinicians and is the drug with which all newly developed agents inevitably are compared for efficacy, safety, and spectrum of activity. Precise mechanism of action of amphotericin B still is not resolved completely. Most investigators have shown the prominence of ergostane sterols. *Leishmania* is remarkably similar to that for fungi such as candida, whose major demethylated sterol is ergosterol. The prescence of fungus like sterols as the primary leishmanial demethylated sterols provide a rationale for the antileishmanial activity of amphotericin B. Ampholericin B increases membrane ergosterol content. Amphotericin B has also been found to increase permeability in vesicles prepared from *leishmania* membranes and in whole promastigotes at drug concentrations comparable to that kills the parasites (8). The results of this study showed that a daily regimen of amphotoricin B was effective and produced no serious toxic reactions. Infusion-related adverse reactions such as shivering, rigor or fever were not common. These reactions are mediated through production of tumour necrosis factor, release of prostaglandins, or the direct effect of the drug on mammalian cells. The incidence of severe reactions including renal complications was not too high. Serum creatinine and potassium levels returned to normal 2 weeks after completion of the treatment (4). It has been suggested that there is a significant correlation between the increase in serum creatinine and the total dose of amphotericin B. Also, renal function abnormalities were more frequent in patients who received a total dose of more than 4g of amphotericin B.

In our study no patient received a total dose of more than 1g, an amount that was based on the results of a pilot study. A total dose of 971 mg of amphotericin B was used by Pratta to treat visceral leishmaniasis in Brazil, and WHO recommends a total dose of 1-3g of the drug for VL (9). It has also been proposed that a daily dose of 1-1.5mg/kg of amphotericin B might cause serum creatinine levels to exceed 3mg/dl. Our findings do not confirm this. Only in one patient serum creatinine level reached to 3mg/dl, and was normalized within 5 days and remained so even when treatment at the same dose was restarted. The cellular effects of amphotericin B are complex and depend on a variety of factors such as the growth phase of the cells, dose, and mode of its adminstration, e.g., single or fractional doses. It appears that more work needs to be carried out to identify the exact mechanism of production and prevention of toxicity by amphotericin B. Possibly because of concerns about its toxicity, amphotericin B

has been mainly advocated as lipid complex. There have been three major commercial formulations: Abelcet (AMB lipid complex), Ambisome (liposomal AMB), and Amphotec (AMB colloidal dispersion). The basic premise behind the development of each variant is that a lipid-encased enclosed/bound formulation of AMB results in lesser toxicity with apparent equal efficacy. Indeed, the literature supports the findings of lesser nephrotoxicity of all three agents compared with AMB, and it also seems clear that much higher doses of these agents may be given. Some published studies administrated liposomal amphotericin B as first-choice treatment of VL in children, but it is not available in Iran and also is expensive (1,10). Amphotericin B had been considered as an alternative treatment for visceral leishmaniasis.It had been used extensively with excellent results in refractory regions in India (4,5,11-13), Nepal (11) and Italy (1). We administrated AMB, 1.5mg/kg daily, with good results, no more toxic effect and good tolerance in antimony-unresponsive VL. Patients have been followed up for one year by the trial design, no long-term adverse effects have emerged.

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