Review Article

Current treatment of visceral leishmaniasis (Kala-azar): an overview

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ABSTRACT

Visceral Leishmaniasis (VL) is also popularly known as kala-azar which was first reported in early forties and since then it continues to affect millions of people. The ranges of common drugs available for the treatment of visceral leishmaniasis are limited. It mainly includes pentavalent antimonials e.g. stibogluconate (SbV), amphotericin B deoxycholate (AB), lipid formulations of amphotericin B (L-AB), miltefosine (MF) and paromomycin (PM) - all of which have limitations in terms of toxicity, variable efficacy, price and inconvenient treatment schedules. Most are parenteral except MF which is administered orally. Due to the parasite’s drug resistance, the most widely used (SbV) of these drugs is now of little use in northern Bihar, India, which alone accounts for 50% of the world's burden of visceral leishmaniasis. In areas of resistance to SbV, AB is highly effective. The formulation of AB in liposomes (L-AB) has been a major advancement in the treatment of visceral leishmaniasis. However, despite a significant reduction in price, this treatment remains very expensive for endemic countries like India. Combination short course therapy has been reported by many researchers who found that it is equally effective as conventional monotherapy with added benefits of less side effects, better compliance and less resistance. The aim of this article is to review the current aspects of the treatment for leishmaniasis, giving an overview of current agents clinically used to new agents & modalities of treatment under development.

Keywords: Leishmaniasis, Kala-azar, Treatment, Drugs

INTRODUCTION

Kala-azar is the most severe form of leishmaniasis and presently prevailing mode of treatment is highly unsatisfactory.¹ The World Health Organisation (WHO) levels visceral leishmaniasis (Kala-azar) as a major tropical disease of great significance and control of it remains a serious problem².

There are currently no vaccines available for leishmaniasis. Chemotherapy is only effective way to treat all forms of disease.³ However current therapy is toxic, expensive and the resistance has emerged as a serious problem and trials are being conducted in many countries in search of new drugs and short term combination therapy which is safe and effective.

In general, treatment of leishmaniasis is far from satisfactory. Most antileishmanial drugs are toxic and have to be given parenterally for prolonged periods. Once successful therapy is instituted, there is a prompt return of temperature to normal, regression of spleen and recovery of blood counts occur towards normal. An apparent or initial cure can be declared if there is clinical improvement and no parasites are seen at the end of treatment. Complete regression of splenomegaly may take several months and best indicator of definite or final cure is freedom from a clinical relapse at six months follow up.
PENTAVALENT ANTIMONIALS

Since early 1940s, stibogluconate (SbV) has been the sheet anchor of treatment for VL and has been used as the first line drug. In most part of the world these compounds are still used to treat all forms of leishmaniasis. There are two pentavalent antimony compounds: sodium stibogluconate and meglumine antimoniate. These drugs can be administered either IV or IM, and inhibit glycolytic enzymes and fatty acid oxidation in the amastigotes of leishmania and there is a dose-dependent inhibition in the net formation of ATP and GTP. Side effects are quite common and include arthralgia, myalgia, nausea, vomiting, metallic taste, local pain and stiffness of injected muscles, increase in AST/ALT and ECG changes (decreased height of T wave or T wave inversion). Occasionally severe cardiotoxicity, manifested by prolongation of QTc to >0.5 ms, ventricular premature complexes, ventricular tachycardia, torsades de pointes, ventricular fibrillation and cardiac arrest can occur. In India, SbV induced cardiotoxicities have been reported in about 10% patients, but mortality attributed to SbV related cardiotoxicity is 5.9%. There is a steady decline in the response rate to treatment with SbV. In early fifties, SbV in a dose of 10 mg/kg body weight (max 600 mg) could cure most patients with Indian kala-azar but it became apparent in late seventies that this traditional dose left a large proportion of patients unresponsive. This led to successive recommendations to increase the dose and duration of the drug. In 1984, WHO recommended the dose to be increased to 20 mg/kg body weight and duration to 20 days which was further increased to 40 days in 1990. The increased dose led to the recovery of cure rate to >90% initially. However, later a decline was noted by most researchers and now up to 60% of patients fail to respond to SbV treatment in Bihar (India), though in other areas in India (Eastern UP, West Bengal) SbV continues to be effective. Emergence of SbV resistance in Bihar has been demonstrated in vitro studies using isolates from SbV unresponsive patients which require 3-5 times greater amount of the drug for similar effects as opposed to those from SbV responsive patients.

PENTAMIDINE ISETHIONATE

It is a polyamine and acts by disrupting kinetoplast DNA. When used at 4 mg/kg parenterally for 10-15 injections, it cured most (99%) patients with kala-azar in early 80s. It is quite toxic and hyperglycemia, Insulin Dependent Diabetes Mellitus (IDDM), shock, myocarditis and death have been reported with its use. Like SbV, its efficacy has also waned and in a study from Bihar (India), up to 33 injections were needed to achieve a cure rate of 78%. Due to its potential toxicities especially IDDM, declining efficacy and high cost, its use has been abandoned in India.

AMPHOTERICIN B

Amphotericin B is a polyene antibiotic that has high affinity to ergosterol like sterols in the membrane, and it inhibits its biosynthesis forming microspores, leading to increased membrane permeability and ultimate killing of leishmania. In visceral leishmaniasis, amphotericin B should be used in doses of 0.75-1 mg/kg body weight for at least 15 injections or till the cure is achieved. The drug, which is available as dry power, is first suspended in 10 ml of water and then diluted in 500 ml of dextrose solution. Initially a test dose of 5 mg is given and then full dose can be administered 6-8 hours later. Infusions are given on alternate days and daily administration is recommended only if intensive monitoring of cardiac status, electrolyte abnormalities, hepatic and renal functions is possible. This is because amphotericin B infusions can cause renal dysfunction, hypokalemia, hepatic dysfunction, bone marrow suppression and myocarditis. All of these can be fatal. Fever with chills, aches and pains all over the body, nausea and vomiting are common and can occur acutely during each infusion. Thrombophlebitis of the injected vein is also common. Response to the treatment is excellent with long term cure rate almost 100%. Major limitation of this treatment is the need for prolonged hospital stay for 4-6 weeks, need for IV infusion, serious toxicities and high cost. With the increasing failure of SbV and pentamidine, many workers advocate amphotericin B to be used as a first line drug in visceral leishmaniasis. Government of India committee on treatment of kala-azar has also recommended amphotericin B to be used as first line agent in the regions with >10% prevalence of SbV resistance. In endemic regions lack of skilled manpower, hospital beds and resources are major limiting factor to treat all kala-azar patients with amphotericin B as the first line drug. Alternative safe and easily administrable treatment regimen are surely needed.

LIPID ASSOCIATED AMPHOTERICIN B

To minimize the adverse reaction associated with amphotericin B, several lipid/liposomal formulations of this drug have been developed in recent years. In these deoxycholate is replaced by other lipids which results in remarkably decreased uptake of the drug by organs like kidney, liver etc. leading to markedly decreased or no toxicity. It has been possible to deliver large amount of amphotericin B in a short time, decreasing the duration of treatment without any significant adverse events or loss of efficacy. Three lipid formulations of amphotericin B are commercially available: 1. Liposomal amphotericin B (AmBisome), 2. Amphotericin B Lipid Complex (ABLC) and 3. Amphotericin B Colloidal Dispersion (ABCD). All these three are commercially available and have been tested successfully in visceral leishmaniasis. In one of Indian clinical trials with ABLC, the drug was found to be very safe and infusion reactions as well as other toxicities were minimal. A total dose of 10-15 mg/kg could cure 90-100% of patients and the duration of
therapy could be compressed to five days. No organ specific toxicity was observed. Liposomal amphotericin B (AmBisome) is approved in several European countries for primary treatment of Visceral Leishmaniasis (VL). The US FDA approved it recently and recommended a total dose of 21 mg/kg (3 mg/kg once daily on days 1-5, 14 and 21), whereas in Europe, Africa and South America, 18 mg/kg or more is considered adequate. AmBisome 0.75 mg/kg for five days (Total dose 3.75 mg/kg) cured 89% of Indian visceral leishmaniasis patients in long term followup. Mild infusion related fever and rigor was seen in one-third of patients and no other systemic toxicity occurred. In a recent Indian study, it was observed that low dose liposomal amphotericin B (5 mg/kg) given either as a five day course or as a single infusion, seems to be effective without any significant difference in the response rate. Amphotericin B Cholesterol Dispersion (ABCD) consists of cholesterol sulphate and amphotericin B (1:1 molar ratio) in disc shaped particles. This drug is being evaluated in India. Results in one lipid associated amphotericin B cannot be extrapolated even in the same region as these different drugs do not share the same structure and composition and it should not be assumed that they will have the same spectrum of activity and toxicities. Most important drawback of lipid formulations of amphotericin B is their high cost. Short duration of therapy leading to reduced hospitalization cost might partially offset the high cost of lipid associated amphotericin B. Still these excellent drugs are unaffordable for most patients in the developing countries.

AMPHOTERICIN B-FAT EMULSION

In Europe, several workers have used amphotericin B mixed in 100 ml of commercially available 20% fat emulsion for treatment of fungal infections in patients with AIDS, malignancy or those critically ill, in an effort to reduce the infusion-related toxicities. When tried in Indian patients with alternate day infusions of amphotericin B (2 mg/kg) mixed with 100 ml of 20% fat emulsion for a total of five doses, 93% responded completely after six months of follow up. The final cost of this regimen was 45% less than re-treatment with either SbV or conventional amphotericin B deoxycholate alone. Another major advantage was a considerably shortened duration of treatment, i.e. 10 versus 20-40 days. Further studies are needed before this combination can be employed.

AMINOSIDINE (PAROMOMYCIN)

It is an aminoglycoside antibiotic which has good leishmanicidal activity in vitro and animal models. Trials have been conducted all over the world using aminosidine alone or in combination with SbV. In India, Thakur et al. used it in 12 mg/kg body weight with SbV 20 mg/kg body weight for 20 days and of the 24 patients, 18 had permanent cure, four improved and two died. Seaman et al. in a large study (200 patients), compared ShV 20 mg/kg body weight alone for 30 days with a combination therapy of ShV and aminosidine (15 mg/kg) for 17 days. In ShV group 81% and in the combination group 95% were cured at the end of 17 days, however at the end of 30 days, cure rate rose to 93.4% in ShV group. Mortality and drug toxicities were similar in the two groups. No serious side effect was observed even though a transient rise in urea and creatinine was seen. Other side effects include nephro and ototoxicity as observed in any other aminoglycoside but these were very uncommon. Main advantage was the short duration of therapy but no significant benefit was achieved as far as cure rate was concerned. Another advantage projected in some studies is that the patients receiving this drug may have lesser degree of infective diarrhoea and pneumonia because of its antibacterial action and its action against intestinal protozoa - giardia and amoeba. A recent study in India by Jha et al. shows that aminosidine alone at 16 mg/kg/day for 21 days cured 93% of patients. However this drug is not available commercially.

CYTOKINE THERAPY

It is now well established that leishmania infection progresses to kala-azar in individuals who are unable to elicit a Th1 type of immune response which is initiated by IL-12 and mediated through IL-2 and IFN-γ. IFN-γ is one of the principle activators of macrophages and has shown its ability to control leishmanial infection in animals. Results of studies in India have been disappointing with IFN-γ with cure rate less than 50%. Decline in the response rate of antimony rendered the addition of IFN-γ ineffective. In a developing country like India field applicability of these products remains a distant possibility.

ORAL AGENTS

Two new oral antileishmanial compounds, miltefosine and WR-6026 (sitamaquine) are available to succeed in the treatment of visceral leishmaniasis.

Miltefosine (hexadecylphosphocholine)

It is an alkyl phosphocholine analogue which was initially developed as an anticancer drug. But later it was found to be having little clinical efficacy against tumour cells when administered orally. However, in vitro and animal studies indicated it to be an effective antileishmanial agent. Exact mechanism of its leishmanicidal activity is not known. Probably it acts by modifying the cell signaling pathways and interfering with membrane synthesis. Miltefosine was first tried in 30 patients phase I dose escalating trial which included 14 patients with SbV failure when administered orally for 28 days in doses of 100-150 mg/day were found to be very safe and resulted in a cure rate of 93%. Doses <100 mg/day were only partially effective and those ≥200 mg/day were poorly tolerated. Three following phase II
studies in adults (including a multicentre trial) established that doses of 100-150 mg for 3–4 weeks were well tolerated and were associated with high cure rates (95%). Gastrointestinal side effects such as vomiting and diarrhoea were frequent but mild to moderate in severity and none of the patients discontinued therapy because of this. Asymptomatic transient elevation of hepatic enzymes was common, however in high doses it was nephrotoxic.

### Table 1: Currently available drugs for visceral leishmaniasis.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Sodium stibogluconate</th>
<th>Pentamidine Isethionate</th>
<th>Amphotericin B deoxycholate</th>
<th>Liposomal amphotericin B (AmBisome)</th>
<th>Miltefosine</th>
<th>Paromomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mechanism of action</strong></td>
<td>Acts by inhibiting glycolytic enzymes and fatty acid oxidation in amastigotes of leishmania and there is a dose-dependent inhibition in the net formation of ATP and GTP&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Acts on the genome of parasite by hampering replication and transcription at the mitochondrial level</td>
<td>Acts by inhibiting the membrane lipids biosynthesis forming microspores, leading to increased membrane permeability and ultimate killing of leishmania&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Same as conventional amphotericin but distribution of drug in body is different</td>
<td>Acts by modifying the cell signalling pathways and interfering with membrane synthesis</td>
<td>Acts by interfering with initiation of protein synthesis by fixing the 30S-50S ribosomal complex at the start codon of mRNA, leading to accumulation of abnormal initiation complex</td>
</tr>
<tr>
<td><strong>Regimen</strong></td>
<td>20 mg/kg daily for 30 days</td>
<td>4 mg/kg three times a week for 3–4 weeks (10–12 injections)</td>
<td>1 mg/kg on alternate days x 15 doses in 30 days</td>
<td>Total dose of 20 mg/kg split over several doses (more required in Africa and HIV+)</td>
<td>1.5-2.5 mg/kg for 28 days (India only)</td>
<td>15 mg/kg for 21 days (India only)</td>
</tr>
<tr>
<td><strong>Administration</strong></td>
<td>i.v. or i.m.</td>
<td>i.v. or i.m.</td>
<td>i.v.</td>
<td>oral</td>
<td>i.m.</td>
<td>i.m.</td>
</tr>
<tr>
<td><strong>Clinical efficacy</strong></td>
<td>35-95% (depending on geographic region)</td>
<td>70-80% (depending on geographic region)</td>
<td>&gt;97% all region</td>
<td>Asia, Brazil; &gt;97%; India: single dose 91%; Africa: not clearly established</td>
<td>India: 94-97% Africa: 94% in immunocompetent South America: not established</td>
<td>India: 94% Africa: unacceptably low Southern Ethiopia and Kenya: efficacy of PM is good. South America: not established</td>
</tr>
<tr>
<td><strong>Resistance</strong></td>
<td>As high as 60% (Bihar, India)</td>
<td>60% (India and adjoining Nepal.)</td>
<td>Not documented</td>
<td>Not documented</td>
<td>Lab isolates</td>
<td>Lab Isolates</td>
</tr>
<tr>
<td><strong>Toxicity</strong></td>
<td>+++ Arrhythmias, reversible pancreatitis, nephrotoxicity, hepatotoxicity, death especially in African HIV/VL</td>
<td>myalgias, pain at the injection site, nausea, headache, metallic taste, burning sensation, numbness, hypotension hypoglycemia</td>
<td>+++ Nephrotoxicity (in-patient care needed), infusion-related fever</td>
<td>+ Minor/no nephrotoxicity, mild infusion-related fever</td>
<td>+ Gastrointestinal (20-55% of patients, usually mild), nephrotoxicity, hepatotoxicity, possible teratogenicity</td>
<td>+ minor/no nephrotoxicity, reversible ototoxicity, hepatotoxicity (all relatively rare)</td>
</tr>
</tbody>
</table>
This drug (Miltefosine) is teratogenic and cannot be used in pregnant females or those who refuse contraception for the duration of therapy plus three months as a safeguard considering its long half-life.

A WHO sponsored phase III trial in India with 100 mg for four weeks has been encouraging. Recently it has been approved for marketing in India for kala-azar in doses of 50 mg/day for those weighing ≤25 kg and 100 mg daily for those weighing >25 kg for four weeks.

WR 6026 (sitamaquine)

It is a primaquine analogue with high antileishmanial activity. It was first developed against malaria in Walter Reed army institute of research (USA) several decades ago. In a phase II clinical trial done in Kenya, it was found that the drug cured 50% in a dose of 1 mg/kg/day.

Methemoglobinemia up to a maximum of 55% and mild elevation of hepatic enzymes are notable adverse reaction. In another phase two dose study in Brazil, the cure rate was 67% (four out of six) at a dose of 2 mg/kg/day. The drug demonstrated some unusual clinical features like lack of increased efficacy against Brazilian kala-azar with increased dosing above 2 mg/kg/day and nephrotoxicity that was not present in previous investigations.

COMBINATION THERAPY

Many trials have been done for role of combination therapy in VL with aim to increase efficacy and decrease resistance with encouraging results. It has been found to play important role in initiation as well as maintenance therapy. In this study, standard conventional therapy in certain type of VL in particular geographical locations, particularly eastern India, however there are no data regarding the efficacy of combined therapy in HIV patients. Nonetheless, many experts are in favour of combined therapy for patients with multiple relapses. Combinations of antimonials with other drugs such as allopurinol, ketoconazole, itraconazole or interferon-c have been used, but with insufficient evidence to consolidate the recommendation. A report from Italy described the safety and efficacy of the combination of liposomal amphotericin B (4 mg/kg/day for 5 consecutive days and on days 10, 14, 17, 31 and 38) and the growth factor recombinant human granulocyte/monocyte colony-stimulating factor (rHuGMCSF) [150 µg subcutaneously twice weekly for 12 consecutive weeks] in an HIV-positive patient with VL.

Sundar et al. from India reported comparative study between conventional therapy and short term combination therapy in VL with better efficacy and safety where in compliance was better and resistance was less in comparison with conventional treatment was given with amphotericin B infusion in dose of 1 mg/kg body weight on alternate days for 30 days (total dose 15 mg/kg) and it was compared with three drug combinations (single injection of 5 mg/kg liposomal amphotericin B and 7-days 50 mg oral miltefosine or single 10-day 11 mg/kg intramuscular paromomycin; or 10 days each of miltefosine and paromomycin) in an open-label, parallel-group, non-inferiority, randomised controlled trial in two hospital sites in Bihar, India. Patients aged 5-60 years with parasitologically confirmed visceral leishmaniasis were randomly assigned one of the four treatments by the trial statistician by use of a computer-generated list. Clinical assessments were done at the end of treatment (15 days on combination treatment; 31 days for standard treatment) and after 45 days and six months. It concluded that combination treatments for visceral leishmaniasis are efficacious as well as safe and it decrease the duration of therapy, thereby encouraging adherence and reducing emergence of drug-resistant parasites. No side effects were observed and after 2 years of follow-up the patients were still free from disease.

MAINTENANCE THERAPY

Maintenance therapy has role in VL with co-existing HIV infection. The data regarding maintenance therapy after a treated episode of VL in HIV-infected patients has fundamentally been developed in Europe, where zoonotic transmission occurs by L. infantum. Combination therapy will ultimately become the best drug regimen for treating VL in many parts of the world. Treatment of VL in HIV co-infected patients is less effective than in HIV-negative patients and relapses are much more frequent. Within the available treatment options, amphotericin B deoxycholate and particularly lipid formulations seem to be the most effective. Meanwhile, it seems that standard treatment with antimonials should be avoided because of their high toxicity in HIV positive patients. Another difficulty that is hard to manage in co-infected patients is the frequent relapses. There is very little evidence regarding which drugs, doses and durations to use for maintenance therapy. The common relapses and prolonged secondary prophylaxis regimens could further add the development of resistant strains and limit the therapeutic options even further and create an epidemiological issue in anthroponotic areas. The only randomized clinical trial which took place in Spain, reported that use of amphotericin B lipid complex as maintenance therapy administered at a dose of 3-5 mg/kg/ day IV every 3 weeks for 12 months, versus no treatment at all, lowered the relapse rate from 50 to 22%. Another prospective study, also carried out in Spain, analyzed the efficacy of maintenance therapy with liposomal amphotericin B at 4 mg/kg/day for 5 days and once weekly for 5 more weeks (total 10 doses), achieving up to 80% patients being free of disease after 12 months of follow up. Pentavalent antimonials were administered every 3-4 weeks as...
maintenance therapy in a study conducted in Spain. The rate of relapses reduced much more significantly than in those patients who either did not receive any treatment or who took allopurinol for the 12 months of follow-up. Pentamidine was used at a dose of 4 mg/kg/day every 2-4 weeks in HIV-positive patients with VL caused by L. infantum, without any evidence of relapses during the follow-up period. Five cases reported in a study performed in Portugal observed that the three patients who received miltefosine as secondary prophylaxis for 21, 14 and 12 months, respectively, remained disease free for a median period of 20 months. Miltefosine could be a good option for maintenance therapy until improvement of immune function (CD4 cell count >250/mm³) because of its long half-life and ease of oral administration. This could allow ambulatory treatment and even dose reductions, using an alternate day regimen. Azole drugs could be effective in maintenance therapy, although there are no clinical trials to support this theory. The experience is based on series of cases where itraconazole was given at a dose of 600 mg/day for up to 24 months of treatment without any relapses. The advantage of these drugs is their good tolerability and low toxicity, although there is a risk of developing resistant fungal infections. Itraconazole or fluconazole combined with allopurinol could be an option. Another relevant aspect to take into account is how long maintenance therapy should be continued. According to different authors, once the patient has recovered their immune function with Highly Active Antiretroviral Therapy (HAART) and the VL is quiescent, suspension of prophylaxis could then be considered when the CD4 count has been maintained at >250 cells/mm³ for more than six months.

NEW VACCINE TRIALS

The first ever vaccine to prevent Visceral Leishmaniasis (VL) entered dual clinical trials in the US at Infectious Disease Research Institute (IDRI) in collaboration with India at IMS BHU in Feb 2012. The vaccine has already completed phase 1 clinical trials in the U.S. to demonstrate safety and the ability to evoke a robust immune response.

The IDRI vaccine, called LEISH-F3 + GLA-SE, is a highly purified, recombinant vaccine which incorporates two fused leishmania parasite proteins, given along with an additive to boost the immune response. It works by activating the immune system to kill the parasite that causes VL, the first of its kind till now developed. As a synthetic vaccine, it can be manufactured at low cost in resource poor settings.

“Only such large trials, conducted in real-life situations of disease exposure, will determine the full effectiveness of the LEISH-F3 + GLA-SE vaccine”.

CONCLUSION

Currently the mainstay of treatment for VL is chemotherapy. The traditional treatment for VL used to be pentavalent antimonials introduced in the late 1940s. However, the development of resistance, especially in India, with failure rates of 60 %, as well as their potential toxicity, made it necessary to seek new treatment options. Thus, since the 1980s, the use of amphotericin B deoxycholate has been introduced, especially in more developed countries. Progressively and because of their efficacy and lower toxicity, lipid formulations of amphotericin have been gaining importance, becoming the first choice treatment established by the US Food and Drug Administration (FDA). However, their elevated cost has somewhat limited their use, but since the introduction of the recent Gilead/WHO AmBisome donation programme to Bangladesh, Nepal, Sudan, South Sudan, Ethiopia and Kenya, this situation has improved. Several studies have demonstrated the efficacy of parenteral paromomycin as a cheap treatment with medium toxicity. Within the range of oral treatments, miltefosine has been fundamentally used in the Indian subcontinent. Combination therapy may ultimately become the best drug regimen for treating Visceral (VL) in many parts of the world in near future.

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