

Review Article

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Visceral leishmaniasis - current therapeutic modalities

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Major therapeutic obstacles in the treatment of visceral leishmaniasis (VL) include the alarming increase in antimonial unresponsiveness especially in Bihar, India and relapses in HIV-*Leishmania* co-infected patients. The therapeutic armamentarium for VL is currently plagued with several limitations as the available drugs are toxic, majority are effective only parenterally and need to be administered for extended periods. The first orally effective drug, miltefosine has been approved for treating VL. In antimony refractory zones, pentavalent antimony has been largely replaced by amphotericin B deoxycholate, but prolonged hospitalization, toxic effects, and requirement for monitoring greatly hamper its widespread application in endemic regions. Lipid formulations of amphotericin B, a remarkable advance in amphotericin B therapy, have greatly reduced toxicity enabling large doses to be delivered over a short period. Even a single dose treatment with liposomal amphotericin B cures >90 per cent patients; however, the stumbling block is its prohibitive cost that precludes its widespread accessibility in endemic countries. Studies using paromomycin in VL are encouraging, and judging by the preliminary results of a recently concluded phase III trial, it could be an extremely useful and affordable antileishmanial drug. Other orally effective drugs include the azoles and allopurinol but these have met with limited success owing to either poor efficacy or unacceptable toxicity. Sitamaquine has undergone limited evaluation, and the data suggest effective antileishmanial activity; its role has to be delineated for which additional developmental studies are proposed. This review highlights the progress made in the treatment of VL, including the multiple mechanisms of action of antileishmanial drugs with a view to enable the researcher to undertake the challenge of providing affordable and effective chemotherapy.

Key words Antileishmanial drugs - chemotherapy -immunomodulators - visceral leishmaniasis

Visceral leishmaniasis (VL, kala-azar) is prevalent in 62 countries with an estimated annual incidence of 500,000. In India, the State of Bihar and adjoining areas of West Bengal, Jharkhand and Uttar Pradesh account for about half the world's burden of VL. Resurgence of VL was noticed in India in the

early seventies and transmission has since remained incessant. The current challenges in its chemotherapy include widespread resistance to pentavalent antimony in India, absence of safe and cost-effective antileishmanial agents and relapses in HIV-*Leishmania* co-infected patients.

Chemotherapeutic agents

Parenteral agents

Sodium stibogluconate: Globally, including India, the treatment of VL has centred around pentavalent antimony compounds (Sb^v) for more than seven decades. Initially Sb^v was used in a dose of 10 mg/kg for 6-10 days, but increasing unresponsiveness in India led to successive upward revisions and currently the amount of drug being used is 10 times more than in earlier years. The last few years have seen the emergence of large scale Sb^v resistance in north Bihar, India, where over 60 per cent of previously untreated patients are unresponsive to Sb^v rendering the drug useless for routine use¹. Resistance seems to be a feature of intensive transmission of anthroponotic *Leishmania donovani* as epidemic turns to endemic in foci where Sb^v has been used as a solo drug, often with poor supervision and compliance. However, there is a regional variation in the response to Sb^v as patients in other States like Uttar Pradesh continue to be responsive¹. Current recommendations are replacement of Sb^v by amphotericin B in these Sb^v refractory zones². However, outside Bihar, Sb^v remains the drug of choice to be used parenterally in a dose of 20 mg/kg daily for 30 days without any upper limit. Till very recently, an unanswered question was whether Sb^v unresponsiveness was linked to the host or parasite. It has now been firmly established that antimonial resistance is an inherent feature of the *Leishmania* parasite³.

To date, the precise mechanism of action of sodium antimony gluconate (SAG) remains an enigma; a general consensus is that Sb^v acts upon several targets that include influencing the bioenergetics of *Leishmania* parasites by inhibiting parasite glycolysis, fatty acid beta-oxidation and inhibition of ADP phosphorylation^{4,6}. It has also been reported to cause non specific blocking of SH groups of amastigote proteins and cause inhibition of DNA topoisomerase I⁷. More recently, it has been demonstrated that antimony can alter the thiol-redox potential in both forms of the parasite by actively promoting efflux of thiols, glutathione and trypanothione, thus rendering the parasite more susceptible to oxidative stress⁸.

The exorbitant cost of brand formulations of Sb^v prompted Medicins Sans Frontieres to commission three studies in Sudan, Kenya and Ethiopia to compare the efficacy in VL of the generic SAG (Albert David, Kolkata India, costs US \$13 per patient) vs. branded SAG (Pentostam, Glaxo-Wellcome, UK, costs US \$200 per patient)⁹. It was conclusively proven that no significant difference existed between the two formulations as generic SAG was equally effective in terms of efficacy and safety in all forms of leishmaniasis and importantly, achievable at a substantially lower cost. However, caution must be exercised before using Sb^v from new manufacturers as bad batches caused fatal cardiotoxicity¹⁰. In two reports from India and Nepal, high incidence of fatal cardiotoxicity was reported with use of antimony made from an unknown manufacturer^{10,11}.

Post kala-azar dermal leishmaniasis (PKDL), a dermatological manifestation generally following VL infection occurs predominantly in India and Sudan. Although in both *L. donovani* is the causative organism, Indian PKDL requires prolonged treatment (>120 days)¹² whereas for the Sudanese variety, two months treatment is considered adequate¹³.

Pentamidine isethionate: Pentamidine, an aromatic diamidine has been previously used as a second line of treatment for VL but its precise mode of action has yet to be elucidated. Since it is a competitive inhibitor of arginine transport and non competitively inhibits putrescine and spermidine, its leishmanicidal activity is possibly mediated via its influence on polyamine biosynthesis and the mitochondrial membrane potential¹⁴.

Pentamidine was initially proven to be useful in Sb^v resistant kala-azar cases in India¹⁵ but the limiting factors were the expense and above all the unacceptable toxicity as it causes irreversible insulin dependent diabetes mellitus and death. Further, its declining efficacy (as only about 70% patients could be cured¹⁶), has led to its being totally abandoned in India.

Amphotericin B and its lipid formulations: Amphotericin B is an antifungal macrolide antibiotic

isolated from *Streptomyces nodosus*. Its antileishmanial activity was first shown in the early 1960s attributed to its selective affinity for 24 substituted sterols, namely ergosterol *vis-a-vis* cholesterol, the primary sterol counterpart in mammalian cells eventually helping to increase drug selectivity towards the microorganism. However, at higher concentrations ($>0.1 \mu M$), it triggers cationic and anionic influx via the formation of aqueous pores resulting in cell lysis¹⁷.

Amphotericin B has excellent leishmanicidal activity. Faced with increasing Sb^V unresponsiveness of VL in India over the last decade, amphotericin in a dose of 0.75-1 mg/kg for 15 to 20 infusions either daily or on alternate days has consistently produced cure rates of about 97 per cent and is now the drug of choice in north Bihar¹⁸. Major limiting factors include an almost universal occurrence of infusion based reactions like high fever with rigor and chills, thrombophlebitis and occasional serious toxicities like myocarditis, severe hypokalaemia, renal dysfunction and even death. Thus, its use at peripheral health posts was prevented by frequent adverse events, the need for prolonged hospitalization and close monitoring.

Toxic effects of amphotericin B deoxycholate have been largely ameliorated with the advent of lipid formulations of amphotericin B. In these formulations, deoxycholate has been replaced by other lipids that mask amphotericin B from susceptible tissues, thus reducing toxicity, and facilitate its preferential uptake by reticuloendothelial cells, thus achieving targeted drug delivery to the parasite resulting in increasing efficacy and reduced toxicity. Three such lipid-associated formulations of amphotericin are commercially available: (i) liposomal amphotericin B (AmBisome; Gilead Sciences, Foster City, CA, USA); (ii) amphotericin B lipid complex [Abelcet (ABLCL); The Liposome Co, Princeton, NJ, USA]; and (iii) amphotericin B colloidal dispersion [Amphocil (ABCD); Sequus Pharmaceutical; Menlo Park, USA].

These preparations have been tested successfully in VL in India, Kenya and Brazil, as also Europe,

where HIV co-infected individuals were included¹⁸. AmBisome was the first to be evaluated and is licensed in several European countries and USA for primary treatment of VL. For immunosuppressed patients, AmBisome in a total dose of 40 mg/kg spread over 38 days is recommended¹⁹, but has not been formally compared with shorter regimens; unfortunately, all co-infected patients relapsed. In immunocompetent patients in Europe and South America, total doses of 18-24 mg/kg, and in Kenya 14-18 mg/kg given over 10 days cured 90-100 per cent patients²⁰. In Indian VL, a dose of 6 mg/kg (2 mg/kg x 3) cured 100 per cent²¹ and 3.75 mg/kg cured 89 per cent patients²². In a subsequent study employing a single dose of 7.5 mg/kg of AmBisome, 90 per cent patients were cured with minimal adverse events²³. Effective single dose treatment makes it possible to treat a large number of patients in a very short time. In India, the cost of a single 5 mg/kg dose of AmBisome for a 30 kg patient is about US\$ 600 (Rs.27000/-), compared with US\$ 60 (Rs. 2700/-) for a typical treatment regimen with conventional amphotericin B. This difference is beyond the reach of most patients in developing countries, despite the shortened hospital stay. It is imperative that the price will have to be substantially reduced if this, the most effective drug of all in VL, is to be made of any use to those who need it most. Similarly, a total dose of ABLCL 10 to 15 mg/kg delivered over 5-10 days cured 90 to 100 per cent of patients^{24,25}. In Brazil, five and seven doses of Amphocil (2 mg/kg) cured 90 and 100 per cent of patients respectively, but side effects were a limiting factor^{26,27}. Of the three lipid formulations, AmBisome is best tolerated.

Results from a recent three armed study in Bihar where a direct comparison was made between conventional amphotericin B (1 mg/kg/day on alternate days for 30 days) and AmBisome and Abelcet (both at a dose of 2 mg/kg/day for 5 days)²⁸ showed that though the overall cure rates of amphotericin B were comparable with AmBisome or Abelcet being 96 vs. 96 vs. 92 per cent, respectively, the lipid formulations had an upper edge as they produced distinctly lower toxicities, notably the absence of nephrotoxicity and significantly lower infusion reactions. However, when the cost factor was taken into consideration, the cost of amphotericin B was

almost half that of AmBisome or Abelcet being US\$ 417 vs. \$872 and \$947 respectively²⁸. Alternatively, single dose regimens for AmBisome (5 mg) was comparable with a similar dose administered for 5 days with similar cure rates of 91 and 93 per cent respectively; this single dosage showed excellent tolerance and safety coupled with a tremendous economic impact as hospital stay would be considerably reduced^{23,29}. However, in India, where the hospital stay cost is low, shortened hospital stay does not offset the high drug cost compared to affluent states like Greece where two infusions each of 10 mg/kg of AmBisome achieved 97.5 per cent cure³⁰.

Oral chemotherapeutic agents

Miltefosine: Several alkylphospholipid derivatives like miltefosine, ilmifosine and edelfosine, originally registered for antineoplastic activities fell out of favour due to severe gastrointestinal toxicities³¹. The entry of miltefosine into the therapeutic armamentarium of leishmaniasis is considered as a landmark event as for the first time, an orally effective antileishmanial agent had been identified. In a phase I/II dose escalation trial in India which established that in adults, a daily dose between 100-150 mg for 28 days was well tolerated and would cure most of the patients³². This was followed by a series of phase II studies confirming results of the pilot study^{33,34}. This led to a multicenter pivotal phase III study in which a high cure rate (94%) unquestionably established it as the first orally effective antileishmanial agent thus revolutionizing antileishmanial therapy³⁵. Its efficacy has also been reported in Sb^v resistant cases³⁵. Its adverse effects were mild to moderate gastrointestinal disturbances that included vomiting and diarrhoea in 40 and 15-20 per cent of patients respectively. Depending on the individual weight, the recommended therapeutic regimen for patients weighing less than 25 kg is a single oral dose of 50 mg for 28 days whereas individuals weighing more than 25 kg require a twice daily dose of 50 mg for 28 days³⁵. Miltefosine, was registered for treatment of VL in India in March 2002. Children constitute about 40 per cent of the patients with VL in India. Since the trials described above included patients in the age group of 12 yr and above, additional trials were conducted to ascertain its safety and efficacy in children. In two multicenter studies

involving 119 paediatric patients, it was established that miltefosine in a daily dose of 2.5 mg/kg for 28 days would cure 94 per cent patients^{36,37}.

The antileishmanial *modus operandi* of this compound can be extrapolated from its effect on mammalian cells where it causes modulation of cell surface receptors, inositol metabolism, phospholipase activation, protein kinase C and other mitogenic pathways eventually culminating in apoptosis^{38,39}.

However, at the end of the day, miltefosine has its limitations in that it induces gastrointestinal disturbances, and renal toxicity. Fortunately, these symptoms are reversible and are not a major cause for concern. As miltefosine is teratogenic, it is contraindicated in pregnancy and women of child bearing age group not observing contraception. A potential problem is the prolonged half-life of miltefosine (150-200 h)⁴⁰ that raises concerns for emergence of resistance.

Paromomycin: Paromomycin (identical to aminosidine), obtained from cultures of *Streptomyces rimosus*, belongs to the class of aminocyclitol-aminoglycosides and possesses both anti-bacterial and antiprotozoal activity. Although developed in the 1960s as an anti-leishmanial agent, it remained neglected until the 1980s when topical formulations were found to be effective in cutaneous leishmaniasis (CL) and a parenteral formulation for VL was also developed.

Paromomycin has been used either alone or in combination with Sb^v for the treatment of VL, and was first reported by Chung *et al*⁴¹, albeit in small number of patients. Its superiority in combination with Sb^v compared to Sb^v alone has clearly been demonstrated in several studies from India⁴²⁻⁴⁴. In a three armed study where paromomycin (12/16/20 mg/kg daily for 20 days) was compared with Sb^v (20 mg/kg/day for 30 days). Paromomycin (16/20 mg/kg) cured 93/97 per cent of VL patients respectively, while antimony alone had a dismal cure rate of 63 per cent⁴⁴. A study from Sudan⁴⁵ also demonstrated that while combining with Sb^v it was possible to reduce the duration of treatment from 30 days to 20 and 17

days respectively, with superior efficacy and decreased mortality. With regard to VL, a monotherapeutic regimen of 12/16/20 mg/kg/day for 20 days had cure rates of 77/93/97 per cent respectively and doses were well tolerated. It was proposed that a 21 day course of aminosidine (16/20 mg/kg/day) could be considered as a first line treatment in Bihar^{44,46}. Unfortunately the clinical development of paromomycin came to a grinding halt as the manufacturers stopped production and only when it was resumed by another company (Pharmamed in Malta), could a pivotal phase III trial to register this drug for VL be undertaken. In 2002, the Gates Foundation funded this project through the Institute of One World Health, USA and the TDR wing of World Health Organization. The trials in VL have just been completed in Bihar, India, and preliminary analysis suggested that its efficacy is comparable to other licensed drugs, and tolerability is excellent. This drug is likely to cost approximately US\$ 10-20 for one adult treatment course, and thus should be considered as the cheapest antileishmanial drug.

The mechanism of action of paromomycin has been linked to the inhibition of cytochrome C reduction in *Candida krusei*⁴⁷, while mechanisms specific to *Leishmania* still require further elucidation. Paromomycin in *L. donovani* promoted ribosomal subunit association of both cytoplasmic and mitochondrial forms, following low Mg²⁺ concentration induced dissociation⁴⁸. Paromomycin also induces respiratory dysfunction in *L. donovani* promastigotes⁴⁹.

Other oral compounds

Azoles: Azoles (Ketoconazole, fluconazole, itraconazole, etc.) are essentially sterol bio-synthesis inhibitors and their efficacy against *L. tropica* was first reported by Berman in 1981⁵⁰. Azoles specifically block ergosterol synthesis and as the presence of ergosterol as a membrane component is shared between fungi and *Leishmania*, it accounts for many antifungal sterol biosynthesis inhibitors (SBIs) to also be leishmanicidal⁵¹. Most SBIs impair the biosynthesis of ergosterol by blocking 14- α -demethylase, leading to the accumulation of 14- α -methylsterols. This results in impaired membrane stability and in growth inhibition of fungi and possibly in *Leishmania* as well. Azoles have been

shown to be active against a wide range of promastigotes and amastigotes⁵²⁻⁵⁴.

Leishmania species differ in their sensitivity to azoles as *L. donovani*, *L. braziliensis* and *L. amazonensis* promastigotes are more sensitive than *L. aethiopica*, *L. major*, *L. tropica* and *L. mexicana*. However, this analogy cannot be extrapolated to clinical studies. Both ketoconazole and fluconazole have undergone evaluation in VL in India^{55,56}. However, despite reports of the former's usefulness, their antileishmanial activity was not enough to induce clinical cure by themselves⁵⁷⁻⁶⁰.

Immunomodulators

Leishmania infection is classically associated with a depression of T helper type 1 cells and preferential expansion of T helper type 2 cells and accordingly, skewing of T helper cells towards a Th1 response is considered as a promising therapeutic strategy⁶¹. Although the macrophage has effective mechanisms to decimate intracellular pathogens by generating toxic metabolites like nitric oxides and reactive oxygen species for which their activation by interferon-gamma (IFN- γ), released by Th1 cells is mandatory, the *Leishmania* is a devious pathogen that evades the immune response by selectively attenuating pro-inflammatory signalling pathways⁶².

Clinical trials with IFN- γ alone and/or in conjunction with Sb^v were undertaken, and with Sb^v it was reported to be useful in treating severe or Sb refractory VL in Brazil⁶³, however, in India in a large (n=156) randomized study comparing Sb^v alone with Sb^v plus IFN- γ for 15 or 30 days had disappointing results as the final cure rate with Sb^v plus IFN- γ for 15 or 30 days was 42 and 49 per cent, respectively⁶⁴.

Sitamaquine: Sitamaquine, an orally active 8-aminoquinoline analog (8-aminoquinoline (8-[6-(diethylamino)hexyl]amino]-6-methoxy-4-methylquinoline), was originally developed as WR6026 by the Walter Reed Army Institute in collaboration with GlaxoSmithKline in response to a pressing need for orally effective agents for VL, its effectiveness was validated in animal models^{65,66}.

Several small phase I or II clinical trials have been undertaken with limited success. The cure rate for VL with sitamaquine in a Kenyan phase II study at a dose of 1 mg/kg/day for 28 days was 50 per cent⁶⁷. Several years later, in a Brazilian phase II trial, the same dose of sitamaquine cured none of the four VL patients while a 2 mg/kg/day for 4 wk gave a maximum efficacy of 67 per cent; surprisingly, a linear correlation could not be sustained as increasing the dose to 2.5 mg/kg/day resulted in decreased efficacy concomitant with enhanced adverse effects such as nephropathy and methaemoglobinaemia⁶⁸. In a multicenter phase II trial in India, sitamaquine demonstrated excellent antileishmanial activity at a daily dose of 1.75 - 2 mg/kg for 28 days (Sundar S, Jha TK, Thakur CP, unpublished observations). However, more studies are needed to evaluate some of the safety issues as this drug appears to have clinical efficacy that warrants further development.

Conclusion

As opposed to two decades ago when Sb^v was the only option for the treatment of patients with VL, considerable therapeutic advances have taken place. The advent of amphotericin B and its lipid formulations can be considered an important breakthrough with increased safety and shorter duration of treatment. Discovery, development and registration of oral miltefosine for the treatment of VL in India has opened up newer vistas. The likely approval of paromomycin and further development of oral sitamaquine will, for the very first time, provide an opportunity to clinicians to look at the combination chemotherapy of VL thus providing a safe and effective shorter course of treatment which would also be affordable.

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