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.

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
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 use\(^1\). 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 responsive\(^1\). Current recommendations are replacement of Sb\(^v\) by amphotericin B in these Sb\(^v\) refractory zones\(^2\). 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.

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 \(^1\). 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 stress\(^8\).

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 cardiotoxicity\(^10\). 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 adequate\(^13\).

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 actively is possibly mediated via its influence on polyamine biosynthesis and the mitochondrial membrane potential\(^14\).

Pentamidine was initially proven to be useful in Sb\(^v\) resistant kala-azar cases in India\(^15\) 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 cured\(^16\)), 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 μM), it triggers cationic and anionic influx via the formation of aqueous pores resulting in cell lysis\(^{17}\).

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 Bihar\(^{18}\). 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 (ABLC); 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 included\(^{18}\). 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 recommended\(^{19}\), 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 patients\(^{20}\). In Indian VL, a dose of 6 mg/kg (2 mg/kg x 3) cured 100 per cent\(^{21}\) and 3.75 mg/kg cured 89 per cent patients\(^{22}\). In a subsequent study employing a single dose of 7.5 mg/kg of AmBisome, 90 per cent patients were cured with minimal adverse events\(^{23}\). 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 ABLC 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 respectively\textsuperscript{28}. 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 was considerably reduced\textsuperscript{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 cure\textsuperscript{30}.

\textbf{Oral chemotherapeutic agents}

\textit{Miltefosine:} Several alkylphospholipid derivatives like miltefosine, ilmifosine and edelfosine, originally registered for antineoplastic activities fell out of favour due to severe gastrointestinal toxicities\textsuperscript{31}. 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 patients\textsuperscript{32}. This was followed by a series of phase II studies confirming results of the pilot study\textsuperscript{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 therapy\textsuperscript{35}. Its efficacy has also been reported in Sb\textsuperscript{V} resistant cases\textsuperscript{35}. 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 days\textsuperscript{35}. 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\textsuperscript{36,37}.

The antileishmanial \textit{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\textsuperscript{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)\textsuperscript{40} that raises concerns for emergence of resistance.

\textit{Paromomycin:} Paromomycin (identical to aminosidine), obtained from cultures of \textit{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\textsuperscript{V} for the treatment of VL, and was first reported by Chunge \textit{et al}\textsuperscript{41}, albeit in small number of patients. Its superiority in combination with Sb\textsuperscript{V} compared to Sb\textsuperscript{V} alone has clearly been demonstrated in several studies from India\textsuperscript{42-44}. In a three armed study where paromomycin (12/16/20 mg/ kg daily for 20 days) was compared with Sb\textsuperscript{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 cent\textsuperscript{44}. A study from Sudan\textsuperscript{45} also demonstrated that while combining with Sb\textsuperscript{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 Bihar44,46. 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 krusei47, 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-â-demethylase, leading to the accumulation of 14-â-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 India55,56. 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-r), 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-r alone and/or in conjunction with Sb' were undertaken, and with Sb' 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' alone with Sb’ plus IFN-r for 15 or 30 days had disappointing results as the final cure rate with Sb' plus IFN-r 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 models65,66.
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 cent. 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 methaemoglobinemia. 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 SbV 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 miltefosine 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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