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Review Article

An insight into the treatment of Leishmaniasis

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Abstract

Leishmaniasis has been classified as a major tropical disease by the World Health Organization. In the absence of an effective vaccine against leishmaniasis, available chemotherapy is the only effective way to treat all forms of the disease. The available drugs, pentavalent antimonials (Sbv) and amphotericin B (AmB) require parenteral administration, prolonged treatment duration, and hospitalization, and are thus inevitably very costly. The development of resistance to Sbv in North Bihar, India, has increased the problems of treatment. Lipid formulations of AmB have lower toxicity, and shorter duration of therapy but prohibitive cost. Miltefosine, the only oral drug, also has long treatment durations and is costly, teratogenic, and requires monitoring for gastrointestinal, hepatic, and nephrotoxicity. Development of resistance against miltefosine is a major concern. Paromomycin sulfate (PM) an aminoglycoside antibiotic has been reported to have antileishmanial efficacy, however, it has some disadvantages like 2 weeks of treatment regimen, injection site pain, and hepatic and ototoxicities. Therefore, the therapeutic armory for the treatment of leishmaniasis is currently obstructed with several limitations such as the toxicity of available drugs, repeated parenteral administration and side effects. The aim of this review is to highlight the treatment of leishmaniasis including the multiple mechanisms of action with a view to enable the researcher to undertake the challenge of providing affordable and effective chemotherapy.

Keywords: Leishmaniasis, Treatment**,** Drugs, Antileishmanial.

Introduction

Leishmaniasis is a disease caused by the bite of *Leishmania* infected *Phlebotomine* sandflies. The disease is included in the list of the world's most neglected diseases, prevalent in developing countries (McCall et al., 2013). It ranks the second only to malaria, and the control remains a serious problem with ever increasing cases worldwide (WHO, 2002). *Leishmania* infection continues to have a major impact on public health inducing significant morbidity and mortality mostly in the poorest populations (Badiee et al., 2013). More than 12 million people are currently infected with Leishmania, and 2 million new cases of Leishmaniasis are reported every year (Kedzierski, 2010, Alvar et al., 2012) and a further, more than 350 million people are living at risk in 98 countries (WHO, 2010). Current control measures rely on chemotherapy to alleviate disease. However, there is consensus that in the longer term, vaccines ought to become a major tool in the control of this group of diseases. Unfortunately, the development of vaccines has been hampered by significant antigenic diversity (Handman, 2001). One of the available anti-leishmanial drugs, pentavalent antimonials are clinically unsatisfactory because many cases are not responsive to it; furthermore, the cases that do respond tend to relapse at a later stage (Sundar et al., 2000). Other drugs recommended for the treatment of visceral leishmaniasis have some limitations including development of resistance, parenteral administration, toxic side effects, high costs and long courses of treatment (Perez-Victoria et al., 2003; Sundar and Chatterjee, 2006) viz; 28 days of oral treatment with miltefosine, 30 days infusion with Amphotericin B and 21 days intramascular injections with paromomycin sulfate. The efficacy of drugs is also compromised due to suppression of immune function during the course of infection (Shakya et al., 2011). Due to all these problems with the existing chemotherapy, there is a need to discover new and more effective antileishmanial compounds.

Treatment of visceral leishmaniasis

Pentavelent Antimonials

For more than 70 years, the first-line treatment in most countries has been injectable pentavalent antimonials (Monzote, 2009). Sodium stibogluconate was the initial drug to be used for the management of VL, in the dose of 10 mg/kg for 6 to 10 days but due to increasing unresponsiveness and treatment failures, its dose was modified and the standard WHO recommendation, at present, is 20 mg/kg for 30 days deep intramuscular injections. However, dose escalation does not prevent the emergence of resistance (Sundar et al., 2000) and in India widespread resistance to these standard drugs still poses a major obstacle in the control of leishmaniasis (Mishra et al., 2013). Moreover, the treatment is lengthy, potentially toxic and painful; it has become ineffective in parts of India and Nepal as resistance has developed (Monzote, 2009). In Bihar, 100 percent resistance in cases of kala azar in two villages of Darbhanga and Sitamarhi districts has been observed who failed to respond to SSG in WHO recommended dosage (Sundar et al., 2000). In *in vitro* studies conducted on SSG resistant cases from some hyperendemic districts of Bihar (Lira et al., 1999) it has been observed that *L. donovani* isolates from 15 non responders required five times more dosage of Sb for killing the parasite than isolates from responders (Jha, 2006).

Sodium stibogluconate (SSG) in phosphatidylcholine (PC)-stearylamine-bearing liposomes (PC-SA-SSG) and PC-cholesterol liposomes (PC-Chol-SSG) have been found to be effective against SSG-resistant *L. donovani* strains (Roychoudhury et al. 2011). In the studies conducted by Bimal et al. (2012) combination of CD2 mAb with conventional antimonial therapy has been found to clear *L. donovani* from infected macrophages. Recently, Fernandes et al. (2013) showed that amphiphilic antimony complexes significantly reduced the parasite load in liver and spleen of *L. infantum* infected BALB/c mice.

Pentavalent antimonials (SbV) are the prodrug, and they should convert to trivalent antimonials (SbIII) in order to demonstrate their antileishmanial activity (Kothari et al., 2007). A parasitic aquaglyceroporin, aquaporin 1 transporter is supposed to be responsible for the transport of antimonials into *Leishmania* infected amastigotes (Gourbal et al., 2004). These drugs are found to influence the bioenergetics of *Leishmania* parasite by inhibiting parasite glycolysis, fatty acid beta oxidation and inhibition of ADP phosphorylation. They may also promote efflux of thiols mainly glutathione and trypanothione (Raguenaud et al., 2007).

Thiol metabolism plays a key role in both clinical and laboratory-generated resistance mechanisms. It has been found that elevated intracellular thiol levels are associated with increased SbIII resistance (Wyllie et al., 2008). In natural antimonial resistance, the impaired thiol metabolism results in inhibition of SbV activation and decreased uptake of the active form SbIII by amastigotes (Kothari et al., 2007). Overexpression of the membrane-bound ATP-binding cassette (ABC) transporters on the surfaces of leishmanias is another mechanism of antimonial resistance (Mandal et al., 2009).

Sinha *et al.* (2011) reported that protein sequences of ABC transporters of *Leishmania spp*. associated with visceral leishmaniasis that are known to play a crucial role in the development of multidrug resistance (MDR) have enormously diverged during the process of evolution even within the identical species strains resulting in insignificant homology. Hence it can be predicted that during the process of evolution a series of frequent mutations might have led to changes in the ABC transporters favorable to effluxing the drug thereby making the *Leishmania* species prone to resistance against SAG.

Amphotericin B deoxycholate

Amphotericin B is an anti-fungal macrolide antibiotic, which has been shown to have anti-leishmanial activity in the late 1960s. Its main mechanism of action, is due to its selective affinity for position-24 substituted sterols mainly ergosterol. It also causes formation of aqueous pores resulting in cell lysis (Ramos et al., 1996). The drug is given at a dose of 1 mg/kg body weight in 5% dextrose intravenously, either on alternate days or daily for 15 days. The cure rate has been shown to be as high as 98% (Mayerhoff, 1999). Major side effects include chills and rigor, thrombophlebitis, occasionally myocarditis, hypokalaemia, renal dysfunction and death. Therefore, its use has been prevented due to frequent adverse effects, prolonged hospitalization and close monitoring. However, toxic effects of amphotericin B have been ameliorated with the advent of lipid formulation of Amp 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 increased efficacy and reduced toxicity (Sundar and Chatterjee, 2006). Three such lipid formulations of amphotericin B are liposomal amphotericin B (ambisome), amphotericin B lipid complex (abelcet) and amphotericin B colloidal dispersion (amphocil). However, in India the cost of a single dose of 5 mg/kg of ambisome for a 30 kg patient is about Rs. 27,000 (US$574.47) as compared to Rs. 2700 (US$57.45) for a typical course of amphotericin B deoxycholate. Thus, the cost difference is beyond the reach of most patients in developing countries (Sundar et al., 1998). Cardiac toxicity is uncommon with amphotericin B and there are only few isolated reports. Co-administration of SAG and amphotericin B has been reported to cause arrhythmia and sudden death in some patients with kala azar (Maheshwari *et al.*, 2011).

Pal et al. (2012) showed that amphotericin B formulated in tripalmitin based nanosize lipid particles, emulsomes and O-palmitoyl mannan at the dose of 1 mg/kg b.wt. eliminated intracellular amastigotes of *L. donovani* within splenic macrophages. Italia et al. (2012) showed that polyster nanoparticles for oral delivery of amphotericin B were more effective than the same dose of amphotericin B solution at suppressing parasite numbers compared to controls in bone marrow derived macrophages infected with *L. donovani*.

Recently, a non-covalent, water-soluble complex of amphotericin B (AMB) and poly (α-glutamic acid) (PGA) with a molecular weight range of 50-70 kDa has been found to be active against intracellular *L. donovani* amastigotes and the ED_{50} values of 0.24±0.03 mg/kg were displayed (Mohamed-Ahmed et al., 2013). Formulation of nanometric amphotericin B (AmB) encapsulated chitosan-nanocapsules (CNC-AmB) using polymer deposition technique mediated by nano-emulsion template fabrication has been found to have an IC_{50} of 0.19±0.04 µg *in vitro* and 86.1±2.08% of parasite inhibition in *Leishmania donovani* infected hamsters (Asthana et al., 2013).

Miltefosine

Miltefosine (hexadecylphosphocholine) is the first orally administered drug for VL and is associated with high efficacy rates, including cases unresponsive to antimonials (Ritmeijer et al., 2006). In phase IV multicenter trial in India of 1132 adults and children treated with miltefosine, cure rates were 82% and 95% per protocol analysis. In this study, 3% of patients developed adverse effects, mainly gastrointestinal toxicity, and elevated hepatic transaminases and creatinine (Bhattacharya et al., 2007). So far, miltefosine is licenced in India, Germany, and Colombia. The dose of miltefosine is 100 mg/kg/day for 28 days in adults weighing ≥50 kg, 50mg/kg/day in adults <50 kg, and 2.5mg/kg/day in children (maximum dose: 100 mg/day). Major concerns for the wide use of miltefosine include its teratogenic potential and its long half-life (approximately 150 hours) which may facilitate the emergence of resistance. Miltefosine is strictly forbidden in women of child-bearing age who may become pregnant up to two months following drug discontinuation. In India miltefosine is available over the counter, a fact that may expose this drug to misuse and emergence of resistance. Once generated, resistant parasites could spread rapidly, endangering the life span of miltefosine in a country where it is needed most (Maltezou, 2010). The exact antileishmanial mechanism of miltefosine remains largely unknown, however, it has been found that miltefosine induces an apoptosis like cell death in *L. donovani* (Perez Victoria et al., 2006).

Miltefosine (5 mg/kg × 5 days, orally) in combination with fluconazole (50 mg/kg × 5 days, orally) and picroliv showed 88% of antileishmanial efficacy against *L. donovani* infected hamsters (Shakya et al., 2011). *L. donovani* infected hamsters treated with combination of ketoconazole (50 mg/kg, 5 days, po)+miltefosine (5 mg/kg, 5 days, po) showed augmentation in efficacy against *Leishmania* parasite (72%) in comparison to those treated with ketoconazole (54.67%) and miltefosine (54.77%) separately. Co-administration of picroliv (10 mg/kg, 12 days, p.o.) further enhanced antileishmanial efficacy from 72% to 82% (Shakya et al., 2011). A combination of Pam3Cys (an inbuilt immunoadjuvant and TLR2 ligand) and miltefosine has been tried recently in BALB/c mouse model and significant enhancement in parasitic inhibition has been observed as compared to groups receiving miltefosine and Pam3Cys separately (Shakya et al., 2012).

Resistance to miltefosine may emerge easily during treatment due to single point mutations. Decrease in drug accumulation is the common cause in all miltefosine resistant *Leishmania* lines (Seifert et al., 2007). Two proteins, miltefosine transporter LdMT and its specific beta subunit LdRos3, form part of the miltefosine translocation machinery at the parasite plasma membrane, and are required for miltefosine uptake. However, experimental mutations at LdMT or

LdRos3 rendered the parasites remarkably less sensitive to miltefosine (Perez- Victoria et al., 2006). The overexpression of ABC transporters is another mechanism for acquisition of miltefosine resistance, through reduction of the drug intracellular accumulation (Castanys-Munoz et al., 2008).

Paromomycin

Paromomycin (aminosidine) is an aminoglycoside with antileishmanial activity. It is one of the drugs being used in chemotherapy of CL and VL (Chawla et al., 2011). Currently, paromomycin is under phase IV clinical trials. (Sundar et al., 2007). A 21-day course of 20 mg/kg/b.wt. should be used as first line treatment for VL (Sundar *et al.*, 2009). In a phase III study of VL in India, this drug was associated with 94.6% cure rates, similar to amphotericin B, however, its use is associated with adverse effects like elevated hepatic transaminases (Sinha et al., 2011), ototoxicity and pain at injection-site (Banerjee et al., 2011). Moreover, problem of drug resistance can grow if there is no control of appropriate use (Wiwanitkit, 2012). Since, monotherapy with PM runs the risk of development of resistance, efforts are needed to develop a combination therapy of PM with other drugs to shorten the duration of treatment and prolong the effective life of the drug. Recently, PM was formulated with leishmanicidal stearylamine (SA)-bearing phosphatidylcholine (PC) liposomes for low-dose therapy. This combination reduced the parasite load with an immunomodulatory effect on CD4+, CD8+ cells, IFN-γ production and downregulation of disease-associated IL-10 and transforming growth factor β (TGF-β) to almost negligible levels (Banerjee et al., 2011).

Paromomycin inhibits protein synthesis and modifies membrane fluidity and permeability. An *in vitro* study showed that following a 72-hour exposure to *L. donovani* promastigotes and amastigotes to paromomycin, the mitochondrial potential was decreased, which indicates that mitochondria are the targets of the drug. (Jhingran et al., 2009). In studies on selected populations of promastigotes, resistance to paromomycin was related to decreased drug uptake in *L. donovani* (Maarouf et al., 1998) but due neither to enzymatic modifications nor to any mutation of the small-subunit rRNA gene in *L. tropica*.

Pentamidine Isethionate

It is an aromatic diamidine and has been previously used as a second line drug. Its anti-leishmanial activity is due possibly to polyamine biosynthesis and its effect on mitochondrial membrane potential (Das et al., 2001). It is reported that the drug enters inside *L. donovani* promastigotes through arginine and polyamine transporters (Kandpal and Tekwani, 1997). Its isethionate and methanesulphonate salts are mainly used for the treatment of VL. The dose is 4 mg/kg intravenously slowly on alternate days for 15 injections and has a cure rate of about 70%. It has been initially used to treat Sb^v refractory patients in India but its declining efficacy and high resistance risk has led to its closure in India (Das et al., 2001). In pentamidine resistant, *L. donovani* and *L. amazonensis* promastigote clones, drug resistance is due to decreased uptake followed by increased efflux of drugs. There is alteration in polyamine carrier that might be responsible for the alteration in surface protein nature and content leading to decreased efflux of drug. Furthermore, this drug gets accumulated in mitochondria and enhances efficacy of mitochondrial respiratory chain complex II inhibitors suggesting its leishmanicidal activities due to decreased mitochondrial membrane potential. It is also reported that it inhibits mitochondrial topoisomerase II (Basselin et al., 1999). Some combinational strategies have also been tried with this drug. A study on antimony unresponsive patient revealed that combination of low dosage of pentamidine and allopurinol as compared to full dosages of pentamidine was more effective and less toxic with ultimate cure rate of 73% and 58 %, respectively (Das et al., 2001).
Pentamidine resistance mechanism is not well understood, but intracellular ABC protein PRP1 can confer resistance to pentamidine in intracellular stage of *Leishmania* (Coelho et al., 2007). However, because of multiple toxic effects like anaphylactic shock, nephrotoxicity and diabetes mellitus (in about 10% of the patients) its use has been restricted (Pandey et al., 2009).

Allopurinol

The antileishmanial activity of the purine analogue allopurinol (Requena et al., 2004) was identified over 30 years ago. Because it had oral bioavailability and it was widely used for other clinical indications, the drug was investigated in clinical trials for CL and VL. However, the results were disappointing. Allopurinol is used as a substrate by various enzymes of the purine salvage pathway of trypanosomatids, and it is selectively incorporated into nucleic acid in the parasite. Later, allopurinol was considered as part of a maintenance therapy for canine leishmaniasis (Koutinas et al., 2001).

Sitamaquine

This is an orally active 8-aminoquinoline analogue and its effectiveness in VL has been validated in animal models. It is still in phase I and II trial stages. In one of the multicentric phase II trials it was being used at the dose of 2 mg/kg/day orally for 21 days. In another previous phase II trial in Kenya as well as India, the cure rate was about 50%. The main toxic effects were renal, like glomerulonephritis and acute tubular necrosis (Wasunna et al., 2005).

Sitamaquine at high concentrations affects parasite motility, morphology and growth (Duenas-Romero et al., 2007). Mechanism of its action involves electrostatic interaction between phospholipid anionic polar head groups and positively charged sitamaquine and then with phospholipid acyl chains leading to drug insertion within biological membranes (Coimbra et al., 2008). Although resistance against this drug has not yet been reported in clinical practices but, in vitro resistance against *Leishmania donovani* promastigote has been reported by selecting drug pressure of sitamaquine at 160 µm concentration (Bories et al., 2008).

Imidazoles/triazoles (ketoconazole, fluconazole, itraconazole)

These antifungal drugs include two distinct classes of compounds: imidazoles (eg, ketoconazole) and triazoles (eg, fluconazole and itraconazole). These two classes of compounds share the same antifungal spectrum and the same mechanism of action, but the metabolism of triazoles is slower. In addition, triazoles interfere less with sterol synthesis in humans and are thus less toxic than imidazoles. A great advantage of the azoles used in leishmaniases is their oral use and lower toxicity relative to pentavalent antimonials (Mitropoulus et al., 2010).

Cisplatin

Cisplatin is a major antineoplastic drug used for the treatment of various types of tumors (Naqshbandi et al., 2012). It is an organic complex formed by an atom of platinum surrounded by chloride and ammonium atoms in the cis position of a horizontal plane (Taguchi et al., 2005). The mechanism of action of cisplatin involves entering the cell, where chloride ion dissociates leaving a reactive complex that reacts with water and then interacts with DNA. It causes intrastrand cross-linking, probably between N^7 and O^6 of the adjacent guanine molecules, which results in local denaturation of the DNA chain (Taguchi et al., 2005). *In vitro* studies have also indicated that interaction between the cisplatin molecule and the DNA may contribute to the generation of superoxide radicals, causing further toxicity to cancer cells (Scarisbrick et al., 2006). It is given either intravenously or intraperitoneally, binds to serum proteins and distributes to most tissues (Royer et al., 2005), however, in the first hour, it accumulates in the kidney, liver, muscle and skin (Maltezou, 2010).

Its antileishmanial potential has been evaluated *in vitro* at a concentration of 0.25-0.64 µM (Tavares et al., 2007). The *in vivo* antileishmanial efficacy of cisplatin has been studied in our laboratory for the first time at two low doses of 0.5 mg and 1 mg/kg b.wt. given for five days, i.p. in *L. donovani* infected BALB/c mice after 30 post infection days. Cisplatin treatment in infected mice resulted in significant reduction in hepatic parasite load with heightened DTH responses, leucopenia with mild hepatotoxicity and nephrotoxicity. It has been found that these doses of cisplatin reduced the parasite burden but could not completely eliminate the parasite (Kaur et al., 2010).

Our further studies have demonstrated that treatment of *L. donovani* infected mice with cisplatin along with herbal drugs (*Tinospora cordifolia, Withania somnifera* and *Asparagus racemosus*) resulted in decreased parasite load with heightened delayed type hypersensitivity responses (DTH), increased levels of IgG2a, IFN-γ, IL-2, CD4+ cells and NK 1.1 cells (and no histopathological changes) over that of IgG1, IL-4, 1L-10, CD8+ and CD19 cell levels in infected mice (Sachdeva et al. 2013, 2014 a,b). Thus, all the above mentioned studies and those still in process aim to obtain new drugs to be used in leishmaniasis treatment with low or no toxic effects compared to that of current treatment. Importantly, the costs of the treatment should be minimized to allow its dissemination and use mainly in poorer countries, where there is a high incidence of this disease.

Conflict of Interest

Authors declare no conflict of interest.

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