Review Article

Resistant leishmaniasis chemotherapy: Present status of drug development

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Abstract

Leishmaniasis or kala-azar, principally emphasising on visceral leishmaniasis (VL) has been one of the most ignored tropical disease for decades despite of its significant epidemic in tropical and subtropical areas. Out of twenty intracellular protozoan parasite species of *Leishmania*, *Leishmania donovani* and *L. infantum* have been the most prevalent species that causes Leishmaniasis. Major systemic toxicities, high ceiling pharmaconomics, non-effectiveness of the existing therapy and emergence of resistance development are the major areas that escalate the treatment failures further. Presently pentavalent antimonials come in first line therapy and failure of which the second line drug like Amphotericin B normally considered. Antineoplastic agent like miltefosine introduced as first effective oral treatment for VL; but development of resistance keeps the therapy in vein. Recently, Andrographolide proven to be the new categories exerts significant antiproliferative agent that exerts significant effects on *Leishmania donovani* parasite life cycle. Initially sodium stibogluconate (Sb) was proven clinically effective but due to increasing number of resistance the existing therapy is becoming harder presently. In this scenario, multiple trials has been tried out for development of new therapy especially emphasising on development of potential formulations using nanotechnology is in the pipe-line, providing site-specific drug delivery with lesser side effects. The present review has being tried out to focus on the development of drug delivery system using nano-formulation against resistant Leishmaniasis.

Keywords: Resistant leishmaniasis, chemotherapy, antileishmanial drug, nano-formulations, andrographolide

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Introduction

Leishmaniasis is composed of group of diseases caused by *Leishmania* protozoan parasite of trypanosomatids family, initiates with mild cutaneous lesions to critical visceral injury. Leishmaniasis effects almost all tropical and subtropical parts of world effecting nearly 1.2 crore people per year [1]. Presently the therapy against this infectious disease is not sufficient. Following are the limitations of current therapy: toxicity, high cost, non-effectiveness, resistance development or even hospitalization.

Injectable pentavalent antimonials (Pentostam® and Glucantime®) are the first line recommended chemotherapy treatments, due to its severe clinical toxicities and increasing incidence of drug resistance has been marked in several parts of the world [2]. Amphotericin B considered a second line drug, has been the first line therapy in Bihar, India, following the loss of effectiveness of antimonial drugs. As it has high toxicity, it requires careful and slow intravenous administration [3]. Liposomal amphotericin B formulations have also been developed in order to improve toxicological and pharmacokinetic properties. Unfortunately, their high cost and relapse in immunocompetent patients are the main restrictions especially in under developing countries [4]. Antineoplastic agent, miltefosine, was introduced as being the first efficient treatment orally for VL, which is also a substitute treatment for HIV patients. But the physical abnormality and increasing number of resistance are the markable limitations in pregnant

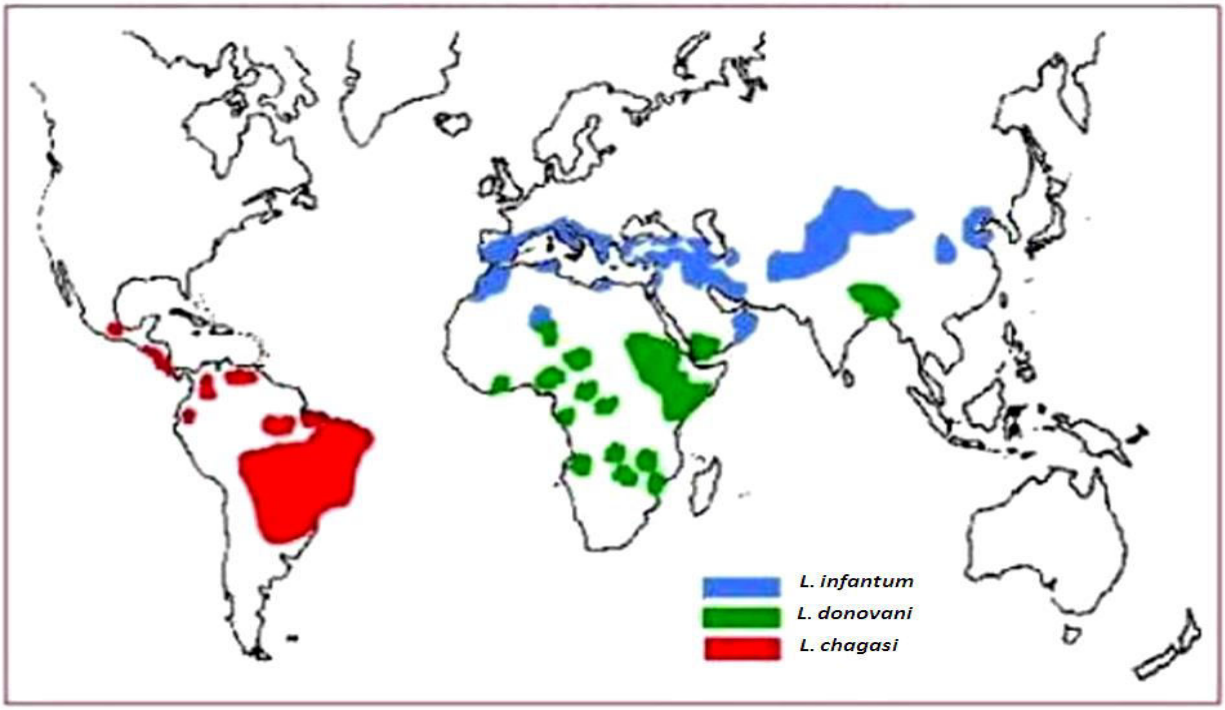


Figure 1: Distribution of visceral leishmaniasis

women [5, 6]. In clinical trial injectable aminoglycoside like paromomycin comes out with 95% efficacy and introduced in India with additional indication for the treatment of VL. In addition to that co-administration of available antileishmanial drugs had also been tried out to offer safer and efficacious treatment of VL [8]. Recently, andrographolide, a labdane diterpenoid isolated from the leaves of *Andrographis paniculata*, showed significant antiproliferative effects on the life cycle of *Leishmania donovani* [9, 10]. However, this compound has some drawbacks, such as low aqueous solubility, poor bioavailability and short plasma half-life making their potential development into chemotherapeutic agent prohibitive. By using nano drug delivery system one can reduce their cytotoxicity side effect, increase site-directed drug delivery and aqueous solubility properties, hence contributing to their bioavailability.
The expected outcome of this review are (i) recently introduced drugs which are still in different phases of clinical trials or registration not yet done, (ii) some compounds and drugs which have shown effectiveness in various animal models, need more concentration for development; and (iii) specific methodologies involved in the establishment of new drugs.

Current situation

There are several proposed theories are available regarding origin of Leishmania. But the actual is not clear till date [11, 12]. One theory focused on the migration from African origin to Americas. In addition, migration from Americas to Old World across Bering Strait Land Bridge millions of years ago. Palearctic origin is also a proposed theory [13]. Successive adaptation results in migration of vector and reservoir. From Mediterranean Basin *Leishmania infantum* migrated to Latin America named *Leishmania chagasi* (Figure 1). Many tropical and subtropical areas are more prone to leishmaniasis transmission. It is found in about 88 countries including Old World and New World [14]. Parasitic diseases continue to be major causes of human misery and death in the world. By a conservative estimate, there are about 65,000 thousand described species of protozoa distributed among seven named phyla [15]. Of those, only a few species cause disease in humans, but these few inflict much misery and death on millions of people. Enteric fever, malaria, trypanosomiasis, leptospirosis, HIV and leishmaniasis are exponentially increasing and life threatening. Among these, malaria, trypanosomiasis and leishmaniasis are caused by parasitic protozoa. These diseases occur in area far from the main stream of medical research of the industrial world, and only substantial contact of the later with these diseases has during military operation [16]. The World Health Organization has established a special research programme into the most prevalent parasitic diseases like malaria, schistosomiasis, trypanosomiasis and leishmaniasis. The working scientific working group is presently emphasing on epidemiology of leishmaniasis, vaccine studies and development of novel compounds addendum with existing therapy [17, 18].

Life cycle of *Leishmania donovani*

Leishmania donovani which is a digenetic parasite needs two hosts to pass its life cycle (Figure 2). The principal agent of transmission in nature is the sand-flies. The female sand-fly is the one to transmit the infection by feeding on blood. The male sand-fly on the contrary does not feed on blood, but sucks plant juices. Hence the male sand-fly does not take part in transmitting the infection. The non-flagellated intracellular amastigotes proliferates in the acid pH of lysosomes of human macrophages [19, 20]. When an infected sand-fly vector stings a human host, the infective promastigotes enter into the subcutaneous tissue of the host. They are converted into intracellular non-flagellated amastigote form after they are phagositosed by mononuclear phagocyte (Figure 3). Inside macrophage the multiplication of promastigotes with the help of cell division carries on until the hosts cell can bear no longer and ruptures.

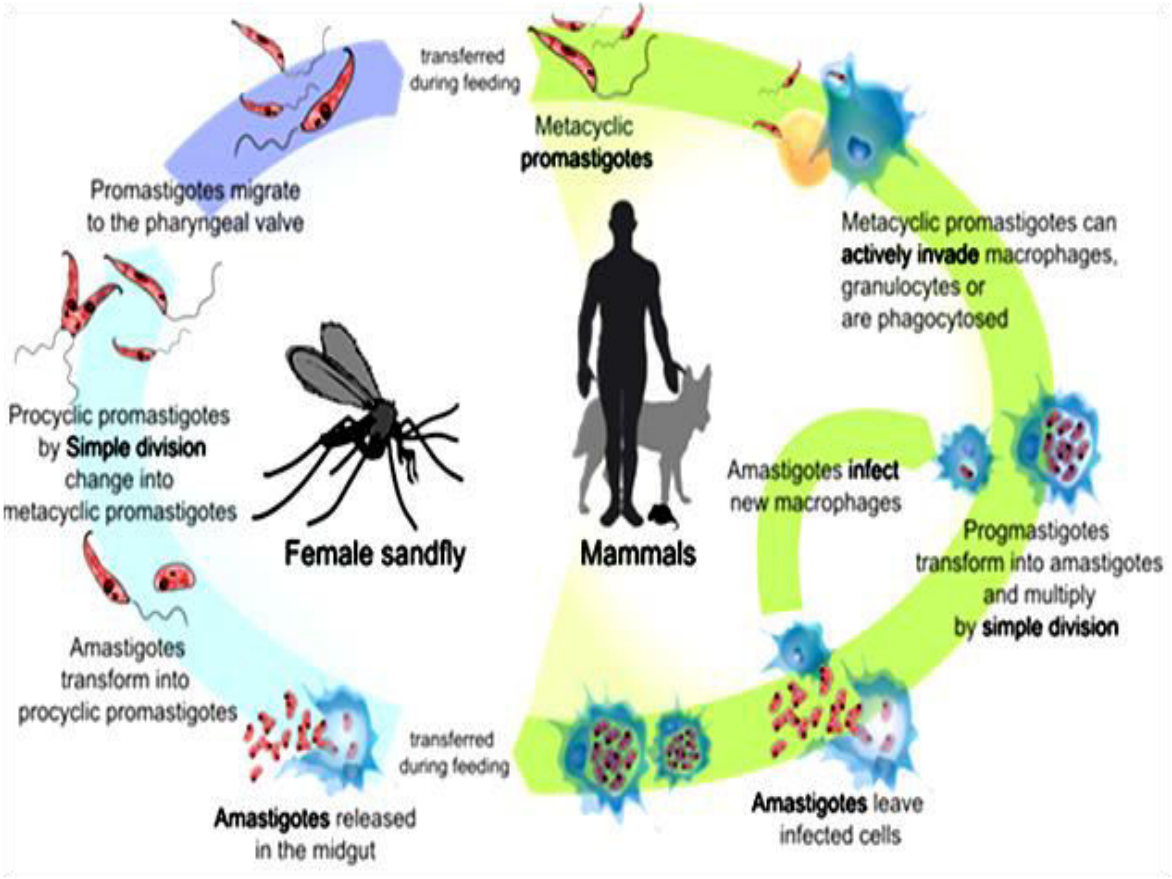


Figure 2: Life cycle of *Leishmania donovani*

Treatment of leishmaniasis

Sodium stibogluconate: In spite of its unknown mechanism of action antimony compounds are used effectively more than 50 years. Moreover the pentavalent form Sb-V required to be reduced to its trivalent form for exerting effective therapeutic efficacy [21]. Another study says that the metal is reduced in the host macrophage [22]. Either enzymatic reaction may cause reduction, or by parasite or host derived thiols [23, 24]. In *in-vitro* studies, it was found that the Sb-V may reduce to Sb-III by Parasite-specific thiols known as trypanothione and macrophage-specific thiols, glycylcysteine non-enzymatically. A current study says that a parasite-specific enzyme thiol dependent reductase, which has similarities to omega glutathione transferases, catalyzes the transformation of Sb-V to Sb-III using a reductant, glutathione [25]. Another report says that a new paracytic antimoniate reductase was found in *Leishmania* shows significant reduction of Sb-V in *Leishmania* cells [26].

Amphotericin B: An antifungal drug amphotericin B
deoxycholate is also became an effective antileishmanial drug and considered as the second-line treatment for visceral leishmaniasis. But the main disadvantage is its acute toxicity attracts in terms of its administration. To overcome this problem, amphotericin B was reformulated and it's pharmacokinetics properties were improved. Lipid was coupled with amphotericin B and the preparation has shown remarkable success and finally the liposomal amphotericin B was developed, is used for the treatment of VL [27]. Later two commercial products, lipid formulation of amphotericin B and preparation of colloidal dispersion were also available but pretend to be inferior in terms of cost of therapy and effectively [28, 29].

Miltefosine: First, miltefosine was established as an anticancer agent. Later it was found effective against VL when administered through oral route [30]. In 1980, Antileishmanial activity of this drug was

identified and the activity was confirmed on various *in vitro* and *in vivo* experimental models [31-33]. Based on the report, clinical trials were successfully performed and finally it was marketed in Indian market in March 2002 [34]. But resistance development was found very soon [35].

Paromomycin: an aminoglycoside antibiotic in the therpy during 1960 later found clinically effective against *Leishmania* sp. but due to its poor bioavailability by oral route kept it aside for treatment of Leishmaniasis; and the improvement of its bioavailability was a challenge. Soon more attention was given to develop an injectable formulation of paromomycin. Phase III clinical trials had been initiated on the preparation. The specific resistance to paromomycin was stable and it may be due to poor uptake of drug [36].

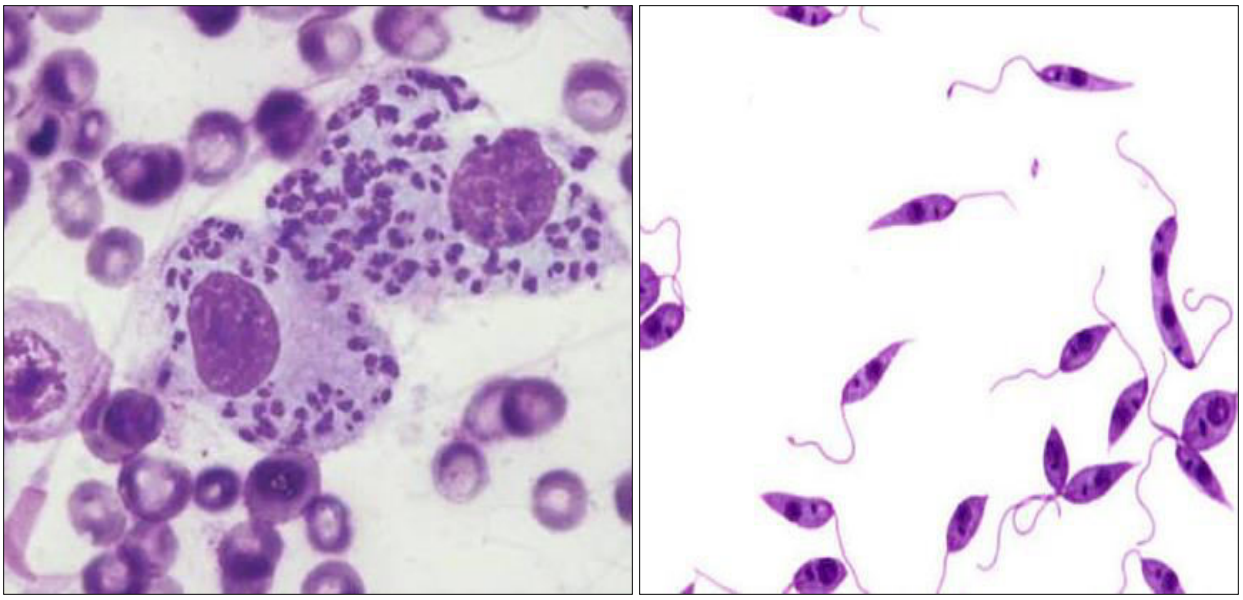
In vitro assays of Antileishmanial drug discovery

Promastigotes: In these assay functional and live promastigotes use to be estimated after treating them with test drug. In spite of the biochemical and structural difference between promastigotes and amastigotes the sensitivity of the test keeps the ahead for primary screening of the antileishmanial drugs [37-40]. In case of bioassay-guided fractionation of plant products, the promastigotes assays are most useful indicators for cytotoxicity.

Macrophage - amastigote models: Most commonly used models to develop antileishmanial drugs is either macrophages from mice peritoneal cavity or monocyte of human, which are transformed as host cells. In respect of drug sensitivity, the species/strain variation was reported for these models [37, 41]. The effectiveness of a drug is determined by either microscopical counting method or percentage of infected is calculated or number of amastigotes/macrophage [42]. The colorimetric or fluorometric methods are also be used to screen the antileishmanial drugs.

Axenic amastigotes: Protocols for axenic *L. donovani* amastigote cultures have been discussed in various articles [43, 44]. The biochemical and immunological markers of amastigotes are used and data interpretation related to the elevated serum concentration must be confirmed as these requires in the method. It was observed that there were significant drug sensitivity differences among the axenic *L. donovani* amastigotes and amastigotes in macrophages [44].

Automated screening: Absence of automation and dependence on microscopical evaluation is a major drawback of the amastigote-macrophage model. Promastigote assays using reazzurin (Alamar Blue) and transfected parasites have been successful but not in clinical model of amastigote-macrophage [45, 46]. Various groups had transfected reporter genes into *Leishmania* successfully; however, the majority of them require drug selection to maintain the plasmid, as in Trypanosoma cruzi85. Recently, drug-screening assay in 96-well plates has been introduced and compared with microscopical evaluation [47] with *L. donovani*.



A B

Figure 3: Microscopic view of *Leishmania* amastigotes in phagolysosomes of mammals [A] and *Leishmania* promastigotes in the sandfly vector [B].

***In vivo* assays**

To observe the drug activity on animal models it is to be resolute in respect to absorption (drug administration route), distribution (infected sites), metabolism and excretion. The toxicity of a drug is also a major concern and its early indications are the most associated parameter for study. Mice are prone to maximum strains and various *Leishmania* species. The animal models were used to ensure the short course effectiveness and to establish a oral dose of a drug with no or less sign of toxicity at the highest doses. The commonly used strain is BALB/c mouse and average body weight should be between 18-20g which will help to get high levels reproducible results. After confirmation of infection, drugs are administered to observe the activity on the liver and the spleen of mice. The infection in individual mouse needs to be checked for particular strains of parasite used to confirm to check the effectively of the drugs [48, 49].

Nano-formulations for antileishmanial drug resistant visceral leishmaniasis

PLGA nanoparticles using Polyvinyl alcohol (PVA)

Andrographolide is a diterpenoid lactone obtained from the *Andrographis paniculata* leaves. It is a effective antileishmanial agent with low-toxicity. Andrographolide has a disadvantage of poor bioavailability, short plasma half-life, and unexpected tissue localization, so chemotherapy application has limitations. So preparation and development of andrographolide nanoparticles has been tried. It was being developed by an emulsion-solvent-evaporation technique. Andrographolide nanoparticles (AGnp) were prepared by loading in 50:50 poly(DL-lactide-co-glycolic acid) for inclusion inside the *leishmania* infected macrophage to treat amastigote parasite [9].

PLGA nanoparticles using vitamin E TPGS

P-gp efflux inhibitor vitamin E TPGS (D-a-tocopheryl polyethyleneglycol 1000 succinate) were being used to develop andrographolide nanoparticles for treating drug resistant visceral leishmaniasis. Andrographolide loaded PLGA (50:50) nanoparticles (AGnps) was stabilized by using vitamin E TPGS, which were prepared to incorporate into macrophage cells infected with drug resistant strain of *Leishmania* parasites. Physicochemical parameters of AGnps were characterized by photon correlation

spectroscopy and an average particle size of 179.6 nm, polydispersity index of 0.245 and zeta potential of 237.6 mV was confirmed. AFM and TEM confirmed smooth spherical nanoparticles. AGnps showed constant release of andrographolide up to 288 hours. Antileishmanial activity was established on wild-type strain and it was found to be significant. The dose was being reduced about tenth times less compared to pure andrographolide. Cytotoxic effects also found to be significantly lesser compared to existing drugs like Amphotericin B, paromomycin or sodium antimony gluconate. Uptake of AGnps was being taken up by macrophages were faster compared to standard drug [10].

Gold nanoparticles

Gold therapy was established for multiple types of infectious diseases including leishmanisis. Considering the severe systemic side effects and erratic bioavailability researchers has tried out for development of its nano formulation. Gold nanoparticles were developed by opting of single pot synthesis which was found faster compared to existing methods resulting uniformity in size in conjugation with quercetin. In this method reduction was carried out in low temperature. As discussed before that the *leishmania* amastigotes suitably multiply in infected macrophages and in maximum cases drug resistance have been developed to available medical therapies. Currently developed gold nanoparticles conjugated with quercetin were effectively evaluated against *leishmania* infected macrophage [50].

Conclusion

Drug resistance and adverse effects to the antileishaminal chemotherapy have forced the scientific research community to involve for the discovery of new improved alternative drugs.
However, the lack of marketable return for the neglected diseases like leishmaniasis, has resulted in disappointing funding for novel antileishmanil drug develoment.
Greater understanding of resistance mechanisms and use of safe and efficient nano-carriers may be a better strategy to alter the whole scenario of leishmaniasis chemotherapy to benefit patients who are mainly neglected poor people in the future.

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Conflict of interest

None

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