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Review

Recent advances in leishmaniasis treatment

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SUMMARY

About 1.5 million new cases of cutaneous leishmaniasis and 500 000 new cases of visceral leishmaniasis occur each year around the world. For over half a century, the clinical forms of the disease have been treated almost exclusively with pentavalent antimonial compounds. In this review, we describe the arsenal available for treating *Leishmania* infections, as well as recent advances from research on plants and synthetic compounds as source drugs for treating the disease. We also review some new drug-delivery systems for the development of novel chemotherapeutics. We observe that the pharmaceutical industry should employ its modern technologies, which could lead to better use of plants and their extracts, as well as to the development of synthetic and semi-synthetic compounds. New studies have highlighted some biopharmaceutical technologies in the design of the delivery strategy, such as nanoparticles, liposomes, cochleates, and non-specific lipid transfer proteins. These observations serve as a basis to indicate novel routes for the development and design of effective anti-*Leishmania* drugs.

1. Introduction

Leishmaniasis is an infectious disease caused by parasites of the genus *Leishmania* in the family Trypanosomatidae. The disease manifests as three types: cutaneous, mucocutaneous, and visceral leishmaniasis, which is also known as kala-azar.[1,2] Cutaneous leishmaniasis, the most common form, is a group of diseases with a varied spectrum of clinical manifestations, which range from small cutaneous nodules to gross mucosal tissue destruction.[3] Visceral leishmaniasis is the most severe form, in which the parasites have migrated to vital organs. It is a severe, debilitating disease, characterized by prolonged fever, splenomegaly, hypergammaglobulinemia, and pancytopenia. Patients gradually become ill over a period of a few months, and nearly always die if untreated.[4]

Leishmaniasis is transmitted through the bite of female phlebotomine sandflies infected with the protozoan. The parasite is then internalized via macrophages in the liver, spleen, and bone marrow.[5] *Leishmania* parasites are dimorphic organisms, i.e., with two morphological forms in their life cycle: amastigotes in the mononuclear phagocytic system of the mammalian host, and promastigotes in the digestive organs of the vector.[6]

About 1.5 million new cases of cutaneous leishmaniasis and 500 000 new cases of visceral disease occur each year. Cutaneous leishmaniasis is endemic in more than 70 countries worldwide, and 90% of cases occur in Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia, and Syria.[3,7,8] Visceral leishmaniasis occurs in 65 countries; the majority (90%) of cases occur in agricultural areas and among the suburban poor of five countries: Bangladesh, India, Nepal, Sudan, and Brazil.[1,9] The number of cases is increasing globally at an alarming rate.[10] Ecological chaos caused by humans has enabled the leishmaniases to expand beyond their natural ecotopes, and this in turn affects the level of human exposure to the sandfly vectors.[11] Cases of *Leishmania* and human immunodeficiency virus (HIV) co-infection have also recently increased.[12,13]

The classical treatment of leishmaniasis requires the administration of toxic and poorly tolerated drugs. The pentavalent antimonials – meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam) – are the first-line compounds used to treat leishmaniasis. Other drugs that may be used include pentamidine and amphotericin B.[14,15] However, parasite resistance greatly reduces the efficacy of conventional medications.[16] In the last 15 years, clinical misapplication of medications has enabled the development of generalized resistance to these agents in Bihar, India, where half of the global visceral leishmaniasis cases occur.[7] Moreover, there are no effective vaccines to prevent leishmaniasis.[17,18]

The purpose of this review is to discuss the current treatment for leishmaniasis and to highlight recent advances in the development of novel chemotherapies.

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2. Current treatment and recent advances

Pentavalent antimonials were developed in 1945, and remain the first-choice treatment for both visceral and cutaneous leishmaniasis in most parts of the world. Amphotericin B and pentamidine are the second-line antileishmanial drugs, although they require long courses of parenteral administration.[19] The choice of treatment also depends on the causative *Leishmania* species.[20] The most common syndrome is localized cutaneous leishmaniasis (CL), which is most frequently caused by *Leishmania major* and *Leishmania tropica* in the Old World (Mediterranean basin, Middle East, and Africa), and by *Leishmania braziliensis*, *Leishmania mexicana*, and related species in the New World (Mexico, Central America, and South America).[21] A study of 103 patients with CL in Peru showed that among patients infected with *Leishmania (Viannia) peruviana* (47.6%), *Leishmania (Viannia) guyanensis* (23.3%), and *Leishmania (Viannia) braziliensis* (22.3%), 21 of them (21.9%) did not respond to pentavalent antimonial chemotherapy. Therefore, accurate identification of the parasite is of great clinical importance, because it will guide the choice of an appropriate treatment.[20] Although spontaneous cure is the rule, the rate of recovery varies depending on the species of *Leishmania*, and may require months or years to complete healing.[3]

Most of the commonly used drugs are toxic and do not cure, i.e., eliminate the parasite, from infected individuals.[19] Failure to treat leishmaniasis successfully is often due to increased chemoresistance of the parasite.[15,22]

Because treatment is a growing problem, the development of new medicines that can replace or complement the presently available therapeutic alternatives is necessary. Encouraging advances in chemotherapy have been made in recent years. Antileishmanial chemotherapy has improved since the development of lipid formulations of amphotericin B, which is a much less toxic treatment for fungal infections, and has been exploited for the treatment of leishmaniasis.[23] The unilamellar liposomal formulation (AmBisome®), lipid complex (Abelcet®), and colloidal dispersion (Amphocil™) have all been evaluated in clinical trials for visceral leishmaniasis and/or mucocutaneous leishmaniasis. Yardley and Croft (2000) found that AmBisome and Amphocil are more effective (50% effective dose (ED_{50}) values 0.3 and 0.7 mg/kg, respectively) than Abelcet (ED_{50} 2.7 mg/kg) against *Leishmania donovani* in a mouse model. AmBisome (25 mg/kg) was the most successful in reducing the size of lesions caused by *L. major*, and Amphocil (12.5 mg/kg) also showed activity, whereas Abelcet was inactive against this species.[24]

However, the high cost of these amphotericin B preparations precludes their widespread use in developing countries. New formulations involving microcapsules made of albumin, which is a cheap and effective carrier system and provides effective protection against phagocytic cells, have been tested. Microspheres of hydrophilic albumin with three amphotericin B aggregation states (monomeric, dimeric, and multiaggregate) and a multiaggregate form encapsulated with two commercial polymers were tested against *Leishmania infantum* (both extracellular promastigote and intracellular amastigote forms). The albumin-encapsulated forms showed no toxicity to murine cells and had lower 50% effective concentration (EC_{50}) values (0.003 μg/ml) for amastigotes than did the free formulations (0.03 μg/ml). These promising results have increased interest in amphotericin B encapsulated in microspheres, and in exploring new chemotherapeutic approaches.[25]

Miltefosine, an alkylphospholipid, was developed as an oral antineoplastic agent (for cutaneous cancers) and has subsequently been applied to treat leishmaniasis.[26] The discovery that miltefosine is effective against *Leishmania* led to the identification of a modern group of antiprotozoal medicines. Following clinical studies, miltefosine was approved as Impavido™ and has become the first oral treatment for leishmaniasis in some countries.[27] It is an effective treatment for visceral and cutaneous leishmaniasis, including for antimony-resistant infections. However, this drug may not necessarily be superior to parenteral therapies for all forms of leishmaniasis.[28] The requirement for a long treatment period (28 days) will necessitate the formulation of strategies for more rational use, in order to prevent patients from developing resistance to the drug. Studies of the resistance mechanisms have shown that possible mechanisms include a decrease in drug uptake, differential plasma membrane permeability, more rapid drug metabolism, and efflux of the drug.[29] Miltefosine was registered in India for the treatment of visceral leishmaniasis in 2002.[30] It has exhibited teratogenic potential, and therefore should not be administered to pregnant women.[31]

Other alkylphospholipids such as edelfosine and ilmofosine,[32] as well as perifosine,[33] have proved to possess potent in vitro antiparasitic activity. In 2008, Cabrera-Serra et al. tested edelfosine and perifosine orally in Balb/c mice infected with *Leishmania amazonensis*. This pre-clinical study showed that perifosine had higher activity in the *in vivo* assay and may be a possible alternative treatment against cutaneous leishmaniasis.[34]

Sitamaquine is a promising oral treatment for visceral leishmaniasis in Africa. A 28-day course of treatment was efficacious and well tolerated in 61 Kenyan patients infected by *L. donovani*, with the tested dose of 2.0 mg/kg/day; however, further studies are required to define the optimal dose. Some adverse effects included abdominal pain, headache, and a severe renal event. The effects of sitamaquine on the kidney need further investigation.[35]

Paromomycin is the only aminoglycoside with clinically important antileishmanial activity. Both visceral and cutaneous forms can be treated with this antibiotic, but poor oral absorption has led to the development of parenteral and topical formulations for the visceral and cutaneous forms, respectively.[36] In a study of patients with a visceral form of leishmaniasis in India, Sundar et al. (2007) found that paromomycin administered by deep gluteal intramuscular injection (11 mg/kg/day) for 21 days was equally effective as infusion of amphotericin B (1 mg/kg/day) for 30 days.[37] In 1992 in central Tunisia, patients with cutaneous leishmaniasis caused by *L. major* were treated with paromomycin ointment, but there was no difference between the treated and control groups.[38] However, new topical formulations of paromomycin have given good results. A randomized, controlled study was undertaken to compare the therapeutic efficacy of two paromomycin topical preparations with meglumine antimoniate. The results showed that topical paromomycin can be a therapeutic alternative for cutaneous leishmaniasis, although a longer period is required for clinical healing.[39] A hydrophilic gel containing 10% paromomycin was evaluated in Balb/c mice infected with *L. amazonensis* and hamsters infected with *L. braziliensis*. Compared to the antimony treatment, the activity of the paromomycin gel was significantly higher against *L. amazonensis*, whereas these two medications were equally effective against *L. braziliensis*. The gel formulation may represent an alternative topical treatment for cutaneous leishmaniasis.[40]

3. Plants as medicine for leishmaniasis

Plants are clearly a potential source of new antiprotozoal drugs. The biological activity of plant extracts has been attributed to compounds belonging to diverse chemical groups including alkaloids, flavonoids, phenylpropanoids, steroids, and terpenoids.[41–43] To obtain a herbal medicine or an isolated active compound, different research strategies can be employed, among them, investigation of the traditional use, the chemical composi-