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Potential therapeutic targets and the role of technology in developing novel antileishmanial drugs

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Leishmaniasis is the most prevalent pathogenic disease in many countries around the world, but there are few drugs available to treat it. Most antileishmanial drugs available are highly toxic, have resistance issues or require hospitalization for their use; therefore, they are not suitable for use in most of the affected countries. Over the past decade, the completion of the genomes of many human pathogens, including that of *Leishmania* spp., has opened new doors for target identification and validation. Here, we focus on the potential drug targets that can be used for the treatment of leishmaniasis and bring to light how recent technological advances, such as structure-based drug design, structural genomics, and molecular dynamics (MD), can be used to our advantage to develop potent and affordable antileishmanial drugs.

Introduction

Leishmaniasis is one of the most neglected tropical diseases in terms of drug discovery. It is a disease caused by protozoan parasites belonging to the genus *Leishmania*, transmitted via the bite of plebotomine sand flies. Leishmaniasis, in each of its three clinical forms, namely cutanous, mucosal and visceral, remains a serious disease in tropical and subtropical areas of the world. In 2012, the World Health Organization (WHO) reported that leishmaniasis threatened approximately 350 million people in 88 countries around the world [1,2]. This disease generally occurs in underdeveloped countries, where most patients do not avail themselves of a complete course of treatment because of the cost, availability, invasive route of administration, and long treatment duration, which, in turn, increases the chance of drug resistance [3]. This generates the need for new treatment methods and the use of recent technologies to develop new chemotherapeutic agents that are easily available to, and affordable by, the affected population. *Leishmania* parasites have a dual-form life cycle, occurring as a promastigote flagellar or an amastigote form. Promastigotes are found in the insect vector and are transmitted to the human host. Once inside the host cell, the promastigote differentiates into amastigote and multiplies until the death of the host cell. This complex life cycle of the parasite provides many targets to explore for drug design and optimization.

Unfortunately, because of a lack of commercial interest, few new drugs are being developed or introduced against this deadly disease. Currently, no effective vaccines have been developed and the control of leishmaniasis primarily relies on chemotherapy. The first-line drug, pentavalent antimony, has long been the cornerstone of antileishmanial chemotherapy, but the development of resistance against it has limited its usefulness [4]. Second-line drugs include pentamidine and amphothericin. However, toxicity and emerging resistance prevents the use of pentamidine, whereas amphothericin B has the potential to induce acute toxicity, requiring patient hospitalization. Amphotericin B in its lipid formulation (Ambisome) has proved to be efficient but the high cost is a major drawback. Miltefosine, which is an alkylphosphocholine and originally an anticancer agent [5], was registered for the treatment of visceral leishmaniasis (VL) in India in 2002 and cutaneous leishmaniasis in Colombia in 2005. It has many advantages, including oral efficiency and a short course of treatment. However, its major limitation is its teratogenicity and long half-life, which could favor the development of resistance [6]. A list of all available antileishmanial drugs, their mode of action, and specific adverse effects are detailed in Table 1.

Combination treatment for VL has been used to increase the efficiency, reduce costs, and tackle the problem of drug resistance,

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TABLE 1

Current antileishmanial drugs and their mechanism of action

Drug	Mechanism of action	Adverse effects	Dosage	Administration route	Cost[a]	Refs
Sodium stibogluconate (Fig. 2g)	Behaves as a prodrug, undergoing biological reduction to a more active/toxic trivalent form of antimony that exhibits antileishmanial activity	Cardiac arrhythmia and hepatitis, leading to reduction or cessation of treatment	20 mg/kg/day for 30 days	Intravenous or intramuscular	US$70–100 per 100-ml vial	[77]
Meglumine antimonite (Fig. 2h)	Inhibits macromolecular biosynthesis in amastigotes possibly via perturbation of energy metabolism because of inhibition of glycolysis and fatty acid beta-oxidation					
Amphotericin B (Fig. 2k)	Selectivity for 24 substituted sterols, mainly ergo sterol, the primary sterol counterpart in mammalian cells, eventually helping to increase drug selectivity toward the microorganism	Thrombophlebitis; occasional serious toxicities, such as myocarditis; severe hypokalaemia; renal dysfunction and even death	1 mg/kg/day for 15 days	Intravenous	US$7.5 per 50-mg vial	[78]
Ambisome (lipid formulation of amphotericin B)	Thought to be drug binding to parasite ergosterol precursors, such as lanosterol, causing disruption of parasite membrane	Fever with rigor and chills; nausea	1 mg/kg/day for 30 days	Intravenous	US$18 per 50-mg vial	[79]
Paromomycin (Fig. 2i)	Binds to 30S ribosomal subunit, interfering with initiation of protein synthesis by fixing the 30S-50S ribosomal complex at start codon of mRNA, leading to accumulation of abnormal initiation complex	Ototoxicity and problems in liver function	15 mg/kg/day for 21 days	Intramuscular	US$15 per course	[80]
Miltefosine (Fig. 2j)	Primary mode of action is uncertain; possible ether remodeling inhibition. Postulated apoptosis and inhibition of cytochrome C oxidase	Teratogenicity (pregnancy must be avoided during treatment and following 2 months)	2.5 mg/day for 28 days	Oral	US$70 for 56 capsules	[6]

[a] Prices as quoted in http://www.who.int/leishmaniasis.

which has been a significant challenge [7]. Combining drugs from different classes could result in shorter treatment duration and fewer adverse effects. A combination therapy of miltefosine with amphotericin B or paromomycin is efficient and could be helpful for treating antimony-resistant VL infections in India [3,8]. Studies on combined drug treatments have shown them to be effective, with fewer adverse effects [7]. The combination treatment approach is now being studied more widely, and some combinations are in clinical trials and are showing promising results [9]. However, combination therapies must be used with care. There is a possibility that, if not applied in a controlled and regulated way, the parasite could develop resistance, resulting in a rapid loss of efficacy of not one but two therapeutic options. Therefore, it is important to design suitable experimental studies to determine whether *Leishmania* parasites are able to develop resistance to the different antileishmanial drug combinations available or in development [10].

Drug discovery for many neglected tropical diseses is carried out using both target-based and phenotypic approaches. Both approaches have their own pros and cons and are being continuously explored by researchers [11]. Phenotypic screening is a powerful method and has been used successfully; this approach does not define any specific molecule or even pathways as a target, but simply selects compounds that are able to eliminate the parasite. The identification of molecular targets from phenotypic approaches can be a way to identify potential new drug targets. Target-based approaches are extensively used in the pharmaceutical industry and involve screening a library of compounds against a protein. The compounds are then optimized for potency against the enzyme, selectivity, and cellular activity [12]. In the case of parasitic diseases, there are few validated molecular targets. This is partly because of the lack of translation from target-based activity to whole-cell assays or *in vivo* activities [13]. The phenotypic screening of smaller subsets of compounds belonging to a specific

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