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Review

Visceral leishmaniasis: Revisiting current treatments and approaches for future discoveries

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ABSTRACT

The current treatments for visceral leishmaniasis are old and toxic with limited routes of administration. The emergence of drug-resistant *Leishmania* threatens the efficacy of the existing reservoir of antileishmanials, leading to an urgent need to develop new treatments. It is particularly important to review and understand how the current treatments act against *Leishmania* in order to identify valid drug targets or essential pathways for next-generation antileishmanials. It is equally important to adapt newly emerging biotechnologies to facilitate the current research on the development of novel antileishmanials in an efficient fashion. This review covers the basic background of the current visceral leishmaniasis treatments with an emphasis on the modes of action. It briefly discusses the role of the immune system in aiding the chemotherapy of leishmaniasis, describes potential new antileishmanial drug targets and pathways, and introduces recent progress on the utilization of high-throughput phenotypic screening assays to identify novel antileishmanial compounds.

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1. Introduction

Leishmaniasis is a parasitic disease caused by *Leishmania* spp., which belong to the kinetoplastids. The parasite is transmitted to humans by species of *Phlebotomine* and *Lutzomyia* sandflies (Kamhawi, 2006). Based on its clinical manifestations, the disease is classified into three types: cutaneous, mucocutaneous, and visceral leishmaniasis (VL)(Murray et al., 2005). The visceral form, also known as kala-azar, causes the most severe disease, which can be fatal if not properly treated (Boelaert et al., 2000). In Latin America and North Africa, *Leishmania infantum* is responsible for VL infections, while *Leishmania donovani* is responsible for VL infections in areas of the Indian subcontinent and of East Africa (Lukes et al., 2007; Mauricio et al., 2000). Although more than 90% of VL infections are concentrated in India, Brazil, Bangladesh, Nepal, and Sudan (Alvar et al., 2012), recent study shows a rapid increase in VL infections all over the world, and climate change is expected to cause VL to severely impact on Europe in the near future (Lindgren et al., 2012).

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L. donovani and *L. infantum* exhibit digenetic proliferation, depending on whether the parasite is in the vector stage or the mammalian stage. In humans, an intracellular, non-motile form without an apparent flagellum, called an amastigote (3–6 μM in length), replicates in macrophage cells. In the insect vector, a motile form with an extended flagellum, called a promastigote (15–30 μM in length), proliferates extracellularly (Burchmore and Barrett, 2001). When a parasite-infected female sandfly feeds on human blood, promastigotes enter into the human bloodstream and spread through the circulation. Macrophages phagocytize the promastigotes, which, instead of being degraded, invade the host cells by transforming into amastigotes. The amastigotes then multiply within parasitophorous vacuoles, resisting the oxidative stress of the lysosome. The parasites later leave the host cells and go through a series of reinfections within the spleen, liver, bone marrow, and lymph nodes, causing damage to the infected organs (van Griensven and Diro, 2012; Harhay et al., 2011). The current arsenal used to fight *Leishmania* is limited to relatively old chemotherapies that involve various forms of toxicity. The recent emergence of drug-resistant parasites is rapidly making the current treatments obsolete. This review discusses the characteristics of the currently available treatments and outlines potential strategies to develop novel chemotherapies against VL.

2. Current treatments

VL is currently treated using five drugs derived from natural and synthetic molecules: pentavalent antimonials, amphotericin B (AmpB), miltefosine, paromomycin, and pentamidine (Fig. 1). Those drugs have different origins of discovery, unique structures, and distinct modes of action (Nagle et al., 2014).

2.1. Pentavalent antimony

The activity of antimony against leishmaniasis was first introduced in the early 1900s when antimony (III) potassium tartrate was found to be effective against mucocutaneous leishmaniasis (Plimmer and Thomson, 1907). Later, efficacy was shown against the visceral form of leishmaniasis. Usage was limited, however, by the severe toxicity caused by the agent (Di Cristina and Caronia, 1915). Pentavalent antimony (V), a safer form, was developed several decades later and has been used since as the first-line treatment for VL (Brahmachari, 1928). At present, two types of organo-antimony (V) complexes are commercially available: Glucantime® (meglumine antimoniate) and Pentostam® (sodium stibogluconate). The mechanism of pentavalent antimony action is not well elucidated. Based on extensive investigations, two major hypotheses are available. The first model is based on the reductive bioconversion of Sb(V) to Sb(III) by the parasite or by the infected host cells to create an active agent (Shaked-Mishan et al., 2001). The second model posits Sb(V) as the active species (Fig. 2A).

In the first model, Sb(V) enters *Leishmania*-infected host cells and is exposed to thioles in the lysosome (cysteine and cysteinyl glycine) (Mego, 1985) and within the parasite (trypanothione) (Fairlamb and Cerami, 1992), causing its bioconversion to Sb(III) (Ferreira Cdos et al., 2003). The conversion process is more efficient at lower pH *in vitro*, which reflects the environment of the intracellular amastigote rather than that of the extracellular promastigote (Ferreira Cdos et al., 2003). Antimonite reductase (Zhou et al., 2004) and thiol-dependent reductase 1 (Denton et al., 2004) are also proposed to be involved in the conversion process. A few studies have described the downstream consequences once Sb(III), the reduced form, is generated. Sb(III) strongly binds biomolecules containing sulfhydryl moieties. Based on that characteristic, Sb(III) is reported to form conjugated pairs with trypanothione (Mukhopadhyay et al., 1996), a virulence factor that helps the parasite to resist the oxidative stress of the host cell environment. The conjugation inactivates the antioxidant activity of trypanothione. The conjugated pairs are later exported by the ATP-binary cassette transporter (Legare et al., 2001). Another proposed mechanism of Sb(III) action involves the inhibition of trypanothione reductase (TryR), an enzyme that recycles oxidized trypanothione to keep the trypanothione in a reducing state (Krauth-Siegel and Comini, 2008). Sb(III)-resistant clinical isolates show an up-regulation of the TryR gene (Rai et al., 2013). The resolved X-ray crystal structure of purified TryR in complex with SB(III) suggests that the mode of TryR inhibition is a direct interaction between TryR and SB(III) (Baiocco et al., 2009). Another proposed scenario involves the binding of Sb(III) to the HEXBP protein, a CCHC zinc finger protein, which replaces the zinc in the active site and interferes with various downstream functions involved in DNA replication and repair (Demicheli et al., 2008; Webb and McMaster, 1993).

In the second model, Sb(V), the pentavalent form, directly exerts activity against *Leishmania*. Using nuclear extracts containing topoisomerase I activity, inhibition by sodium stibogluconate was confirmed to be three times more selective against the topoisomerase of the promastigote than against the topoisomerase of the monocyte (Walker and Saravia, 2004). Sb(V) was also reported to bind to ribonucleosides, such as adenosine and guanosine, at low pH, an environment similar to that within lysosomes (Demicheli et al., 2002). The downstream effect of the binding in both promastigotes and amastigotes was shown to be a net decrease of ATP and GTP relative to ADP and GDP (Berman et al., 1987).

2.2. AmpB

AmpB, a natural antibiotic first isolated in 1955 from *Streptomyces noclosus* from Venezuela, is a macrolide polyene metabolite with antifungal and antiparasitic activities. Currently, AmpB is widely used to treat systemic *Candida albicans* and *Aspergillus fumigatus* infections and is especially effective against fungal infections in immunodeficient patients. The use of AmpB is limited, however, by severe side effects such as nephrotoxicity (Fanos and Cataldi, 2001) and hematotoxicity (Wong-Beringer et al., 1998). Recently, various types of formulations, including some that lower the free AmpB concentration in the blood stream, have been investigated to reduce the toxicity. The first antileishmanial activity of AmpB was reported in the early 1960s. Currently, the liposomal formulation of AmpB (AmBisome®) is administered intravenously to treat VL and has a 95% of cure rate for a single-course therapy (Sundar et al., 2010). Even with the high cure rate, the cost of AmBisome® treatment is a limiting factor for patients in developing countries. Despite negotiations between the World Health Organization and Gilead, the producer of AmBisome®, to reduce the price of the treatment ($18 per 50-mg ampoule), the treatment is still expensive (Meheus et al., 2010).

AmpB comprises two main core-structural components: a macrolactone ring and a mycosamine glycosylated at the C19 position. In the macrolactone ring, seven conjugated double bonds serve as a rigid backbone, while hydrophilic hydroxyl groups characterize the other side of the ring. The mycosamine group contains an amine group at the C3' position and, together with a carboxylic acid at the C16 position in the lactone ring, gives rise to the amphoteric property of the compound (Fig. 1). Those structural qualities are strongly related to the action of AmpB. A very similar compound, Amphotericin A, which differs only by a double bond between the C28 and C29 positions in the lactone ring, has significantly decreased anti-infective activity (Aszalos et al., 1985). AmpB preferentially binds to the ergosterol in the fungus or the parasite rather than to human cholesterol. In general, AmpB binds to ergosterol through a hydrophobic interaction between