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REVIEW

CONSIDERATIONS ON LEISHMANIASIS AND THE CURRENT SCENARIO FOR DEVELOPING NEW FORMS OF TREATMENT

Vanessa Nunes dos Santos Silva[1,2], Aline Caroline Da Silva Santos[2], Valéria Rêgo Alves Pereira[2] and Maria Carolina Accioly Brelaz-de-Castro[1,2]

ABSTRACT

Leishmaniasis is a neglected disease that affects millions of people around the world, mainly socially vulnerable populations and is considered a serious public health problem. Caused by several species of the flagellated protozoa of the *Leishmania* genus, it is transmitted to man through female sand fly bites. The disease can present the cutaneous, mucocutaneous and visceral clinical forms, varying according to the parasite species and depending on host immune response. Depending on its evolution, the disease may pose serious risks to the afflicted individual's health. In general, treatment for Leishmaniasis is with pentavalent antimonials, in use for approximately 70 years. However, the existing treatment for Leishmaniasis presents drawbacks such as high toxicity, several side effects, cases of resistance, highlighting the need for new efficient therapeutic approaches. Given all the problems that involve the current treatment of leishmaniasis, it is of paramount importance to seek and screen new molecules that have leishmanicidal activity, meet the safety criteria, while presenting low toxicity, low cost, easy administration and that cure efficiently. This review presents some considerations on the leishmaniasis situation, its treatment and the current panorama for the development of new therapies.

KEY WORDS: *Leishmania* spp.; therapeutics; drug development; immune response.

INTRODUCTION

Leishmaniasis comprises a group of diseases present in about 102 countries posing a serious public health problem, mainly affecting socially vulnerable populations. It is a disease with 1.3 million new cases and 20,000 to

1. Federal University of Pernambuco, Parasitology Laboratory, Academic Center of Vitória (UFPE- CAV), Vitória de Santo Antão, Pernambuco, Brazil.
2. Oswaldo Cruz Pernambuco Foundation (Fiocruz/PE), Department of Immunology, Recife, Pernambuco, Brazil.

Vanessa Nunes dos Santos Silva: https://orcid.org/0000-0002-9985-2427; Aline Caroline Da Silva Santos: https://orcid.org/0000-0002-8592-395X; Valéria Rêgo Alves Pereira: https://orcid.org/0000-0002-7995-7360; Maria Carolina Accioly Brelaz-de-Castro: https://orcid.org/0000-0003-2205-0618

Corresponding author: Maria Carolina Accioly Brelaz-de-Castro, Parasitology Laboratory, Federal University of Pernambuco-Academic Center of Vitória (UFPE- CAV), Vitória de Santo Antão, Rua do Alto do Reservatório s/n, Bela Vista, CEP: 55608-680, Vitória de Santo Antão, PE, Brasil. E-mail: carolina.brelaz@gmail.com

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30,000 deaths each year (PAHO, 2019). In addition, the number of people residing in endemic areas, at imminent risk of transmission, amounts to approximately 1.7 billion individuals, which makes the disease even more distressing (Pigott et al., 2014). These data also imply that there may be many underreported cases (Brazil, 2017; Negrão& Ferreira, 2014).

The disease is caused by several species of flagellated protozoa of the *Leishmania* genus transmitted to man through female sand fly bites, and may present the cutaneous, mucocutaneous and visceral clinical forms, varying according to the species of parasite and depending on the host immune response. According to its evolution, the disease may pose serious risks to the individual's health (da Silva et al., 2017; de Oliveira et al., 2020; Gurung &Kanneganti, 2015).

This genus presents a diversity of species and about 22 are capable of infecting humans and other animals. In Brazil, three species are considered the main causal agents for the development of the tegumentary form: *L. (V.) braziliensis, L. (V.) guyanensis* and *L. (L.) amazonensis. L. (L.) infantum* is associated with the visceral form (PAHO, 2019).

The clinical forms observed in leishmaniasis can develop according to certain factors, such as the geographical distribution of the parasite and insect vectors, as well as those directly linked to the individual, such as the relationship between the parasite species and host immune response, age, nutritional status, co-infections such as HIV and genetic factors. These parasites develop in the cells of the mononuclear phagocytic system of the infected individual (Aires, 2017; Brelaz-de-Castro, 2013).

Depending on the aforementioned factors, the clinical forms can be classified as cutaneous (CL) and mucocutaneous (MCL) leishmaniasis, which occurs when the parasite species present tissue tropism for skin and mucocutaneous tissue macrophages, and the visceral clinical form (VL), when they present tropism for tissues of the mononuclear phagocyte system in visceral organs (Kulkarni et al., 2014; Santos, 2017). The visceral form is the most severe form of the disease, and can be lethal when not treated (Kevric et al., 2015; WHO, 2017).

Regarding CL, epidemiological data show that cases are spread worldwide, with an estimated 0.6 to 1.0 million cases. The American continent is foremost regarding these numbers, with records from the extreme south of the United States to the north of Argentina, with the exception of Chile and Uruguay (Brazil, 2017).

In Brazil, there are CL cases in all regions of the country and, according to the Ministry of Health; from 2003 to 2018 more than 300,000 cases were recorded, averaging 21,158 cases per year. This further highlights the major public health problem posed by this parasitosis. The Northeast region stands out in this scenario, with highly prevalent numbers. This may be due to the extensive areas of Atlantic Forest presenting a great diversity of vectors and reservoirs with different transmission patterns (Brazil, 2017; Brito et al., 2015; Guedes Farias et al., 2019).

Regarding VL, numerous cases are also spread throughout the world and recurrent outbreaks have been occurring in eastern Africa, causing high morbidity and mortality, highlighting this clinical form of the disease, in view of the high lethality that is observed in immunocompromised individuals and cases of co-infection with HIV. Epidemiological data suggest that there is an incidence of about 0.2 to 0.4 million cases of VL in the world, but the vast majority of cases occur mainly in rural and suburban areas of the following six countries: Bangladesh, Brazil, Ethiopia, India, Sudan and South Sudan (Okwor & Uzonna, 2016; WHO, 2021).

In Brazil, records on the number of VL cases are mainly restricted to rural areas with few urban regions, but over the last few years the disease has also been expanding to urban centers. Currently, all regions of the country confirm cases annually, especially the North, Northeast, Southeast and Midwest regions. The latest data showed that this rise in the number of cases was mainly due to the peri-urbanization and urbanization of the disease, a scenario that is strongly related to the migratory flow of people between these regions and the environmental changes that modify the ecosystem (Aguiar & Rodrigues, 2017; Brazil, 2017).

CURRENT LEISHMANIASIS TREATMENT

First line therapy adopted for leishmaniasis consists of pentavalent antimonials (Sb^{+5}), which have been used for over 70 years. Nowadays, there are two formulations available on the market, N-methylglucamine antimoniate (Glucantime®), considered the first choice for treatment in Brazil, and sodium stibogluconate (Pentostan®) whose commercialization is not allowed in the country (Brazil, 2017; Zulfiqar et. al., 2017).

According to the World Health Organization (WHO), the therapeutic dose of these antimonials should be calculated in milligrams according to body weight per day (20 mg Sb^{+5}/kg/day). This is a contraindicated treatment for pregnant women, patients over 50 years of age, patients with heart disease, kidney disease, liver disease and hypersensitivity to medication components (Brazil, 2017).

Pentavalent antimonials are used to treat all clinical forms of the disease, being administered intramuscularly or intravenously at the recommended therapeutic dose for 20 to 30 days. During treatment, several adverse effects may develop, such as: arthralgia, myalgia, anorexia, nausea, vomiting, gastric fullness, epigastric pain, heartburn, abdominal pain, pancreatitis, itching, fever, weakness, headaches, dizziness, palpitations, insomnia, nervousness, pyrogenic shock, edema and acute renal failure. In the most severe forms, these may cause cardiac, liver and pancreatic alterations leading to treatment interruption (Mcgwire & Satoskar, 2014).The mechanism of action of these

drugs, in spite of being long-established forms of treatment, are not yet fully known. However, it is believed that the pentavalent molecule (Sb^{+5}) acts as a prodrug, being converted into trivalent antimony (Sb^{+3}), conferring greater toxicity and allowing interference in bioenergetic pathways, such as the fatty acid oxidation process and glycolysis of amastigote forms (Queiroz, 2019; Rath et al., 2003).

A recent alternative regarding this drug is the intralesional treatment with meglumine antimoniate, with restricted use in cases of localized cutaneous leishmaniasis and for recurrent CL. This form of drug application presents much milder side effects than those observed in the systemic administration. A study by Yesilova et al. (2015) showed the effectiveness of this treatment in patients infected with *L. tropica* and/or *L. major* who were cured, presenting total wound healing (Duque et al., 2016; Yesilova et al., 2016).

Despite potent action against the parasite, pentavalent antimonials have several factors limiting their use. Among the great challenges described are high toxicity, high cost, the mode of administration and length of use, requiring several doses to reach the effective therapeutic concentration (Chakravarty & Sundar, 2019). This can lead to withdrawal by the patient and the emergence of resistant strains, which have been reported, indicating treatment failure (Meheus et al., 2010). Studies highlight that India presents the most reports of resistance due to the indiscriminate use of this drug (Haldar et al., 2011; Zulfiqar et al., 2017).

For cases that are not successful after treatment with these first-line drugs or if they are contraindicated, second-line drugs are available with Amphotericin B (AmB) and Pentamidine (de Vries et al., 2015; Brazil, 2017). Amphotericin B presents excellent activity against the evolutionary forms of *Leishmania*. The parasite membrane contains ergosterol and the mechanism of action of this drug is associated with binding to these sterols, implying an increased cell permeability, which leads to the loss of cations such as K+ and causes parasitic death (Chávez-Fumagalli et al., 2015). The administration dosage recommended by the Brazilian Ministry of Health is 0.7 to 1.0 mg/kg/day, with a total dose of 25 to 40 mg/kg, administered intravenously. The side effects observed are related to its toxicity and include infusion reactions, fever, hypokalemia, anorexia, hypotension, myocarditis and aggravated impairment of liver and kidney functions, being contraindicated for patients with renal failure (Burza et al., 2018).

In order to reduce these toxicity levels, physicochemical modifications were carried out in the AmB structure, and liposomal amphotericin B (AmBisome®), amphotericin B in colloidal dispersion (Amphocil®) and amphotericin B in lipid complex (Abelcet®) were developed. These formulations use a slower release of the drug in nanostructured systems capable of forming micelles that, when captured by macrophages, release the drug inside the infected cells. The WHO recommends it for the treatment

of the disease, due to its effectiveness and reduced toxicity compared to free AmB. Side effects were also reduced, including the nephrotoxic effect presented by the conventional formulation. Although liposomal AmpB presents satisfactory results for the treatment of the disease when compared to conventional treatment, therapy based on this new formulation implies high cost and hospitalization, often making its use unfeasible (Chávez-Fumagalli et al., 2015; Oliveira et al., 2013; Singh et al., 2016).

Pentamidine, another second choice drug in the therapeutic scheme for the treatment of leishmaniasis, is a class of aromatic diamines that has also shown great leishmanicidal efficacy. However, it is highly toxic, presenting severe cardiac and gastrointestinal side effects including pancreatitis, cardiac arrhythmias, leukopenia, acute renal failure, hypocalcemia, and ventricular tachycardia (Ghorbani & Farhoudi, 2018; Brazil, 2017). Besides these side effects, there is still the risk of developing diabetes mellitus due to the drug's acute toxic action on beta-pancreatic cells (Brazil, 2017). The recommended dose is 4mg/Kg/day, by intramuscular or intravenous administration. As well as AmpB, the use of this drug is costly and there is greater need for more complex medical services for its administration (Brazil, 2017).

Another drug used is Miltefosine (ImpavidoTM), a drug that was initially developed as an antineoplastic and was redirected to treat leishmaniasis, due to its high effectiveness against the parasite. This drug became the first oral treatment of the disease in some countries, being effective in all clinical forms, serving as another option in cases of resistance to antimonials (Santos et al., 2020; Tiuman et al., 2011). The Unified Health System authorized Miltefosine in 2018 for therapeutic use in Brazil and it is indicated for the treatment of Tegumentary Leishmaniasis. The recommended dose is 2.5 mg/kg/day orally, divided into 2 to 3 daily doses with a limit of 150 mg/day. Despite its efficient action, similar to other established drugs, miltefosine also presents high toxicity with gastrointestinal and teratogenic effects, contraindicated for pregnant women. This drug should be administered preferably after meals, to reduce gastrointestinal effects (Brazil, 2020).A formulation of this drug for veterinary purposes (Milteforan® - Virbac) is also available in Brazil for the treatment of canine visceral leishmaniasis (Queiroz, 2019). Even though its effectiveness is reported in countries like India, in other countries such as Colombia, Bolivia and Brazil this efficiency can vary according to the species of *Leishmania* (Santos et al., 2020; Soto et al., 2004).

Drugs such as Paromomycin, azithromycin, ketoconazole, fluconazole and other antifungals have presented good leishmanicidal activity and are used to treat cases of CL. However, results are not so conclusive, as the mechanisms of action against the parasite are not yet totally clear (Brelaz-de-Castro, 2013; Goto & Lindoso, 2010).

Given the flaws observed in the current therapeutic arsenal available, it is important to search for new molecules with effective leishmanicidal action while less toxic (more selective to the parasite and less toxic to the infected individual) coupled with low cost to fight this disease (Queiroz, 2019). A possible improvement may be to change the chemical constituents used in the synthesis of drugs, and in the development of therapies associated with the modulation of the host immune system, as it plays an important role in the development of the disease (Singh et al., 2012).

THE CHALLENGE IN THE STUDY OF NEW COMPOUNDS

Given the scenario of currently available drugs and according to the Drugs for Neglected Diseases Initiative (DNDi, 2016), the quest for new molecules for treating leishmaniasis is a great challenge, mainly due to the need to overcome limitations in current treatments. It is essential to search for compounds with low toxicity, effective for all clinical forms of the disease and, above all, with minimal side effects to the individual (DNDi, 2016).

Based on the Target Product Profile (TPP) strategies, the DNDi adopts some criteria that can be considered a basis for meeting the challenges in the study of new molecules. These criteria are divided into what is considered ideal and what is acceptable in new forms of treatment for tegumentary and visceral leishmaniasis (DNDi, 2016) (Table).

Along with these factors, it is also important to search for new compounds that may act as immune modulators, stimulating an immune response that can induce the therapeutic objective. This is important considering that evolution towards cure in cases of leishmaniasis is linked to several aspects, one of which is the individual's immune response (Field et al., 2017; Singh et al., 2016).

The immune response plays an important role in the healing process and the course of disease development. Studies report the role of T cell-mediated immune responses to this parasitosis, with cytokines associated with a Th1 profile (IFN-gamma, TNF and IL-12) leading to the activation of macrophages and parasite death, as well as functioning as protective immunity, as well as the production of Th2 cytokines (IL-4, IL - 5 and IL-10) causing the evolution of the disease with the replication and persistence of the parasite (Dayakar et al., 2019; Maspi et al., 2016; Scorza et al., 2017). Therefore, it is important to consider this response when developing new drugs, searching for new therapeutic strategies that aim to modulate these immune mechanisms positively in favor of the host (Novais et al., 2021).

Table. Target Product Profile for the development of new drugs to fight leishmaniasis

Cutaneous and visceral Leishmaniasis		
Criteria	Ideal	Acceptable
Target species	All species	(Cutaneous form) *L. tropica* or *L. braziliensis* (Visceral form) *L. donovani*
Target population	All	> 9 months of age and Immunocompetent
Clinical effectiveness	> 95%	>90%
Formulation	(Cutaneous form) Oral /Topical (Visceral form) Oral / Intramuscular	Not parenteral, or with few doses if parenteral or intramuscular
Treatment regimen	(Cutaneous form) Oral <7 days Topical ≤ 14 days (Visceral form) Oral: 1 / day for 10 days Intramuscular: 3 doses/10 days	(Cutaneous form) Oral: twice daily for 28 days Topical: 28 days (Visceral form) <10 days orally > 3 intra-muscle injections in 10 days
Contraindications	None	Pregnancy / Lactation
Safety / tolerability	No tolerance for adverse effects requiring monitoring	Safety monitoring at the primary health care level

Source: Adapted from DNDi (2020), Borsari et. al. (2018).

New drugs active against *Leishmania* species, must present efficacy against the parasite, and be planned for a short-term treatment model with safety and tolerability for patients. Even the study and modification of synthetic molecules whose biological properties are already known or studied can help in the discovery of effective drugs for the treatment of leishmaniasis (DNDi, 2016).

Some parameters adopted by DNDi are used to better target studies of new therapeutic approaches and are shown in the Table. A study by Don & Ioset (2014) also addresses some of these characteristics. The study brings indicators by which a new molecule should be considered promising. These parameters are mainly related to the effectiveness of these new compounds already in the initial phase, that is, in *in vitro* tests. Those with IC50 values ≤10 μM are considered promising, especially for the amastigote form, and allied to a 10-fold higher selectivity to the parasite than mammalian cell lines (Don & Ioset, 2014).

CONCLUDING REMARKS

Due to the absence of compounds that selectively act on the parasite that causes leishmaniasis with minimal harm to humans, strategies and studies on new therapeutic approaches, especially those in which the compound is associated with immune modulation, are very important for public health and should be considered in the development of drugs for the disease.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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