Applied Microbiology and Biotechnology (2020) 104:8965–8977
https://doi.org/10.1007/s00253-020-10856-w

MINI-REVIEW

Recent advances and new strategies on leishmaniasis treatment

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Received: 21 May 2020 / Revised: 13 August 2020 / Accepted: 23 August 2020 / Published online: 2 September 2020

Abstract

Leishmaniasis is one of the most important tropical neglected diseases according to the World Health Organization. Even after more than a century, we still have few drugs for the disease therapy and their great toxicity and side effects put in check the treatment control program around the world. Moreover, the emergence of strains resistant to conventional drugs, co-infections such as HIV/*Leishmania* spp., the small therapeutic arsenal (pentavalent antimonials, amphotericin B and formulations, and miltefosine), and the low investment for the discovery/development of new drugs force researchers and world health agencies to seek new strategies to combat and control this important neglected disease. In this context, the aim of this review is to summarize new advances and new strategies used on leishmaniasis therapy addressing alternative and innovative treatment paths such as physical and local/topical therapies, combination or multi-drug uses, immunomodulation, drug repurposing, and the nanotechnology-based drug delivery systems.

Key points

- *The treatment of leishmaniasis is a challenge for global health agencies.*
- *Toxicity, side effects, reduced therapeutic arsenal, and drug resistance are the main problems.*
- *New strategies and recent advances on leishmaniasis treatment are urgent.*
- *Immunomodulators, nanotechnology, and drug repurposing are the future of leishmaniasis treatment.*

Keywords Leishmaniasis · Conventional chemotherapy · Multi-drug therapy · Immunomodulators · Drug repurposing · Nanotechnology

Introduction

Leishmaniasis constitutes a group of human and animal diseases caused by *Leishmania*, a protozoan parasite from the *Trypanosomatidae* family. More than 20 *Leishmania* species, around the world, are known to be transmitted to humans by the bite of infected phlebotomine sandflies during their blood meal. Distinct species of *Leishmania* spp. cause different clinical manifestations of the disease, which can be characterized by at least three syndromes: visceral leishmaniasis (VL, also known as kala-azar); cutaneous leishmaniasis (CL); and mucocutaneous leishmaniasis (MCL) (Burza et al. 2018). Post-kala-azar dermal leishmaniasis (PKDL) constitutes a complication from visceral leishmaniasis after treatment, with macular, maculopapular, and nodular rash as clinical manifestations. These diseases mainly affect the poorest regions where health services are the most precarious (Alvar et al. 2006).

Since the report of the first cases of leishmaniasis associated with infection by the human immunodeficiency virus (HIV) in Europe, Mediterranean, Brazil, and East African countries, an increasing number of co-infection cases have been described (Desjeux 1995). There are regions in Ethiopia where approximately 40% of leishmaniasis patients are co-infected with HIV (Alvar et al. 2008). Facing this scenario, the WHO Department of Neglected Tropical Diseases

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Table 1 Conventional strategies for leishmaniasis treatment recommended by the World Health Organization

Visceral leishmaniasis treatment
Anthroponotic visceral leishmaniasis caused by *L. donovani* in the Indian subcontinent
Liposomal amphotericin B: dose of 3–5 mg/kg daily by infusion given over 3–5 days period (total dose of 15 mg/kg) or 10 mg/kg as a single dose by infusion
Visceral leishmaniasis caused by *L. donovani* in East Africa
Pentavalent antimonials (20 mg Sb^v/kg/day intramuscularly or intravenously) + paromomycin (15 mg (11 mg base)/kg/day intramuscularly), both for 17 days
Visceral leishmaniasis caused by *L. infantum*: Mediterranean Basin, Middle East, Central Asia, South America
Pentavalent antimonials (20 mg Sb^v/kg/day intramuscularly or intravenously) for 28 days
Cutaneous leishmaniasis treatment*
Old World leishmaniasis
L. major, *L. tropica*, *L. aethiopica***, and *L. infantum***
Local therapy: 15% paromomycin/12% methylbenzethonium chloride ointment twice daily for 20 days or intralesional antimonials (1–5 ml per session) every 3–7 days (1–5 sessions)
Systemic therapy: pentavalent antimonials (as above) for 10–20 days
L. aethiopica
Systemic therapy: pentavalent antimonials (as above) for 10–20 days + paromomycin (as above) for 60 days or longer to treat diffuse cutaneous leishmaniasis (C)
New World Leishmaniasis
L. mexicana, *L. guyanensis*, *L. panamensis*, *L. braziliensis*, *L. amazonensis*, *L. peruviana*, *L. venezuelensis*
Local therapy: 15% paromomycin/12% methylbenzethonium chloride ointment twice daily for 20 days
Systemic therapy: pentavalent antimonials (as above) for 20 days
Mucocutaneous leishmaniasis
All species***
Pentavalent antimonials (as above) for 30 days

*The most used treatment strategy for CL. However, the treatment of CL varies from observation, locality, local, or systemic therapy depending on gravity of lesions, etiological species, and its potential to develop into mucosal leishmaniasis

**Few data are available on therapy for CL caused by *L. infantum* and *L. aethiopica*

***Few data are available for the therapy of MCL due to *L. aethiopica*

brought together a group of experts aiming to develop evidence-based guidelines for the treatment of co-infection HIV-VL in East Africa and Southeast Asia, since treatment of leishmaniasis in HIV-infected patients is a special condition with reduced therapeutic options (WHO 2020).

In CL, despite being less severe than VL, in untreated patients, the lesions may worsen due to secondary infections and ulcerations. Another complication is the disfiguring scars and psychosocial suffering due to the stigma and social isolation caused by the disease (Kassi et al. 2008; Bailey et al. 2017). The lesions in CL are usually self-healing; however, they can lead to mucosal involvement or diffuse form, depending on the species of parasite, immunological status, and genetically determined responses of patients (Reithinger et al. 2007).

Since the beginning of the treatment of VL and CL with pentavalent antimonials, resistance of the population to the treatment has been observed, due to its great toxicity and side effects. These factors impair the patient's adherence to treatment and in turn select resistant strains of the parasite. The improvement of amphotericin B deoxycholate in liposomal form reduced the side effects; however, the financial impact in poor regions is worrying. Other drugs have been developed, among them miltefosine; however, there are resistant strains, and in the tegumentary form of the disease, the response varies according to the species of the parasite. In this sense, it is important to develop new treatment strategies for the affected population. Thus, the present review addresses important points in conventional therapies and therapeutic advances in the present and future of leishmaniasis treatment.

Conventional antileishmanial therapy

Pentavalent antimonials

Pentavalent antimonial (Sb^v) compounds have been available for almost seven decades and constitute the first-line treatment against leishmaniasis. The two main formulations are meglumine antimoniate (MA) and sodium stibogluconate (SSG). Sb^v would behave as a prodrug that is reduced within the organism into active Sb^{III} form, a borderline metal ion with high affinity towards nitrogen- and sulfhydryl-containing

ligands. The antileishmanial mechanisms of Sb^{III} are probably related to its interaction with sulfhydryl-containing biomolecules, including thiols, peptides, proteins, and enzymes (Frézard et al. 2009). Although effective, the use of these compounds is many times limited by the need for daily parenteral administration, severe toxicity and side effects, and treatment failures. With emerging resistance to this drug in Bihar, alternative treatment strategy has been adopted for these areas. In the eastern Africa, the combination of SSG and paromomycin has been recommended as first-line drug for VL treatment (Table 1) (Musa et al. 2012; Kimutai et al. 2017). In cutaneous forms, Sb^{v} has shown varied responses in different etiological species. Despite that, they are the mainstays of treatment for CL in several regions of the world. Currently, in Old World cutaneous leishmaniasis (OWCL), most patients are treated with intralesional Sb^{v} infiltration. Systemic use can be considered for only mucocutaneous lesions (Table 1). In New World cutaneous leishmaniasis (NWCL), most patients are treated with systemic MA (20 mg of Sb^{v}/kg/day for 20 days), even though it has already been recommended the use of intralesional MA has already been recommended by the Pan American Health Organization (PAHO). Side effects of Sb^{v} include local irritation, cardiac and hepatic alterations, anorexia, nausea, and vomiting (Sundar and Chakravarty 2015). Further, HIV/leishmaniasis co-infected-treated patients present pronounced side effects, high failure and relapse rates, and increased mortality during Sb^{v} therapy (Cota et al. 2013).

Amphotericin B deoxycholate and liposomal amphotericin B

Amphotericin B deoxycholate (AmB-D) is a broad-spectrum antifungal and active against protozoan *Leishmania* species. Following the loss of effectiveness of antimonial drugs in India, AmB-D was elected as the first-line drug of choice for VL. This drug binds to membrane sterols with particular affinity towards ergosterol, forming complexes that arrange into ion channels and increase membrane permeability (Gray et al. 2012). Despite its efficacy, the treated patients present frequent adverse effects such as fever, hypokalemia, myocarditis, and mainly nephrotoxicity, which demand hospital monitoring (Sundar et al. 2007a, b). Many lipid formulations of amphotericin were developed in recent years to minimize the side effects of AmB-D. But the liposomal amphotericin B (AmB-L—AmBisome®) is the only one approved by US Food and Drug Administration (FDA). The total dose requirements of lipid formulations for treatment of VL vary by region (Sundar et al. 2007a, b) (Table 1). Although it has expensive cost, the high effectiveness of AmB-L ensures that it is the first treatment choice for HIV co-infection patients, pregnant, and transplanted individuals in most countries (Sundar and Chakravarty 2013). In cutaneous forms, AmB-D treatment shows at least equivalent results to Sb^{v} treatment. Therefore, it is not considered an alternative treatment due to considerably serious side effects. Thus, there are only a few studies with AmB-D, as well as the liposomal form in CL and MCL patients, with absent randomized clinical trials (Wortmann et al. 2010; Balasegaram et al. 2012).

Miltefosine

The antileishmanial activity of miltefosine, an antineoplastic drug, was first identified in the 1980s and until today is the only oral drug approved for leishmaniasis treatment (Croft et al. 1987; Sundar and Chakravarty 2015). This drug is able to kill parasites in vitro and in vivo by changing signaling pathways and cell membrane synthesis, thus leading to apoptosis (Verma and Dey 2004). Following a phase III clinical trial, in which 50–100 mg/day dose for 28 days resulted in a cure rate of 94%, miltefosine was first registered for VL treatment in India (Sundar et al. 2002; Sundar et al. 2008a, b). Unfortunately, after a decade of use, there was an increase in resistance with reduced effectiveness doubling the relapse rate (Sundar et al. 2012; Dorlo et al. 2014; Srivastava et al. 2017). This caused the exchange of miltefosine by single-dose AmB-L as therapeutic strategy of VL elimination program in the India subcontinent (Table 1). Currently, its use has been recommended only in combinations with other drugs in this region (Chakravarty and Sundar 2019; van Griensven and Diro 2019). In other countries, the studies with miltefosine have shown varied performance demonstrating the need of higher drug doses (Ritmeijer et al. 2006; Wasunna et al. 2016). For cutaneous leishmaniasis, the recommended dose is 2.5 mg/kg/day orally for 28 days. However, miltefosine has varied species-dependent effectiveness. A clinical trial demonstrated that miltefosine is useful against CL caused by *Leishmania panamensis* in Colombia but not against CL caused by *Leishmania braziliensis* in Guatemala (Soto et al. 2004). On the other hand, Machado et al. (2010) observed that miltefosine therapy was more effective and safer than standard Sb^{v} for the treatment of CL caused by *L. braziliensis* in Bahia, Brazil. Adverse effects of miltefosine include gastrointestinal alterations and less often hepatotoxicity. Moreover, it presents teratogenic effects, so women of child-bearing age are advised contraception during the treatment regimen (Sundar and Chakravarty 2015).

Paromomycin

Paromomycin, an aminoglycoside antibiotic that interferes in protein synthesis and modifies mitochondrial membrane fluidity inhibiting respiration, has also been shown to be an effective antileishmanial agent. This drug is used in topical and systemic treatment for CL and as a systemic drug for VL in some regions of the Indian continent (Sundar and Chakravarty

2008; Jhingran et al. 2009). Paromomycin was licensed by the Indian government for VL treatment in 2006, and a phase IV trial demonstrated cure rates of 94% (Sinha et al. 2011). However, paromomycin has limited effectiveness as monotherapy in East Africa (Hailu et al. 2010; Musa et al. 2012). Adverse effects of systemic therapy are uncommon and include pain and burning on application site, low hepatotoxicity, and ototoxicity (Sundar et al. 2007b). Paromomycin has been evaluated in topical formulations with variable clinical results in OW and NW *Leishmania* species. In general, the efficacy of topical paromomycin did not differ from that of intralesional Sb^v in the OWCL, whereas its efficacy was inferior to parenteral Sb^v in the NWCL (Kim et al. 2009). For CL cases, the decision to use local or systemic drugs usually depends on the lesion characteristic, etiological species, and imminent risk of progressing to mucocutaneous disease (PAHO 2018) (Table 1).

Other antileishmanial therapies

Pentamidine

Since it was first synthesized in the 1940s, the pentamidine has been studied for its anti-*Leishmania* action, mainly after the cases of antimonial resistance in India subcontinent (Jha et al. 1991). However, its use was abandoned due to toxicity, such as pancreatitis leading to diabetes mellitus, hypoglycemia, hypotension, cardiac alterations, and hyperkalemia (Sundar and Chakravarty 2015). Recently, the monthly use of 4 mg/kg dose for 12 months has been recommended as a secondary prophylaxis HIV-VL co-infected patients in Ethiopia, if the T CD4 lymphocyte count is below 200 cells/μl (Diro et al. 2015; Diro et al. 2019). Despite scarce studies demonstrating the use of pentamidine in OWCL, its use has been demonstrated in NWCL. However, the effectiveness of these studies varies according the *Leishmania* species, duration and composition of therapy, and geographic region (Soto et al. 1994; Lai et al. 2002; Paula et al. 2003; Roussel et al. 2006; Amato et al. 2009; Soto et al. 2016; Christen et al. 2018; Gadelha et al. 2018).

Azoles

Azole antifungal agents have been evaluated as therapeutic strategies, mainly for cutaneous forms. In OWCL, the oral fluconazole therapy presented cure rates of approximately 60%, with substantial increase in effectiveness when the drug concentration was doubled. However, this treatment was associated with side effects, such as hepatotoxicity and cardiotoxicity (Alrajhi et al. 2002; Emad et al. 2011). High-dose oral fluconazole therapy in *L. braziliensis*-infected patients demonstrated cure rate of 75–100% (Sousa et al. 2011). However, a randomized clinical trial evaluated the efficacy of oral fluconazole, in a similar therapeutic scheme, for the CL treatment caused by *L. braziliensis*, evidencing cure rates of only 22% (Prates et al. 2017). Itraconazole demonstrated low effectiveness for the MCL treatment in Brazil, and the complete resolution of MCL lesions was observed in only three (23%) high-dose itraconazole-treated patients in Ecuador (Amato et al. 2000; Calvopina et al. 2004). A recent systematic meta-analysis identified that none of the azoles is effective for the treatment of cutaneous forms of leishmaniasis, and its use should be associated only with other drugs (Galvão et al. 2017).

New strategies and recent advances on leishmaniasis treatment

Emergence of resistant strains to conventional drugs (Sundar 2000; Mueller et al. 2007), high toxicity, co-infections such as HIV/*Leishmania* spp., the small therapeutic arsenal available for treatment of the disease, and the low investment for the discovery/development of new drugs force researchers and world health agencies to seek new strategies to combat and control this important neglected disease. In this sense, the following section is a brief summary of recent advances and new strategies used to treat leishmaniasis (Table 2).

Cutaneous and mucocutaneous leishmaniasis

Physical and local therapies

CO_2 laser administration and thermotherapy Based on the principle to directly destroy the *Leishmania* parasites, the CO_2 laser and thermotherapy are a simple way to deliver external heat on infected tissues, causing damage to specific areas with parasitism (Asilian et al. 2004; Valencia et al. 2013). The direct application of heat can accelerate the cure of the cutaneous lesions (Navin et al. 1990). In OWCL, some studies have demonstrated that thermotherapy showed better results in relation to cure rate compared with intralesional treatment with antimonials with similar or reduced side effects (Sadeghian et al. 2007; Aronson et al. 2010). An improvement was described using a CO_2 laser thermotherapy technology, demonstrating in a clinical trial 93.7% of cure rate compared with combined therapy using intralesional antimonials (78% of cure rate) (Shamsi et al. 2011). For CL, these strategies have been used with relative success, both for old and new world infections.

Cryotherapy Cryotherapy also known as cryosurgery was firstly evaluated in Saudi Arabia patients infected with *Leishmania major* and obtained 100% of cure using a CO_2 cryomachine (Bassiouny et al. 1992). Nowadays, cryotherapy

Table 2 New advances and new strategies for leishmaniasis treatment

Advance/strategy	Disease	Benefits compare to conventional therapy	Treatment efficacy	References
Thermotherapy and CO_2 laser administration	Cutaneous and mucocutaneous leishmaniasis	Reduction of side effects and accelerated cure of the cutaneous lesions	Marked	Sadeghian et al. (2007); Aronson et al. (2010); Asilian et al. (2004); Valencia et al. (2013); Shamsi et al. (2011)
Cryotherapy and electrotherapy	Cutaneous and mucocutaneous leishmaniasis	Reduction of side effects and accelerated cure of the cutaneous lesions	Moderate	Bassiouny et al. (1992); Mosleh et al. (2008); Negera et al. (2012); Chakravarty and Sundar (2019)
Topical drug therapy using nitric oxide derivates	Cutaneous and mucocutaneous leishmaniasis	Accelerated heal of the cutaneous lesions and reduction of side effects	Moderate/low	Lopez-Jaramillo et al. (1998); Lopez-Jaramillo et al. (2010)
Intralesional drug administration	Cutaneous and mucocutaneous leishmaniasis	Reduction of side effects and relapses, accelerated heal of the cutaneous lesions, high percentage of cure, and low cost	Marked	Uzun et al. (2004); Bashir et al. (2019); Ramalho et al. (2018); Arboleda et al. (2019); Masmoudi et al. (2006)
Multi-drug or combination therapy	Cutaneous, mucocutaneous, and visceral leishmaniasis	Reduction of side effects and relapses, high percentage of cure, low risk of developing resistant parasites, and low cost	Marked	Sundar et al. (2008b); Melaku et al. (2007); Mahajan et al. (2015)
Immunomodulators	Cutaneous, mucocutaneous, and visceral leishmaniasis	Reduction of side effects and relapses, high percentage of cure, and low risk of developing resistant parasites	Marked	Sundar and Murray (1995); Squires et al. (1993); Convit et al. (2003); Badaro et al. (1999); Sundar et al. (1994); Mastroianni (2004); Monjour et al. (1994); Mayrink et al. (2006)
Nanotechnology	Cutaneous, mucocutaneous, and visceral leishmaniasis	Reduction of side effects and relapses, high percentage of cure, and low risk of developing resistant parasites	In vitro and in vivo testing	Sazgarnia et al. (2013); Jebali and Kazemi (2013); Costa Lima et al. (2012); De Mattos et al. (2015); Italia et al. (2012); Kumar et al. (2019)
Drug repurposing	Cutaneous, mucocutaneous, and visceral leishmaniasis	Reduction of side effects and relapses, high percentage of cure, and low risk of developing resistant parasites	In silico, in vitro, and in vivo testing	Bustamante et al. (2019); Braga (2019)

uses liquid nitrogen (at − 195 °C), and applied once or twice weekly in *Leishmania* lesions, it can achieve an efficiency rate over 95% (Mosleh et al. 2008; Negera et al. 2012). The mechanism of killing parasites was described by the formation of ice intracellularly causing the disruption of cells leading to localized ischemic necrosis. The secondary side effects were mainly associated with edema and erythema at the site, hyper- or hypopigmentation (Chakravarty and Sundar 2019).

Electrotherapy Electric field stimulation, a non-drug treatment, has been described as a potential tool to control microbial infection. Previous test using mice infected with *Pseudomonas aeruginosa* resulted in significantly inhibiting bacteria in lung infections (Giladi et al. 2010). Authors have shown that exposure to electrical currents could lead to healing the skin lesions and intractable ulcers and the new skin produced has better tensile properties compared with that skin produced naturally (Wolcott et al. 1969). In leishmaniasis, the use of therapeutic electricity applied on infected mice with *L. major* showed important death of parasites at the lesion sites (Hejazi et al. 2004). Recent advances have demonstrated that electric fields affected *Leishmania tarentolae* promastigote motility, clumping, and viability in vitro (Dorsey et al. 2018). However, these studies are in an initial phase requiring more data related to what currents, potentials, numbers of stimulations, and durations are safe but effective for clinical use against CL.

Topical drug therapies

Nitric oxide derivates This is a promise strategy used against some *Leishmania* species from Americas (*L. braziliensis* and *L. panamensis*). A *S*-nitroso-*N*-acetylpenicillamine (SNAP) cream, compound that generates NO, was firstly tested for 10 days showing, after 30 days of treatment, the healing of all lesions and the formation of new skin in patients presenting CL (Lopez-Jaramillo et al. 1998). Moreover, a most recent study using a topical nanofiber nitric oxide (NO)-releasing patch administered for 12 h a day for 20 days showed ineffectiveness, with only 37.1% of cure rates in CL Colombian patients (Lopez-Jaramillo et al. 2010). The authors suggest

that therapeutic failure can be reversed by increasing drug concentration or treatment time.

Intralesional drug administration Thinking of reducing the adverse effects while maintaining efficacy and safety of the form of conventional use (intramuscular or intravenous infusion), this new way to treat CL using pentavalent antimonials has gained prominence. Since 2013, the use of intralesional antimonial therapy has been recommended by the PAHO guidelines (OPS 2013) when systemic treatment is not indicated or if local treatment for CL is required. Intralesional pentavalent antimonials achieved cure rates over 90% against Asia and Mediterranean species of the parasite (*L. major* and *Leishmania tropica*) (Uzun et al. 2004; Bashir et al. 2019). For new world *Leishmania* species, open-label phase II clinical trials showed elevated cure rates (87–91.6%) after 180 days of treatment (Ramalho et al. 2018; Arboleda et al. 2019). The most common side effects observed were bacterial secondary infection, erythema, local itching, and pain during administration, which tend to disappear few days after the end of the treatment (Masmoudi et al. 2006; Ramalho et al. 2018).

Visceral leishmaniasis

Multi-drug or combination therapy

It is well known that untreated symptomatic VL is almost always fatal. Moreover, it is observed a large range on the variability in the effectiveness of antileishmanial drugs associated with the region where the *Leishmania* infection occurred and the host immune status (van Griensven and Diro 2019). In this sense, combined therapy has the following objectives: shortening the treatment duration (reducing side effects and improved adherence to the regimen by the patient), controlling the development of parasite resistance, lowering the costs, and encouraging a cure, especially in complicated cases of VL (Monge-Maillo and López-Vélez 2013).

The combined therapy is mainly recommended for patients who had not responded to monotherapy with Sb^v. Most of the studies were conducted in India especially in *Leishmania donovani*-infected patients. Using AmB-L at 5 mg/kg/day in a single dose followed by different regimens of miltefosine, the authors demonstrated greater efficacy with combinations compared with AmB-L at 5 mg/kg/day in a single dose (98% versus 91%) (Sundar et al. 2008b). In Eastern Africa, some studies have demonstrated that the combination of paromomycin with SSG increased the cure rate response in comparison with SSG as monotherapy (Melaku et al. 2007). A recent study in VL/HIV co-infection demonstrated reduced rates of mortality and VL relapse when AmB-L (AmBisome) and miltefosine (Impavido) were combined and administered. Moreover, the authors concluded that combination therapy appeared to be well tolerated, safe, and effective and may be considered as an important option for treatment of VL in HIV co-infected patients (Mahajan et al. 2015).

Immunomodulators

Leishmania parasites have stated systematic resistance against the immune system manipulating different mechanisms to survive into the host. In this way, treatments with substances that promote the restoration of the immune response against the parasite are an alternative approach to combat the infection (Musa et al. 2010; Roatt et al. 2014; Taslimi et al. 2018).

The IFN-γ is well recognized as a cytokine capable of inducing macrophages to kill *Leishmania* parasites. In VL patients, the use of IFN-γ as immunotherapy promoted accelerated parasitological control (Squires et al. 1993; Sundar and Murray 1995) and enhanced the clinical efficacy of conventional Sb^v therapy, promoting more than 80% cure rate (Badaro et al. 1999; Sundar et al. 1994; Sundar and Murray 1995). More recently, in a case report on HIV/VL co-infection in Italy, the combination treatment using rHuGM-CSF (recombinant human granulocyte macrophage colony-stimulating factor) showed to be effective leading to a reduction on the spleen size, disappearance of symptoms, and clinical cure of the patient (Mastroianni 2004).

Taking into account the use of immunomodulators, the most used strategy in leishmaniasis is therapeutic vaccines. In literature, many studies around the world describe important results obtained using vaccines as immunotherapeutic tool. More than 5000 patients were treated against CL with heat-killed *Leishmania* parasites plus BCG in Venezuela with an incredible 95.7% of cure rate achieved (Convit et al. 2003). In the same way, patients infected with *L. braziliensis* were treated with a therapeutic vaccine composed of parasite-derived antigen Fraction 2 (LbbF2) that promoted secretion of key cytokines by T cells leading to clinical cure of the infected patients (Monjour et al. 1994). In Brazil, more than 500 patients with CL were treated either with pentavalent antimony, killed *Leishmania* vaccine plus BCG, BCG, or a combination. The cure rates in therapeutic vaccine or pentavalent antimony chemotherapy were the same, but with fewer adverse effects and shorter recovery time (Mayrink et al. 2006). As observed in these studies, activation of the immune system through immunotherapy associated with application of antileishmanial drugs can solve the complicated cases of the disease mainly in patients with drug refractory *Leishmania* infections.

Nanotechnology

Recently, nanotechnology-based drug delivery systems have advanced to efficiently deliver different types of drugs to specific tissues and cells. In leishmaniasis, the combination of

nanocarrier system with antileishmanial drugs enables targeted and efficient delivery improving the on-target effect, bioavailability, reducing toxicity, and side effects (Jebali and Kazemi 2013; Wagner et al. 2019). In this horizon, new advances in development of nanostructured lipid carriers, liposomes, nanoemulsions, solid-lipid nanoparticles, niosomes, nanocapsules, nanoparticles, metallic nanosphere, polymeric nanoparticles, and nanostructured layered films have been done to trigger efficient drug delivery for leishmaniasis treatment (Costa Lima et al. 2012; Italia et al. 2012; De Mattos et al. 2015; Kumar et al. 2019; Wagner et al. 2019; Saleem et al. 2019).

Various nanocarrier strategies have been used for leishmaniasis treatment demonstrating their own advantages and disadvantages. As it is known, the main cell targets in leishmaniasis are macrophages. Thence, the most employed nanoparticles in the disease are polymeric nanoparticles and liposomes because of the easy and fast way of internalization by the infected cells. Liposomes are nanocarriers that have unique properties to load and deliver hydrophobic and/or hydrophilic molecules by surface activation. Moreover, because they are positively charged, liposomes are promptly internalized by the macrophages. Due to these properties, they are the most commonly nanocarriers employed in leishmaniasis (Saleem et al. 2019).

Nanoemulsions are considered one of the best drug delivery systems due to their simple preparation, their ability to solubilize hydrophobic drugs, and their physicochemical stability over several years, and they can be easily scaled-up (Bilia et al. 2014). Similarly, polymeric nanoparticles are also widely used, besides having small size, capacity to deliver more than one drug, and present low toxicity, and they are cost effective. The main characteristics of nanoparticles are the possibility of functionalizing their surface and the use of components with excellent biodegradation capacity and biocompatibility (poly lactide-co-glycolide (PLGA)) (Saleem et al. 2019). Metal-based nanoparticle systems also offer significant advantages as drug delivery against leishmaniasis including enlargement of the biological action of the carried molecule and decrease in side effects. Moreover, nanostructure metal compounds show enhanced selectivity for parasite biomolecules, like cysteine protease that is a promising family of *Leishmania* enzymes. It is important to note that many nanotherapeutic agents have been approved by the FDA and will be available for clinical use as soon as possible (Eifler and Thaxton 2011). Indeed, for leishmaniasis treatment, most of the nanostructured drugs are still undergoing in vitro and in vivo testing. Only few nanostructures of amphotericin B are available for human use until now.

A recent study of in vitro and preclinical evaluation of amine functionalized carbon-based nanoparticle loaded with AmB (f-Comp-AmB) showed a remarkable antileishmanial efficacy in comparison to AmB-D. In golden Syrian hamsters' model, the authors observed 98% of suppression of the parasite replication in the spleen in comparison with the untreated animals (Gedda et al. 2020). In CL, the development of an effective topical drug formulation is desirable mainly by its non-invasive nature, which may potentially enhance the treatment accessibility and patient adherence. In this sense, the use of nanotechnology has brought great advances. In a preclinical study, a combination of amphotericin B (AmB) and miltefosine (MTF) co-loaded in ultra-deformable liposomes (SGUDLs) was evaluated. An important antileishmanial activity of co-loaded SGUDLs was observed against amastigotes of *Leishmania mexicana* and the in vivo study demonstrated a significant reduction in the parasitic burden in BALB/c model of CL (Dar et al. 2020). The authors suggest that the great efficacy of the co-loaded SGUDLs is directly associated with synergistic interaction between AmB and MTF and concluded that AmB-MTF co-loaded SGUDLs could be an effective topical treatment option against CL (Dar et al. 2020).

Drug repurposing

Drug repurposing is regarded as one of the most important strategy for the rational use of drugs, especially against neglected diseases. Called as drug reprofiling or repositioning is a modern strategy to identify and develop new uses for existing drugs (Ashburn and Thor 2004). In addition, repositioning has main advantages, including lower risk of drug failure, reduced time frame for development/application of the drug, and reduced costs, and can reveal new pathways and targets that may be further explored (Pushpakom et al. 2019).

Focusing on leishmaniasis drug repurposing, computational approaches are the main strategies that have been applied with relative success among the diseases. These computational techniques involve systematic analysis of any data type such as bioinformatics targeting gene expression, chemical structure, and genotype or proteomic data. In this sense, molecular docking is a computational strategy to predict binding sites between the ligands (drugs, for example) and the target (a receptor) (Bustamante et al. 2019). In conventional docking, one receptor/protein target is chosen so that multiple drugs could be tested against that target. In this case, the knowledge about the target/protein or the drug class may favor the choice of a possible ideal drug with a greater chance of success in subsequent trials (Pushpakom et al. 2019). Another important tool is called signature matching that is based on the comparison between the characteristic (signature) of a drug or molecule compared with that of another drug or molecule (Keiser et al. 2009). The signature analyses are derived from some general data such as metabolomic, proteomic, transcriptomic, or chemical structures. These two drug-repositioning tools are the most used strategies against leishmaniasis currently.

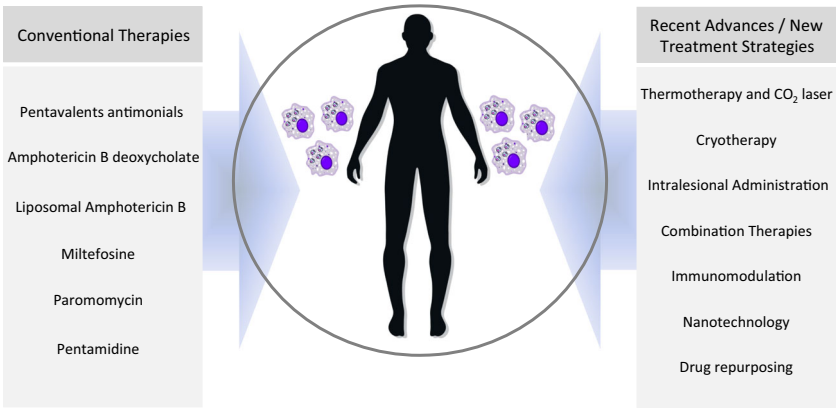

Fig. 1 Conventional therapies, recent advances, and new treatment approaches against leishmaniasis

In a recent study, Bustamante et al. (2019) used bioinformatic predictions to detect some repurposing drugs for leishmaniasis treatment. In this study, the authors performed some simulations to identify and predict these drugs with in vitro validations and pharmacokinetic simulations. As strategy, the bioinformatic predictions were used to detect potential homologs between targeted proteins by approved drugs and other proteins of the *Leishmania* spp. parasites. In this study, 33 drugs were identified with potential target prediction with in vitro action (rifabutin and perphenazine) (Bustamante et al. 2019).

Metallodrugs have been identified with important antitumor, anti-inflammatory, and antimicrobial actions. Auranofin (Ridaura), a gold(I) triethylphosphine thiosugar drug, has been described as having antileishmanial activity with the ability to inhibit trypanothione reductase (TR). In this sense, a recent study performed a preclinical evaluation of gold(I) triphenylphosphine- and triethylphosphine-based complexes showing their activity against *Leishmania infantum* and *L. braziliensis* intracellular amastigotes (Tunes et al. 2020). Using bioimaging, the authors observed reduced lesion size and parasite burden in BALB/c mice infected with luciferase-expressing *L. braziliensis* or *Leishmania amazonensis* and orally treated with gold(I) complexes. According to the authors, the gold(I) complexes are promising antileishmanial agents, with a potential for therapeutic use (Tunes et al. 2020).

An interesting review discussing drug repurposing has recently been published and describes multi-target drugs active against leishmaniasis (Braga 2019). In this review, it is shown that the azoles presented growth inhibitory activity against both fungi and *Leishmania*. Some compounds such as posaconazole, fluconazole, and itraconazole act against the same target, lanosterol 14-a-demethylase enzyme. Similarly, it is shown that both amphotericin B and miltefosine act on small molecules, proteins, genes, and even organelles showing their profile of multi-target agents as known. The authors concluded that some steps towards drug repurposing for multi-target strategy will be the future in the search for leishmanicidal drug candidates (Braga 2019) (Table 2).

Conclusions and prospects

There are few drugs for the leishmaniasis treatment, and the great toxicity and side effects put in check the international treatment control. Moreover, the emergence of resistant strains to conventional drugs, co-infections such as HIV/*Leishmania* spp., the small therapeutic arsenal, and the low investment for the discovery/development of new drugs force researchers and world health agencies to seek new strategies to combat and control this important neglected disease. In this context, new strategies with important advances in physical and local therapies including thermotherapy and CO_2 laser administration and topical drug therapies using NO compounds and intralesional drug administration have given a better perspective of cure in patients with CL. Moreover, the use of combination therapy or multi-drug therapy and activation of immune system using immunomodulators have helped to solve problems in relation to parasitic resistance and serious cases of HIV/*Leishmania* spp. infection. Finally, being considered as the future of leishmaniasis treatment, the drug repurposing and the nanotechnology-based drug delivery systems bring the opportunity to use computational tools for the identification of existing drugs which are used in the treatment of the disease with less time, cost, and using nanotechnology that promotes an efficient delivery of different types of drugs to specific tissues and cells infected by the *Leishmania* parasites (Fig. 1). Thus, efforts need to be directed for the rational investment in new therapies and treatment strategies against the disease, in order to seek therapies with less side effects, lower costs, and better efficacy against these parasites.

Author contributions All the authors participated with suggestions during the development of this manuscript; B.M.R and J.M.O.C. performed all the literature search and participated in drafting the manuscript. All the authors participated in the critical revising process for important intellectual content and created the tables. The authors B.M.R., J.M.O.C., R.D.O.A.-S, R.C.F.B., W.C-V., and A.B.R. participated in the study conception and critical revision of the article. B.M.R and A.B.R. contributed to the supervision.

Funding This work was financially supported by the Brazilian agencies, CNPq (grant number 435224/2018-2), FAPEMIG (grant number APQ-03505-13 - PROGRAMA DE PESQUISA PARA O SUS–PPSUS, APQ-01373-14 – PRONEX, APQ 02577-18 and APQ-02556-18), CAPES (this study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES) - Finance Code 001), and Universidade Federal de Ouro Preto – UFOP for funding (TO 072018020, TO 222019019). R.C.F.B. are grateful for CAPES fellowship, and A.B.R. and B.M.R. are also grateful to CNPq for fellowships.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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