CURRENT DRUGS AND NEW THERAPEUTIC APPROACHES FOR THE LEISHMANIASIS TREATMENT

DROGAS ATUAIS E NOVAS ABORDAGENS TERAPÊUTICAS PARA O TRATAMENTO DA LEISHMANIOSE

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ABSTRACT

Leishmaniasis is an endemic disease in 98 countries and 5 continents and approximately 1.3 million new cases occur annually. This work describes the main therapies used to treat the diverse clinical manifestations of leishmaniasis. Considering that the currently available anti-*Leishmania* drugs present several problems such as severe side effects and the possibility of parasitic resistance to the drug, among others, there is a constant search for new therapies for this disease. In addition to the drugs commonly used for the treatment of leishmaniasis, therapeutic alternatives such as metallic complexes were highlighted.

Key words: Treatment of Leishmaniasis; therapeutic alternatives; metallic complexes.

RESUMO

A leishmaniose é endêmica em 98 países e 5 continentes e aproximadamente 1,3 milhões de novos casos ocorrem anualmente. Neste trabalho estão descritas as principais terapias utilizadas para o tratamento das diversas manifestações clínicas da leishmaniose. Considerando que as drogas anti-*Leishmania* atualmente disponíveis apresentam vários problemas como graves efeitos colaterais e possibilidade de resistência parasitária ao medicamento, dentre outros, existe uma constante busca pelo desenvolvimento de novas terapias para essa doença. Além das moléculas comumente utilizadas no tratamento da leishmaniose, alternativas terapêuticas promissoras como os complexos metálicos foram destacadas.

Palavras-chave: Tratamento de Leishmaniose; alternativas terapêuticas; complexos metálicos.

INTRODUCTION

Leishmaniasis comprises a group of parasitic diseases caused by protozoa belonging to the order Kinetoplastida, family Trypanosomatidae and *Leishmania* genus[(1)]. Its transmission occurs through the bite of females pheblotomine sand flies

infected with parasites (Figure 1), being possible the transmission of more than 21 species of *Leishmania* to man[2]. The various clinical manifestations of the disease depend on the *Leishmania* species involved in the infection, the immune status and the age of the affected patient. Thus, children and the elderly are more likely to present complications[3 - 4]. The disease can be classified as: Visceral Leishmaniasis (VL), a more severe clinical form of the disease that affects multiple organs, especially the liver and spleen, and when untreated it can be fatal[5]; and Tegumentar Leishmaniasis (TL; cutaneous, CL or mucocutaneous, MCL), which affects the skin and/or mucous membranes generating ulcers in different areas of the body and may result in permanent and disfiguring scars, with total or partial mutilation of the nasopharyngeal mucosa[2, 4,6-7].

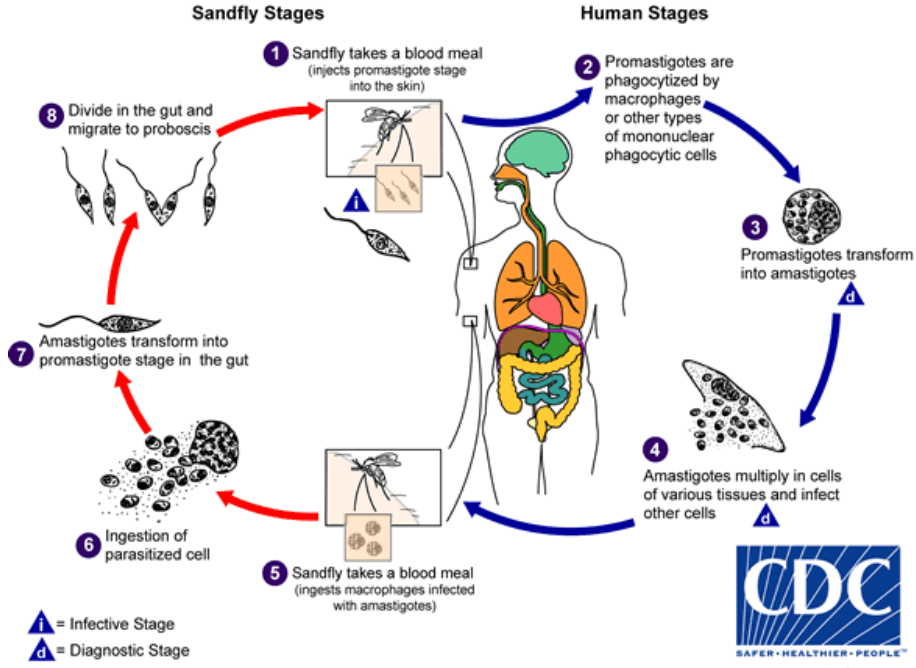


Figure 1 – Life cycle of *Leishmania* parasites. Source: Public Health Image Library (https://www.cdc.gov/parasites/leishmaniasis/biology.html)

According to the World Health Organization (WHO), 98 countries, 3 territories and 5 continents are endemic for leishmaniasis and approximately 1.3 million new cases occur annually. VL affects mainly regions such as Bangladesh,

Brazil, Ethiopia, India, Nepal, South Sudan and Sudan and it is related to the infection of approximately 300 thousand people annually, which can cause 20 to 50 thousand deaths. The TL related to the development of cutaneous form is more common in regions like Afghanistan, Algeria, Brazil, Colombia, Islamic Republic of Iran, Pakistan, Peru, Saudi Arabia, Syrian Arab Republic and Tunisia. The TL related to the development of the mucocutaneous form affects mostly Brazil, Peru and Plurinational State of Bolivia. Both TL (cutaneous and mucocutaneous) are associated with the infection of approximately 1 million people per year[3, 6, 8].

Parasites of the *Leishmania* genus have been distributed in an expansive way in the last decades and, thus, the number of cases of the disease has increased exponentially. However, actual epidemiology is still unknown due to underreporting and lack of adequate epidemiological surveillance systems [8]. Furthermore, cases notification of this disease is mandatory in only 34% of endemic countries. In this way, most of data are based on estimates [8] and it is assumed that the actual values of leishmaniasis cases are higher than the data previously reported[9,10].

The few drugs currently available for leishmaniasis treatment present several problems associated with side effects, cost, safety, increasing emergence of parasitic drug resistance, which limit their use [2]. To further aggravate this situation, unlike other diseases associated to deaths, which have the focus of attention and funding for research in developing countries, neglected tropical diseases, including leishmaniasis, comprise a category of infectious diseases with little investment to the search for new drugs and as well as for actions aimed at the control of the disease[4,11-13]. In this sense, the present work describes the main drugs associated with TL and VL treatments in Brazil, as well as the use of some promising molecules for the treatment of the disease, since they have an important anti-*Leishmania* activity.

METHODOLOGY

The methodology used was a literature review, searching for specific information about the current drugs and new therapeutic approaches for leishmaniasis treatment.

DEVELOPMENT

The choice of appropriate therapy for each clinical manifestation of leishmaniasis is based on some important criteria: [1] host factors such as genetics, immune response and clinical presentation of the disease; [2] treatment resources, such as quality of the drug, dosage, and duration and completion of the therapy; and [3] characteristics of the parasite, such as intrinsic sensitivity of the species and lack of resistance to the medication[2]. The following drugs are recommended by WHO as pharmacological treatment: pentavalent antimony, amphotericin B, pentamidine, miltefosine and paramomycin (Table 1).

PENTAVALENT ANTIMONY

Pentavalent antimonials have been extensively used in leishmaniasis therapy and have been indicated as first choice drug since 1945 in several places in the world[14]. The first report on the use of antimonials in *Leishmania* was performed by Brazilian physician Gaspar Vianna, who showed the use of (Sb^{III}) for the treatment of mucocutaneous leishmaniasis [15]. The anti-*Leishmania* activity was later confirmed also for visceral leishmaniasis in Italy and Africa [16 – 17]. However, due to the high toxicity produced by this drug, years later the pentavalent form of antimony (Sb^{V}) was discovered and is used to date for the treatment of most leishmaniasis[18].

Pentavalent antimonials are marketed as N-methylglucamine antimoniate (NMG) (trade name Glucantime®) in Latin America and Africa and as sodium stibogluconate (SGS) (trade name Pentostam®) in Europe and the United States [5, 19]. Both present similar results for the treatment of clinical forms of American

tegumentary leishmaniasis (ATL), and this equivalent efficacy can be explained due to they belong to the same pharmacological class and have similar mechanism of action and pharmacokinetics[19]. In addition, they exhibit a low oral absorption, therefore their administration is by parenteral route (intramuscularly or intravenously)[18, 20]. Although antimonials have been used for more than 70 years to treat leishmaniasis, there are still important gaps concerning the precise mode these drugs action. Probably, it is related to multifactorial actions involving effect on molecular processes of the parasite and influence on the parasiticidal activity of macrophages[5]. Three hypothetical models are proposed regarding the action mechanism of these drugs:

(1) The Sb^{V} behaves as a prodrug and undergoes biological reduction to a more active/toxic form, Sb^{III}, which has leishmanicidal activity. However, the site and mechanism of reduction remain controversial since thiol compounds from both the mammalian host (glutathione) and the parasite (trypanothione) could be involved in the reduction of Sb^{V} to Sb^{III}. Regardless of the reduction mechanism involved, trivalent antimony would promote the inhibition of trypanothione reductase, an enzyme that regenerates the reduced trypanothione, an important biomolecule for the metabolism of hydrogen peroxide in trypanosomatids. Furthermore, Sb^{III} could bind to proteins involved in DNA structure, replication and repair[21 - 22].

(2) The Sb^{V} has intrinsic anti-*Leishmania* activity. The drug would cause ATP depletion probably via inhibition of parasite glycolysis and β-oxidation. It has also been reported that Sb^{V} could form complexes with ribonucleosides, interfering in the purine metabolism[2, 22].

(3) Antimony acts in the macrophages activation, inducing the production of reactive oxygen species, nitric oxide and cytokines, which would culminate with the death of intracellular parasites[22].

The main adverse effect of pentavalent antimonials is their action on the cardiovascular system causing cardiac arrhythmias, ventricular systole, tachycardia and fibrillation and changes in the electrocardiogram[14; 23]. Other adverse effects include arthralgia, myalgia, anorexia, nausea, vomiting, gastric fullness, abdominal

pain, pancreatitis, itch, fever, weakness, headache, dizziness, palpitation, insomnia, nervousness, edema, liver enzyme disorders and acute renal failure [23]. Moreover, antimonials are contraindicated in pregnant women, since the drug crosses the transplacental barrier and may lead to severe mental retardation syndromes. There are also restrictions on the use of the drug in patients over 50 years of age, who have cardiopathy, nephropathy, liver disease and Chagas ones[2]. Other limitations to the antimonials efficacy include an increase in drug parasite resistance [24] and its preferably parenteral route of administration, which makes the treatment expensive and difficult, especially in rural areas[24].

In Brazil, the pentavalent antimony use as a first choice drug is recommended for both TL and VL. The recommended dose of pentavalent antimony ranges from 10 to 20 mg/kg/day over a period of 20 to 30 days, and the treatment time may be extended to some clinical forms of leishmaniasis [25 - 26]. In case of contraindication or unsatisfactory response to treatment, second-line drugs, described in the following, may be used.

AMPHOTERICIN B AND LIPOSOMAL AMPHOTERICIN B

Amphotericin B is a polyene antibiotic initially isolated from the microorganism *Streptomyces nodosus* in the 1950s with potent antifungal action. The first report of therapeutic success in the use of Amphotericin B in patients with leishmaniasis occurred in Brazil in 1963 with three individuals who presented the visceral form of the disease[27].

It is a potent leishmanicide drug that presents selective toxicity by interfering with the esters (preferably ergosterol) of parasites cytoplasmic membrane forming pores that increase the permeability of the parasite membrane and promote an influx of ions. The change in ionic balance leads to the death of promastigotes and amastigotes [19, 24]. The Amphotericin B deoxycholate (AmBD) was first licensed in 1959 for use in fatal fungal infections.

Because of intravenous application and the high toxicity and extensive adverse effects caused by amphotericin B, new presentations of this drug has been developed including liposomal amphotericin B (AmBisome®; Gilead Sciences),

Amphotericin B lipid complex (ABLCcet®, Enzon Pharmaceuticals) and amphotericin B colloidal dispersion (ABCD; Amphotec ™, Intermune Corp.) have been produced [28]. The liposomal amphotericin B is a pharmaceutical formulation in which amphotericin B is incorporated into liposomes containing phosphatidylcholine, cholesterol and disterolfosphatidylglycerol. In Brazil, the drug is registered in the National Agency of Sanitary Surveillance (ANVISA) for use in the treatment of visceral leishmaniasis, and it is considered an off-label drug for tegumentary leishmaniasis. The off-label use of any medicine can be performed at the risk of the prescribing physician. Literature data indicate the use of liposomal amphotericin B for TL treatment in cases in which all other therapeutic options have been contraindicated or used without success[29].

Amphotericin B is commonly used for treatment of VL[23] and to treat leishmaniasis where antimony resistance is widespread. Its use has also been successful for the treatment of cutaneous leishmaniasis in the "New World" caused by *Leishmania (Viannia) braziliensis*, a species that is known to have high levels of antimony resistance. A study carried out in Bolivia with *L. (V.) braziliensis* showed that liposomal amphotericin B was more efficient (cure rate 85%), better tolerated, and more cost-effective than antimonials[30]. In Brazil, another study with infections mainly caused by *L. (V.) braziliensis* showed a cure rate of 81% and few side effects[31]. There is high variety of total treatment dosages and it shows that there is no consensus on the optimal dosage or schedule of application. Typically, liposomal amphotericin B is used in the dose of 2.5 – 5.0 mg/kg/day and the treatment periodicity depends on the clinical manifestation and the therapy (combination of drugs or use alone)[5, 7]. This formulation reduces the treatment total time by half when compared to the non-liposomal form[5].

Serious adverse effects are related to the intravenous administration of amphotericin B, such as: fever, headache, nausea, vomiting, anorexia, tremors, chills, cyanosis, hypotension, hypopotassemia, hypomagnesemia, impaired renal function, behavioral disorders [5, 14, 24, 28]. Then, the drug is contraindicated for cardiac patients, hepatopathies and especially nephropathies. Furthermore, it

requires intravenous administration[28]. Liposomal amphotericin B also has limitations, such as high cost and renal toxicity[5, 32].

PENTAMIDINE

Pentamidine, an aromatic diamine, was originally used for the treatment of African trypanosomiasis [33 – 34]. Its first use for the treatment of VL was reported in 1949 in India and in 1950 in Spain [35 - 36] and since then it has been used as a second-choice drug for the treatment of CL in endemic areas of the American, Asian and African continents. Pentamidines are marketed for human use as Isethionate (di-B-hydroxyethane sulfonate) and Mesylate (di-B-hydroxymethylsulfonate) formulations [37] with the following trade names 'Pentacrinat' and 'Pentam'[5].

It is known that pentamidines interfere with glucose metabolism. The drug can induce cytolysis of β-pancreatic cells and, consequently, insulin-dependent diabetes [2]. In Brazil, the pentamidine has been used as a second-choice drug for the treatment of TL/VL [38]. The drug has also been used as first choice for cutaneous leishmaniasis caused by *Leishmania (Viannia) guyanensis* in French Guiana and Suriname[39].

The most frequent adverse reactions are pain, induration and sterile abscesses at the site of application, as well as nausea, vomiting, dizziness, myalgia, headache, hypotension, syncope, hypoglycemia and hyperglycaemia[5, 23]. There is contraindication to use in case of gestation, diabetes, renal and hepatic insufficiency, heart disease and for children weighing less than 48 kg[2].

Few studies using pentamidine as a therapy for ATL were conducted in the Americas. Classically, the recommended dose is 4.0 mg/kg/day, by deep intramuscular route, every two days and it is not recommended to exceed the total dose of 2.0 g[29]. For the treatment of CL caused by *L. (V.) guyanensis* and *Leishmania (Viannia) panamensis* in South America, the 4.0 mg/kg dose has been used for 3 – 5 days, intravenously [40]. In Brazil, the *L. (V.) braziliensis* infection treated at a dose of 4.0 mg/kg/day on alternate days for one week resulted in healing of 71% of patients[37].

MILTEFOSINE

Originally investigated as an antineoplastic agent, the miltefosine had the leishmanicidal action tested in 1996 when its *in vitro* activity against *Leishmania (Leishmania) donovani* amastigote forms and later against *Trypanosoma cruzi* was verified[32,41]. Miltefosine (hexadecylphosphocholine) is derived from alkyl-lysophospholipids and it is available under the trade names Impavido® and Miltex®[5].

The mode the drug works in *Leishmania* is still not well understood. However, it is believed the mechanism of action is similar to that of tumor cells, involving apoptosis and disruption of the lipid-dependent cell signaling pathway[42].

Miltefosine has been shown to have good results, mainly for oral TL treatment, since it has a low toxicity when compared to antimonials[43]. The drug is also used as a second-choice for the treatment of VL in some countries[32]. As the drug is very well tolerated, the main adverse effects include nausea and vomiting. Limitations for the use of miltefosine include its relatively high cost, need for monitoring of gastrointestinal side effects and occasionally liver and nephrotoxicity. In addition, it is a teratogenic agent and therefore contraindicated for pregnant women[2,5,23].

Miltefosine has been used for the treatment of "New World" cutaneous leishmaniasis caused by several species and different levels of efficacy were observed[44]. In the "New World" CL caused by *Leishmania (Leishmania) mexicana*, *L. (V.) guyanensis* and *L. (V.) panamensis*, the drug is effective at 2.5 mg/kg/day dose for 28 days, but is not effective for the treatment of *L. (V.) braziliensis* infections[5, 23]. Bolivian CML responded to treatment with miltefosine for 4 – 6 weeks, with a cure rate of up to 75%[45].

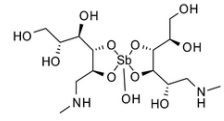
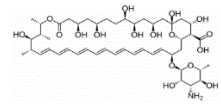
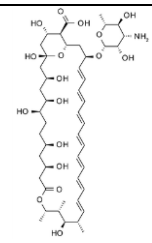
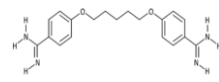
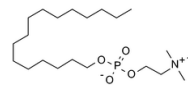
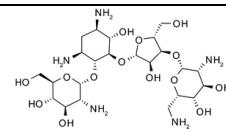
PARAMOMYCIN

It is a broad spectrum aminoglycoside antibiotic isolated from the *Streptomyces krestomuceticus* bacterium, which blocks proteins synthesis due to the binding to ribosomal RNA[32]. It has been used in topical and parenteral formulations for the treatment of CL, but its systemic use is rare[23]. Data on the use of paramomycin are

fundamentally limited to its topical use because the lack of knowledge about its efficacy when administered parenterally[44].

Local adverse effects associated with the use of paramomycin in topical formulation include itch, rash, and burning [20]. Adverse effects due to systemic use include ototoxicity, vestibular instability, and nephrotoxicity[5,19].

Table 1 – Drugs used to treat leishmaniasis.

Drug	Structure	Trade names	Current situation	Adverse effects
Antimony	 (47)	Glucantime® (*N-methylglucamine antimoniate) Pentostan® (*Sodium stibogluconate)	First-choice drug for VL and TL	Cardiotoxicity; nephrotoxicity and hepatotoxicity
Amphotericin B	 (48)	Anforicin B®, Anfolip B®, Abelcet®, Funtex B®, Unianf®, Amphocil®	Second-choice drug for VL and TL	Hypopasemia; anorexia; renal insufficiency; anemia; leucopenia and cardiac changes
Liposomal Amphotericin B	 (47)	Ambisome®	Restricted use for cases where other therapies have not shown therapeutic success	Renal toxicity
Pentamidine	 (49)	Fauldpenta® Pentacarinat®	Second-choice drug for VL and TL.	Hypoglycemia; hyperglycemia; nausea and vomiting and hypotension
Miltefosine	 (47)	Milteforan® Miltex®	Second-choice drug for VL and TL.	Nausea and vomiting and casionally nephrotoxicity and hepatotoxicity
Paramomycin	 (47)	Humatin®	Second-choice drug for TL.	Pruritus; rash; ototoxicity and nephrotoxicity

Paramomycin is available in several topical formulations, which have shown variable results for CL. Topical treatment for CL in the "New World" consists of 15% paramomycin ointment with 12% methyl benzethonium chloride applied once daily for 20 days[19,24]. Methyl benzethonium acts as a skin penetration

facilitating agent[19]. The topical formulation is easy to administrate and its side effects include itch, burning and vesicles formation[24]. The combination of paramomycin with gentamicin has also been used[19]. The systemic use at 20 mg/kg/day dose for 20 days showed an excellent cure rate (greater than 90%) in Brazil[46].

OTHER THERAPEUTIC APPROACHES – POTENTIAL OF METAL COMPLEXES: RUTHENIUM COMPLEXES

Bioinorganic chemistry reached its milestone in the 1960s through studies by Rosenberg and colleagues, which described the antitumor properties exhibited by *cis*-platinum, a Pt (III) complex[50]. The success of cisplatin triggered an intense search for metalloproteinases that had biological activity. Thus, several studies have shown the use of metallic complexes in different pathologies such as cancer[51-52], cardiovascular problems [53] and parasitic diseases[54 - 56].

There are similarities between protozoa of the Trypanosomatidae family and tumor cells, since both present highly proliferative cells, thus requiring a great demand of nutrients. Considering that many antitumor drugs are based on preclusion of obtaining nutrients or generating enough energy for this high metabolic demand, chemotherapeutic agents are expected to present action against protozoan trypanosomatids, such as *Leishmania*[57].

The first reports involving the application of metallic complexes in parasitic diseases caused by trypanosomatids were performed by Williamson and Farrell, who verified the *in vitro* activity of *cis*-platin complexes against *Trypanosoma rhodesiense*[58-59]. Several attempts to develop metallodrugs with antiparasitic action are underway and take into account some strategies[60-61], such as: (I) inclusion of a metallic center in the antiparasitic drug structure to promote an increase in pharmacological properties due to a synergistic action between drug and metal, through multiple mechanisms of action; (II) coordination of the metal with DNA intercalators to promote inhibition of parasite multiplication; and (III) coordination of the metal with binders or inhibitors that specifically act on parasitic enzymes allowing the targeting of the metal to the parasite in a selective way.

The Ruthenium (Ru) is a transition metal and, like platinum, presents different stages of oxidation: Ru (II), Ru (III) and Ru (IV). Some ruthenium complexes are already in clinical screening for cancer therapy[62-63]. The pioneering work of Sanchez-Delgado, more than 20 years ago, represented the starting point for the use of ruthenium complexes as metallodrugs for the parasitic infections treatment[54].

The intent of the study was to elucidate the synergism between drug and metal and the multiplicity of pharmacological targets. In this sense, ruthenium complexes coordinated with clotrimazole (CTZ) – $[RuCl_2(CTZ)_2]$ and subsequently with ketoconazole (KTZ) drugs were developed[54, 64]. CTZ and KTZ are antifungal drugs that act by inhibiting the sterol biosynthesis pathway and also exhibit moderate activity against trypanosomatids. $[RuCl_2(CTZ)_2]$ showed a 10-fold more potent *in vitro* activity against *T. cruzi* when compared to the free CTZ action[54].

Furthermore, in relation to the targets multiplicity hypothesis, it was determined that binding of $[RuCl_2(CTZ)_2]$ to DNA would promote the release of CTZ, which would present its classical action in the biosynthesis pathway of sterols[54,65–66]. Ruthenium complexes coordinated with CTZ also showed synergism in *Leishmania*. *In vitro* studies showed that 8 CTZ-associated complexes exhibited toxicity against *Leishmania (Leishmania) major* promastigote forms and that this action was remarkably superior to free CTZ activity. Another study showed the coordination of ruthenium complexes with lapachol, which has antitumor and antimicrobicide action, resulted in complexes with anti-*Leishmania* (promastigote and intracellular amastigote) activity more potent than free lapachol[56]. Activity against trypanosomatids data, obtained with the ruthenium complexes associated to drugs, confirm the synergistic action between the metal and the drug and highlight the therapeutic potential of these metallic complexes.

A remarkable anti-*Leishmania* activity has recently been described by Costa and colleagues (2017) for the use of new ruthenium (II) complexes against *Leishmania (Leishmania) amazonensis*, *L. (V.) braziliensis* and *Leishmania (Leishmania) infantum* species. Such complexes, referred to as complex 1 – cis-

[$Ru^{II}(\eta^2O_2CC_{10}H_{13})$ $(dppm)_2$] PF_6, complex 2 – cis-[$Ru^{II}(\eta^2$-$O_2CC_7H_7S)$ $(dppm)_2$] PF_6 and complex 3 – cis-[$Ru^{II}(\eta^2$-$O_2CC_7H_7O_2)(dppm)_2$] PF_6, exhibited potent cytotoxic activity against promastigotes of the three species analyzed, presenting IC_{50} values ranging from 0.52 – 12.49 µM.

Additionally, the effect of ruthenium complexes on the parasite-host interaction was verified by the infectivity test performed on RAW 264.7 macrophages, using two different concentrations: (1) cytotoxic concentration for 50% of promastigotes (IC_{50}) and (2) non-toxic concentration to 90% of macrophages. Complexes **1 – 3** at both concentrations tested were able to significantly reduce the infectivity index (37 – 87%) for all species of *Leishmania* analyzed. Interestingly, there was no statistically significant difference between the concentrations tested, except for complex **1** against the *L. (L.) infantum* species[67].

The antiproliferative activity of novel ruthenium complexes associated with purine analogues, 5,6,7-trimethyl-1,2,4-triazolo [1,5-a] pyrimidine (tmtp): cis, fac-[$RuCl_2$ (dmso)$_3$ (tmtp)] (complex 1), mer-[$RuCl_3$(dmso)(H_2O)(tmtp)]•$2H_2O$ (complex 2) and fac,cis- [$RuCl_3$ (H_2O) $(tmtp)_2$] (complex 3) was recently reported against *Leishmania* spp (*L. (V.) braziliensis*, *L. (L.) donovani* and *L. (L.) infantum*) promastigote and *Trypanosoma cruzi* epimastigote forms. Complexes 1 – 3 exhibited IC_{50} values ranging from 9.2 – 202.5 µM for different *Leishmania* promastigote forms analyzed and 32.4 – 51.5 µM for the epimastigote form of *T. cruzi*[68].

The *in vitro* and in *vivo* leishmanicidal activity of nitrosyl complex **(***cis*[Ru(bpy)$_2$(SO_3)(NO)](PF_6) was recently evaluated against *L. (V.) braziliensis. In vitro*, low concentrations of the complex were able to significantly decrease the cellular infection after 24h of treatment, leading to a total reduction of infectivity with the use of 50 µM of the complex. In the *in vivo* infection model, the daily treatment with 300 µg/ kg/day of the complex was able to reduce the size of the lesion in a significant way (51%), eliminating almost all (99.9%) the parasites found in the lymph nodes[69].

The mechanism of death involved in the action of a drug has been increasingly explored, since its knowledge enables a better understanding of the cellular response involved, configuring an important tool for the prospection of new therapeutic agents. Recently, Costa and colleagues (2019) demonstrated the mechanism of death involved in the action of the complex of ruthenium called hmxbato (*cis*-[Ru^{II}(η^2-$O_2CC_7H_7O_2$)(dppm)$_2$]PF_6) against *L. (L.) amazonensis* promastigotes. The complex was able to induce death by apoptosis, which was demonstrated by the following changes: (1) depolarization of the mitochondrial membrane, (2) increase in reactive oxygen species production, (3) DNA fragmentation, (4) formation of to pre-apoptotic peak, (5) alterations in parasite morphology and (6) formation of autophagic vacuoles[70].

Taken together, these results show that ruthenium complexes may be considered excellent candidates for the new pharmacological targets prospection against *Leishmania* parasites. Although promising, there are few studies demonstrating the probable mechanism of parasite death. Then, new studies will be needed aiming at elucidation of the promising potential of ruthenium complexes for the treatment of leishmaniasis.

FINAL CONSIDERATIONS

The present work describes the main therapies used to treat the various clinical manifestations of leishmaniasis. Given that the anti-*Leishmania* drugs available are often associated with problems as serious side effects and the possibility of parasitic drug resistance, there is an intense search for the development of new therapies for this disease. Some therapeutic alternatives and new molecules with anti-*Leishmania* activity were also highlighted.

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REFERENCES

(1) Lainson, R.; Shaw, J. Evolution, classification and geographical distribution. In: Peters, W.; Killick-Kendrick, R. eds. The leishmaniasis in Biology and Medicine, Biology and Epidemiology. London: Academic Press. 1987, 1120 p.

(2) Anversa, L.; Tiburcio, M. G. S.; Richini-Pereira, V. B.; Ramirez, L. E. 2018. Human leishmaniasis in Brazil: a general review. Revista da Asssociação Médica Brasileira,64(3): 281-289.

(3) Von Stebut, E. 2015. Leishmaniasis. JDDG: Journal der Deutschen Dermatologischen Gesellschaft, 13: 191-201.

(4) Sunyoto, T.; Boelaert, M.; Meheus, F. 2019. Understanding the economic impact of leishmaniasis on households in endemic countries: a systematic review. Expert Review of Anti-infective Therapy, 17: 57-69.

(5) Mcgwire, B. S.; Satoskar, A. R. 2014. Leishmaniasis: clinical syndromes and treatment. International Journal of Medicine, 107: 7-14.

(6) Bi, K.; Chen, Y.; Zhao, S.; Kuang, Y.; Wu, C. H. J. 2018. Current visceral leishmaniasis research: a research review to inspire future study. BioMed Research International, 2018: 1-13.

(7) Mosimann, V.; Neumayr, A.; Paris, D. H.; Blum, J. 2018. Liposomal amphotericin B treatment of Old World cutaneous and mucosal leishmaniasis: A literature review. Acta Tropica, 182: 246-250.

(8) Pace, D. Leishmaniasis. 2014. Journal of infection, 69: 510-518.

(9) Aagaard-Hansen, J.; Nombela, N.; Alvar, J. 2010. Population movement: a key factor in the epidemiology of neglected tropical diseases. Tropical Medicine & International Health, 15(11): 1281-1218.

(10) Argaw, D.; Mulugeta, A.; Herreo, M.; Nombela, N.; Teklu, T.; Tefera, T.; Zewdu, B.; Alvar, J.; Bern, C. 2013. Risk factors for visceral leishmaniasis among residents and migrants in Kaft a-Humera, Ethiopia. PLOS Neglected Tropical Diseases, 7(11): 1-9.

(11) Lindoso, J. A. L.; Lindoso, A. A. B. P. 2009. Neglected tropical diseases in Brazil. Revista do Instituto de Medicina Tropical de São Paulo, 51(5): 247-253.

(12) Sarteriale, A.; Bessoff, K.; Sarkar, I. N.; Huston, C. D. 2014. Drug repurposing: mining protozoan proteomes for targets of known bioactive compounds. The Journal of American Medical Informatics Association, 21: 238-244.

(13) Vias, S. J.; Stolk, W. A.; Rutte, E. A.; Hontelez, J. A. C.; Bakker, R.; Blok, D. J.; Cai, R.; Houweling, T. A. J.; Kulik, M. C.; Lenk, E. J.; Luyendijk, M.; Matthijsse, S. M.; Redecop, W. K.; Wagenaar, I.; Jacobson, J.; Nagelkerke, N. J. D.; Richardus, J. H. 2016. Concerted efforts to control or eliminate neglected tropical diseases: how much health will be gained? Plos Neglected Tropical Diseases, 10(2).

(14) Rama, M.; Kumar, N. V. A.; Balaji, S. 2015. A comprehensive review of patented antileishmanial agents. Pharmaceutical Patent Analyst, 4(1): 37-56.

(15) Vianna, G. 1912. Comunicação à Sociedade Brasileira de Dermatologia. Arquivo Brasileiro de Medicina, 2: 426.

(16) Di Cristina, G; Caronia, G. 1915. "Sulla terapia della leishmaniosis interna. Pathologica, 7: 82-83.

(17) Cole, A.C.E. 1944. Kala-azar in east Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene, 37: 409-435.

(18) Rath, S; Trivelin, L. A; Imbrunito, T. R; Tomazela, D. M; de Jesus, M. N; Marzal, P. C; Junior, H. F. A; Tempene, A.G. 2003. Antimoniais empregados no tratamento da leishmaniose: estado da arte. Química Nova, 6(4): 550-555.

(19) Lima, E. B.; Motta, J. O. C.; Porto, C.; Sampaio, R. N. R. 2007. Tratamento da leishaniose tegumentar americana. Anais Brasileiros de Dermatologia, 82(2): 111-124.

(20) Balaña-Fource, R.; Reguera, R. M.; Cubría, J. C.; Ordóñez, D. 1998. The pharmacology of leishmaniasis. General Pharmacology, 30(4): 435-443.

(21) Turrens, J. F. 2004. Oxidative stress and antioxidant defenses: a target for the treatment of diseases caused by parasitic protozoa. Molecular Aspects of Medicine, 25: 211-220.

(22) Haldar, A. K.; Sen, P.; Roy, S. 2011. Use of antimony in the treatment of leishmaniasis: current status and future directions. Molecular Biology International, 2011: 1-23.

(23) Sundar, S.; Chakravarty, J. 2015. An update oh pharmacotherapy for leishmaniasis. Expert Opinion on Pharmacotherapy, 16(2): 237-252.

(24) Handler, M. Z.;Patel, P. A.; Kapila, R.; Al-Qubati, Y.; Schwartz, R. A. 2015. Cutaneous and mucocutaneous leishmaniasis: differential diagnosis, diagnosis, histopathology and management. Journal of the American Academy of Dermatology, 73(6): 911-926.

(25) Herwaldt, B. L.; Berman, J. D. 1992. Recommendations for treating leishmaniasis with sodium stibogluconate (Pentostam) and review of pertinent clinical studies. American Journal of Tropical Medicine & Hygiene, 46(3): 296-306.

(26) Abramowicz, M. 1993. Drugs for parasitic infections. The Medical letter on drugs and therapeutics, 1993(33): 111-122.

(27) Prata, A. Treatment of kala-azar with amphotericin B. 1963. Transactions of the Royal Society of Tropical Medicine and Hygiene, 57(4): 266-268.

(28) Andrews, K. T.; Fisher, G.; Skinner-Adams, T. S. Drug repurposing and human parasitic protozoan diseases. 2014. International Journal for Parasitology: Drug and Drug Resistance, 4: 95-111.

(29) Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de Vigilância da Leishmaniose Tegumentar Americana/Ministério da Saúde. Brasília: Editora do Ministério da Saúde, 2013.

(30) Solomon, M. P. F.; Barzilai, A.; Schwartz, E. 2013. Liposomal amphotericin B in comparison to sodium stibogluconate for *Leishmania braziliensis* cutaneous leishmaniasis in travelers. Journal of the American Academy of Dermatology, 68(2): 284-289.

(31) Motta, J. O.; Sampaio, R. N. 2012. A pilot study comparing low-dose liposomal amphotericin B with N-methyl glucamine for the treatment of American cutaneous leishmaniasis. Journal of the European Academy of Dermatology and Venereology, 26: 331-335.

(32) Nagle, A. S.; Khare, S..; Kumar, A. B.; Supek, F.; Buchynskyy.; Mathison, C. J.; Chennamaneni, N. K.; Pendem, N.; Buckner, F. S.; Gelb, M. H.; Molteni, V. 2014. Recent developments in drug discovery for leishmaniasis and human African trypanosomiasis. Chemical Reviews, 26(114): 11305-11347.

(33) Lourie, E. M.; Orke, W. 1939. Studies in chemotherapy. XXI. The trypanocidal action of Certain aromatic diamidines. Annals of Tropical Medicine Parasitology, 33: 289-304.

(34) Lourie, E. M. 1942.Treatment of sleeping sickness in Sierra Leone. Annals of Tropical Medicine Parasitology, 36: 113-131.

(35) Hazarika, A. N. 1949. Treatment of kala-azar with pentamidine isothionate; a study of 55 cases. The Indian Medical Gazette, 84(4): 140-145.

(36) Martínez, A.; Carreon, T.; Iniguez, E.; Anzelotti, A.; Sánchez, A.; Tyan, M.; Sattler, A.; Herrera, L.; Maldonado, R. A.; Sáchez-Delgado, R. A. 2012. Searching for new chemotherapies for tropical diseases: Ruthenium – Clotrimazole complexes display high *in vitro* activity against *Leishmania major* and *Trypanosome cruzi* and low toxicity toward normal mammalian cells. Journal of Medicinal Chemistry, 55: 3867-3877.

(37) de Paula, C. D.; Sampaio, J. H.; Cardoso, D. R.; Sampaio, R. N. 2003. A comparative study between the efficacy of pentamidine isothionate given in three doses for one week and N-methil-glucamine in a dose of 20mgSbV/day for 20 days to treat cutaneous leishmaniasis. Revista da Sociedade Brasileira de Medicina Tropical, 36(3): 365-371.

(38) Mitropoulos, P.; Konidas, P.; Durkin-Konidas, M. 2010. New World cutaneous leishmaniasis: updated review of current and future diagnosis and treatment. Journal of the American Academy of Dermatology, 63(2): 309-322.

(39) Roussel, M; Nacher, M; Fremont, G; Roturneau, B; Clyti, E; Sainte-Marie, D; Carme, B; Pradinaud, R; Couppie, P. 2006. Comparison between one and two injections of pentamidine isethionate, at 7 mg/kg in each injection, in the treatment of cutaneous leishmaniasis in French Guiana. Annals of Tropical Medicine and Public Health, 100(4): 307-314.

(40) Hellier, I.; Dereure, O.; Tournillac, I.; Pratlong, F.; Guillot, B.; Dedet, J. P.; Guilhou, J. J. 2000. Treatment of Old World cutaneous leishmaniasis by pentamidine isethionate. An open study of 11 patients. Dermatology, 200(2): 120-12.

(41) Croft, S. L.; Snowdon, D.; Yardley, V. 1996. The activities of four anticancer alkyllysophospholipids against *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei*. Journal of Antimicrobial Chemotherapy, 38(6): 1041-1047.

(42) Singh, N.; Kumar, M.: Singh, R. K. 2012. Leishmaniasis: current status of available drugs and new potential srug targets. Asian Pacific Journal of Tropical Medicine, 5(6): 485-497.

(43) Machado, P. R.; Ampuero, J.; Guimarães, L. H.; Villasboas, L.; Rocha, A. T.; Schriefer, A.; Souza, R. S.; Talhari, A.; Penna, G.; Carvalho, E. M. 2010. Miltefosine in the treatment of cutaneous leishmaniasis caused by *Leishmania braziliensis* in Brazil: a randomized and controlled trial. PLOS Neglected Tropical Diseases, 21(4): 1-6.

(44) Monge-Maillo, B.; López-Vélez, R. 2013. Therapeutic options for Old World cutaneous Leishmaniasis and New World Cutaneous and Mucocutaneous Leishmaniasis. Drugs, 73: 1889-1920.

(45) Soto, J.; Rea, J.; Valderrama, M.; Toledo, J.; Valda, L.; Ardiles, J.; Berman, J. 2009. Efficacy of extended (six weeks) treatment with miltefosine for mucosal leishmaniasis in Bolivia. American Journal of Tropical Medicine & Hygiene, 81: 387-379.

(46) Correia, D.; Macedo, V. O.; Carvalho, E. M.; Barral, A.; Magalhaes, A. V.; de Abreu, M. V.; Orge, M. L., Marsden, P. 1996. Comparative study of meglumine antimoniate, pentamidine isethionate and aminosidine sulphate in the treatment of primary skin lesions caused by *Leishmania (Viannia) braziliensis*. Revista da Socidade Brasileira de Medicina Tropical, 29(5): 447-453.

(47) Burrows, J. N.; Elliott, R. L.; Kaneko, T.; Mowbray C. E. Waterson, D. 2014. The role of modern drug discovery in the fight against neglected and tropical diseases. Medicinal Chemistry Communications, 5: 688-700.

(48) Falci, D. R.; Pasqualotto, A. C. 2015. Anfotericina B: uma revisão sobre suas diferentes formulaões, efeitos adversos e toxicidade. Clinical & Biomedical Research, 35(2): 65-82.

(49) Dorlo, T. P. C.; Kager, P. A. 2008. Pentamidine dosage: a base/salt confusion. PLOS Neglected Tropical Diseases, 2(5): 1- 3.

(50) Rosenberg, B.; Vancamp, L.; Krigas, T. 1965. Inhibition of cell division in *Escherichia Coli* by electrolysis products from a platinum electrode. Nature, 205: 698-699.

(51) Komeda, S.; Casini, A. 2012. Next-Generation Anticancer Metallodrugs. Current Topics in Medicinal Chemistry, 12(3): 219-235.

(52) Pereira, F. C; Lima, B. A. V; de Lima, A. P; Pires, W. C; Monteiro, T; Magalhaes, L. F; Costa, W; Graminha, A. E; Batista, A. A, Ellena, J; Silveira-Lacerda, E. P. 2015. Cis-[RuCl(BzCN)(N-N)(P-P)]PF6 complexes: Synthesis and in vitro antitumor activity: (BzCN=benzonitrile; N-N=2,2'-bipyridine; 1,10-phenanthroline; P-P=1,4-bis(diphenylphosphino) butane, 1,2-bis(diphenylphosphino)ethane, or 1,1'-(diphenylphosphino)ferrocene). Journal of Inorganic Biochemistry, 149: 91-101.

(53) Bates, J. N; Baker, M. T; Guerra, R; HÁRRISON, D. G. 1991. Nitric oxide generation from nitroprusside by vascular tissue: Evidence that reduction of the nitroprusside anion and cyanide loss are required. Biochemical Pharmacology, 42(1): 157-165.

(54) Sánchez-Delgado, R. A.; Lazardi, K.; Rincón, L.; Urbina, J. A. 1993. Toward a novel metal-based chemotherapy against tropical diseases. 1. Enhancement of the efficacy of clotrimazole against *Trypanosoma cruzi* by complexation to ruthenium in $RuCl_2$(clotrimazole)$_2$. Journal of Medicinal Chemistry, 36(14): 2041-2043.

(55) Martinez Garcia, P.; Guasch, J.; Llaurado, F. 1950. First cases of infantile kala-azar treated in Spain with diamidines (stilbamidine and pentamidine). Revista Española Pediatría, 6(3): 319-346.

(56) Barbosa, M. I; Correa, R. S; DE Oliveira, K. M; Rodrigues, C; Ellena, J; Nascimento, O. R; Rocha, V. P; Nonato, F. R; Macedo, T. S; Barbosa-Filjo, J. M; Soares, M.B; Batista, A. A. 2014. Antiparasitic activities of novel ruthenium/lapachol complexes. Journal of Inorganic Biochemistry, 136: 33-39.

(57) Borst, P. 1977. Metabolism and chemotherapy of African trypanosomes, Transactions of the Royal Society of Tropical Medicine and Hygiene, 71: 3-4.

(58) Farrell, N. P; Williamson, J, Mclaren, D. J. M. 1984. Trypanocidal and antitumour activity of platinum-metal and platinum-metal-drug dual-function complexes. Biochemical Pharmacology, 33: 961-971.

(59) Farrell, N. P. Transition metal complexes as drugs and chemotherapeutic agents. Dordrecht: Kluwer Academic Publishers. 1989.

(60) Navarro, M.; Gabbiani, C.; Messori, L.; Gambino, D.2010. Metal-based drugs for malaria, trypanosomiasis and leishmaniasis: recent achievements and perspectives. Drug Discovery Today, 15: 1070-1078.

(61) Gambino, D.; Otero, L. 2012. Perspectives on what ruthenium-based compounds could offer in the development of potential antiparasitic drugs. Inorganica Chimica Acta, 393: 103-114.

(62) Bratsos, I.; Jedner, S.; Gianferrara, T.; Alessio, E. 2007. Ruthenium anticancer compounds: challenges and expectations. International Journal of Chemistry, 61: 692-697.

(63) Hartinger, C. G.; Jakupec, M. A.; Zorbas-Seifried, S.; Groessi, M.; Egger, A.; Berger, W.; Zorbas, H.; Dyson, P. J.; Keppler, B. K. 2008. KP1019, a new redox-active anticancer agent--preclinical development and results of a clinical phase I study in tumor patients. Chemistry & Biodiversity, 5(10): 2140-2155.

(64) Sánchez-Delgado, R. A.; Anzellotti, A. 2004. Metal complexes as chemotherapeutic agents against tropical diseases: Trypanosomiasis, Malaria and Leishmaniasis. Mini-Reviews in Medicinal Chemistry, 4(1): 23-30.

(65) Sánchez-Delgado, R. A.; Navarro, M.; Lazardi, K.; Atencio, R.; Capparelli, M.; Vargas, F.; Urbina, J. A.; Bouillez, A.; Noels, A. F.; Masi, D. 1998. Toward a novel metal based chemotherapy against tropical diseases 4. Synthesis and characterization of new metal-clotrimazole complexes and evaluation of their activity against *Trypanosoma cruzi*. Inorganica Chimica Acta, 275-276.

(66) Navarro, M.; Lehmann, T. E.; Cisneros-Farjado, E. J.; Fuentes, A.; Sánchez-Delgado, R. A.; Silva, P.; Urbina, J. A. 2000. Toward a novel metal based chemotherapy against tropical diseases. Synthesis and characterization of new Ru(II) and Ru(III) clotrimazole and ketoconazole complexes and evaluation of their activity against *Trypanosoma Cruz*i. Polyhedron, 19: 2319-2325.

(67) Costa, M. S.; Gonçalves, Y. G.; Nunes, D. C. O.; Napolitano, D. R.; Maia, P. I. S.; Rodrigues, R. S.; Rodrigues, V. M.; Von Poelhsitz, G.; Yoneyama, K. A. G. 2017. Anti- *Leishmania* activity of new ruthenium(II) complexes: Effect on parasite-host interaction. Journal of Inorganic Biochemistry, 175: 225-231.

(68) Frandzloch, M.; Arriaga, J. M. M.; Sánchez-Moreno, M.; Wojtczak, A.; Jezierska, J.; Sitkowski, J.; Wisniewska, J.; Salas, J.M.; Lakomska, I. 2017. Strategies of overcoming tropical disease by Ruthenium Complexes with purine analog: application against *leishmania spp* and *Trypanosoma cruzi*. Journal of Inorganic Biochemistry, 176: 144-155.

(69) Do Nascimento, N. R. F.; De Aguiar, F. L. N. Santos, C.F., Costa, A. M. L., Hardoim, D. J., Calabrese, K. S., Almeida-Souza, F., De Sousa, E. H. S., Lopes, L. G. F., Teixeira, M. J., Pereira, V.S., Brilhante, R. S. N., Rocha, M. F. G. 2019. *In vitro* and *in vivo* leishmanicidal activity of a ruthenium nitrosyl complex against *Leishmania (Viannia) braziliensis.* Acta Tropica, 192: 61 – 65.

(70) Costa, M.S., Gonçalves, Y.G., Teixeira, S.C.,Nunes, D. C. O., Lopes, D. S., Da Silva, C. V., Da Silva, M. S., Borges, B. C.,Silva, M. J. B., Rodrigues, R. S., Rodrigues, V. M., Von Poelhsitz, G., Yoneyama, K,A. G. 2019. Increased ROS generation causes apoptosis-like death: Mechanistic insights into the anti-*Leishmania* activity of a potent ruthenium(II) complex. Journal of Inorganic Biochemistry,195: 1-12.