Leishmaniasis and Chagas Disease Chemotherapy: a Critical Review

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Leishmaniose e doença de Chagas, causadas pelos parasitas kinetoplastideos Leishmania spp e Trypanosoma cruzi, respectivamente, estão entre as doenças parasitárias mais importantes, afetando milhões de pessoas e consideradas dentro do grupo mais relevante de doenças tropicais negligenciadas. A principal alternativa para controlar essas parasitoses é a quimioterapia. No entanto, os atuais tratamentos quimioterápicos estão longe de serem satisfatórios. Esta revisão delinea o entendimento atual de diferentes fármacos contra leishmaniose e doença de Chagas e seus mecanismos de ação. As abordagens recentes na área de terapias anti-Leishmania e tripanocida também são enumerados, assim como a busca por novas drogas.

Leishmaniasis and Chagas disease, caused by the kinetoplastid parasites Leishmania spp and Trypanosoma cruzi, respectively, are among the most important parasitic diseases, affecting millions of people and considered to be within the most relevant group of neglected tropical diseases. Chemotherapy is the main alternative to control such parasites, nevertheless, current treatments are far from satisfactory. This review outlines the current understanding on different drugs against leishmaniasis and Chagas disease and their mechanism of action. Recent approaches in the area of anti-leishmanial and trypanocidal therapies are also enumerated, as well as the search for new drugs.

Keywords: Visceral leishmaniasis, American tegumentary leishmaniasis, Chagas disease, Leishmania, Trypanosoma cruzi, chemotherapy

1. Leishmaniasis

Visceral leishmaniasis (VL), or kala-azar, is considered one of the oldest diseases of humanity, according to the World Health Organization (WHO). 1 It has long been confused with other diseases, such as malaria. It was described for the first time only in 1822 and in 1903 its etiologic agent was identified. 2 Charles Donovan found the parasite in the spleen of a Hindu child with irregular fever, but confused it for another protozoan, Trypanosoma brucei. After some false descriptions, Ronald Ross created the genus Leishmania and named the causative agent of visceral leishmaniasis, Leishmania donovani, in honor of William Boog Leishman and Charles Donovan. 3

The possible role of dogs in the epidemiology of leishmaniasis was suggested by Nicolle and Comte in 1908, in Tunisia, after the detection of VL in animals. 4 The first human case described in Brazil was a patient originally infected in the Brazilian state of Mato Grosso and that migrated to Assunción in Paraguay. 5 Penna 6 in 1934 described the parasite in liver of patients with yellow fever coming from the north and northeast of Brazil and one of the first observations of canine Leishmania infection was made by Chagas et al., 7 when he demonstrated the existence of the disease in man, dogs and Lutzomyia longipalpis, classifying the parasite as Leishmania chagasi.

Leishmaniasis is found in the tropical and subtropical regions and areas close to the Mediterranean. It is estimated that 350 million people are considered at risk of becoming infected with leishmaniasis and approximately 2 million
new cases are notified every year. The transmission of *Leishmania* spp is through the bite of female sand flies from the Phlebotomus genus in the Old World (Europe, Asia, Africa) and Lutzomyia genus in the New World (Americas). VL is mainly the result of infection by *Leishmania donovani* and *Leishmania infantum* (known as *Leishmania chagasi* in South America); sometimes, in few cases, *Leishmania tropica* in the Middle East and *Leishmania amazonensis* in South America can result in VL.\(^8\) Currently, VL is estimated to cause 12 million cases worldwide, with 200,000 to 400,000 new cases notified each year.\(^9\) Epidemiological studies have shown that more than 90% of VL are concentrated in only six countries: Bangladesh, Brazil, India, Sudan, South Sudan and Ethiopia, however, the prevalence of the disease is increasing in the Mediterranean region, Spain and France. Thus, VL is endemic in nine countries of the European Union (EU).\(^10\) Also, VL cases are reported in all continents, with exception of Antarctica.\(^1\)

VL is a fatal form of leishmaniasis, due to the involvement of several organs, such as liver, spleen and bone marrow,\(^1,9,10\) with mortality rates ranging from 70 to 95%, before the present chemotherapy was made available. Such mortality rates are among the highest reported for infectious diseases and show the severity of the parasite infection.

VL pathogenesis is initiated by the invasion of the mononuclear phagocyte system of some organs by *Leishmania*. In several cases it is accompanied by significant pathological alterations. Clinical manifestations are splenomegaly, hepatomegaly, as well as bone marrow involvement, and they result from the hypertrophy and hyperplasia of the macrophage system. Some organs with a high content of macrophage cells, such as the spleen, can affect lymphoid follicles and the circulation in the capillaries, causing severe congestion resulting in areas of ischemia. In the liver, considerable hypertrophy of Kupffer cells, crowding around the sinusoids or portal space, significantly affects this organ. Anemia is the result of the gradual replacement of the hematopoietic tissue by infected bone marrow macrophages.\(^16,17\) The clinical symptoms of VL infection are fever, weight loss, splenomegaly, hepatomegaly, anemia, leukopenia and thrombocytopenia. Such symptoms can be easily mistaken for other diseases if the clinician is not aware of the possibility of a *Leishmania* infection.\(^18\)

Immunosuppression is a potential outcome of VL infection resulting in a reduction of the patient responsiveness and resistance to antigens from other infections. In chronic cases fibrosis of the spleen tissue is observed, which gradually leads to a complete change in the organ architecture accompanied by portal hypertension and ascites, among other events that lead to a gradual, and often fatal, organ failure. Co-infection of VL and human immunodeficiency virus (HIV) is considered of great importance to public health due to its mortality rates and geographical incidence.\(^19,20\)

Dermal leishmaniasis post-kala-azar is a potential secondary manifestation of *L. donovani* infection, especially in individuals who did not receive leishmaniasis treatment, resulting in skin lesions in the form of small nodules, erythematous macules, containing *Leishmania* cells in large amounts.\(^21\)

Besides the described VL, usually fatal if untreated, several different clinical forms of leishmaniasis are described. The most common forms are cutaneous leishmaniasis, which cause skin sores leading to disfiguring lesions.\(^22,23\) In South America, cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis (DCL) and mucocutaneous leishmaniasis (MCL) are together termed the American tegumentary leishmaniasis (ATL).\(^24\)

ATL can be characterized by chronic skin ulcers. When they develop strictly to the site of the bite of the insect vector they are characterized as CL and can take several months to heal. MCL may be present as skin lesions similar to those of CL that heal spontaneously and reappear mainly in the mucosa of the nose and mouth. MCL is usually accompanied by secondary infections and destruction of the tissue. DCL, a rare form of leishmaniasis, causes infiltrative and non-ulcerating lesions in anergic individuals who do not respond to parasite antigens.\(^24-27\)

The pathogenesis of ATL is associated with host immune responses mediated by T cell and virulence of the infecting *Leishmania* species. The interaction between the different species of *Leishmania* and the immune response mechanisms result in a wide spectrum of clinical, histopathological and immunopathological manifestations in humans.\(^28\) In Brazil, ATL is caused by species of both Leishmania subgenera (*L. amazonensis*) and Viannia subgenera (*L. braziliensis, L. guyanensis*) and, exceptionally, *L. shawi, L. naiffi, L. lainsoni* and *L. lindenbergi*,\(^29,30\) posing an additional difficulty in ATL treatment due to the different drug response of each species to chemotherapy.

### 1.1. Current VL and ATL chemotherapy

Despite the long history of the discovery of human infection with *Leishmania* (VL and ATL), the main form of treatment for these diseases is still chemotherapy (Table 1). There is no effective vaccine available. Especially in the case of CL, the treatment is important to accelerate cure,
to reduce scars and to prevent parasite dissemination to mucosal sites, or relapse.\textsuperscript{51} Some characteristics of Leishmania parasites may affect treatment efficacy, such as different drug sensitivities of the several species that infect humans and the influence of immune suppression associated with leishmaniasis.\textsuperscript{52-55}

Available antileishmanial drugs still depend on high doses of pentavalent antimonials, such as glucantime, meglumine antimoniate, pentostam and sodium stibogluconate (Figure 1) that result in severe side effects and require long-time treatment.\textsuperscript{56-58} After administration, pentavalent antimonials are rapidly absorbed and are converted into trivalent antimonite inside the macrophage, which is the active form of the drug.\textsuperscript{31,32} The reduction of pentavalent to trivalent antimony takes place either in the macrophages or in the parasite.\textsuperscript{33} A specific parasite enzyme involved in this reduction process was identified as thiol-dependent reductase (TDR1) and is capable of catalyzing the conversion of the pentavalent form of antimony to the trivalent one using glutathione as the reducing agent.\textsuperscript{34-36} Myalgia, nausea, liver and cardiac disorders, abdominal pain, headache, and asthenia are side effects often associated with such drugs.\textsuperscript{37} Antimonials are also contraindicated in pregnancy and for patients with kidney, liver and heart diseases,\textsuperscript{38} reducing the effective use of the drugs.

A disadvantage of antimony is its rapid excretion by the kidneys, which makes a long-term administration of the drug necessary to achieve satisfactory therapeutic levels.\textsuperscript{39} The efficacy of treatment currently available is also compromised when there is immunosuppression, in particular due to co-infection with HIV, leading to exacerbation of the disease or emergence of latent infection.\textsuperscript{39} The antimonials have reduced activity in the absence of immune response mediated by T cells.\textsuperscript{60}

Meglumine antimoniate (Figure 1) mechanism of action remains poorly known.\textsuperscript{40,41} It is believed that the mechanism

\begin{table}[h!]
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\begin{tabular}{|l|l|l|l|}
\hline
Drugs & Mechanism of action & Outcome & Disadvantage & Reference \\
\hline
Pentavalent antimony (Parenterally, daily, for at least three weeks (20 mg kg\textsuperscript{-1} day\textsuperscript{-1} for 20-30 days) & Rapid absorption and inside the macrophage are converted into trivalent antimonite, which is the active form of the drug & TDR1\textsuperscript{t} is capable of catalyzing the conversion of the pentavalent form of antimony to trivalent one using glutathione as the reducing agent & Side effects: myalgia, liver and cardiac disorders, abdominal pain, headache, asthenia. Its rapid excretion by the kidneys, which makes long-term administration of the drug necessary & 31-39 \\
\hline
Meglumine antimoniate (Glucantime\textsuperscript{a}) (15 mg kg\textsuperscript{-1} day\textsuperscript{-1} for 20 days) & Interference on amastigote bioenergetic process. Inhibits parasite proteins, as enzymes involved in glycolysis and fatty acid oxidation, reduction in the production of ATP\textsuperscript{a} and GTP\textsuperscript{a} & Precaution for use in the elderly; acute pancreatitis, acute renal failure, leukopenia & High level of toxicity & 40-43 \\
\hline
Sodium stibogluconate (Pentostam\textsuperscript{a}) (20 mg kg\textsuperscript{-1} day\textsuperscript{-1} i.v.\textsuperscript{a} for 10 or 20 days) & Inhibits type I DNA topoisomerase & Low concentrations induce increases in ROS\textsuperscript{a} formation, alter the state of phagocyte activation; affects the production of superoxide, indicating that the activity of NADPH oxidase is enhanced & May cause diabetes mellitus, proteinuria, hypotension, myalgia, headache & 42, 44-46 \\
\hline
Amphotericin B/liposomal amphotericin B (10 mg kg\textsuperscript{-1} day\textsuperscript{-1}) & Macrocyclic, polyene antifungal agent, it is thought to act by binding to ergosterol, the principal sterol in fungal cell membranes and Leishmania cells & Change in membrane permeability, causing metabolic disturbance, leakage of small molecules and, as a consequence, cell death & Hyperpyrexia, severe malaise, hypotension, thrombophlebitis, azotemia, renal tubular damage, hypokalemia, anemia and hepatitis & 47, 48 \\
\hline
Miltefosine (One 50 mg capsule twice daily with food (breakfast and dinner)) & Involves interaction with lipids (phospholipids and sterols), including membrane lipids, inhibition of cytochrome C oxidase (mitochondrial function), and apoptosis & Protein kinase B (Akt) inhibitor, a serine/threonine-specific protein kinase that plays an important role in several cellular mechanisms, such as glucose metabolism, cell proliferation and migration, regulating cellular survival & Low therapeutic effect suggested resistance, high cost, teratogenic and severe gastrointestinal side effects, such as vomiting and nausea & 49, 50 \\
\hline
\end{tabular}
\caption{Summary of antileishmanial drugs}
\end{table}

\textsuperscript{t}TDR1: thiol dependent reductase; ATP: adenosine triphosphate; GTP: guanosine triphosphate; i.v.: intravenously; ROS: reactive oxygen species.
of action of antimonials is based on the interference of amastigote bioenergetic process. Metabolic compounds of the drug inhibit different parasite proteins, particularly enzymes involved in glycolysis and fatty acid oxidation, resulting in a reduction in the production of adenosine triphosphate (ATP) and guanosine triphosphate (GTP), and reports have raised the possibility that antimony could trigger apoptosis. In addition to antimonials, other drugs have been used as alternatives in the treatment of leishmaniasis, among which amphotericin B and pentamidine stand out.

Pentamidine (Figure 1) is an aromatic diamine, which can be used in the treatment of visceral and mucocutaneous leishmaniasis resistant to antimonials or in individuals intolerant to antimony treatment. It is a molecule of great interest in the treatment of antimony refractory visceral and mucocutaneous leishmaniasis. In Brazil it has been used mainly to treat infections caused by L. (V.) guyanensis, which generally respond poorly to antimony treatment. The use of pentamidine in both VL and ATL treatment is limited by toxicity. The side effects are severe and prolonged, such as hypoglycemia, arrhythmia, renal failure, pancreatitis, and diabetes mellitus. Its mechanism of action is not well defined. It is possible that pentamidine inhibits synthesis of polyamines, putrescine and spermidine. This drug may also act binding to kinetoplast DNA. Studies have shown that the mitochondria is an important target of the drug, which may act inhibiting mitochondrial type I DNA topoisomerase, as well as affecting membrane potential.

Amphotericin B (Figure 1) is a macrolide antibiotic derived from a strain of Streptomyces nodosus, belongs to the group of second generation leishmanicidal drugs and is extensively used in case of failures in the treatment with antimony compounds. Despite its high toxicity and the requirement of parenteral administration, amphotericin B has been proposed as a therapeutic agent of choice for MCL and VL. In the last decades, several new lipid formulations of amphotericin B have been developed to reduce toxicity. These formulations include liposomal amphotericin B (Ambisome), amphotericin B colloidal dispersion (Amphocil) and amphotericin B lipid complex (abelcet).

Its mechanism of action is related to its binding to the fungal membrane steroid, acting on the cell membrane ergosterol. As the membrane of Leishmania also contains ergosterol, a lipid not present in the human host, the drug alters their permeability, with loss of small cations,
particularly K⁺, causing cell death. But their use is limited by adverse effects, such as anaphylaxis, thrombocytopenia, generalized pain, convulsions, fever, phlebitis, anemia, anorexia, and decreased renal tubular function.

Miltefosine (Figure 1), an alkylphosphocholine (commercial names Impavido and Milte), was approved for the treatment of human VL infections in 1996. Described in the 1980s as an anti-cancer agent, it was later found to have antileishmanial activity and introduced for treatment of VL in the late 2002 as the first oral drug for treatment of human leishmaniasis. Miltefosine is a protein kinase B (Akt) inhibitor, a serine/threonine-specific protein kinase that plays an important role in several cellular mechanisms, such as glucose metabolism, cell proliferation and migration, regulating cellular survival. However, the long half-life (100 to 200 h) of miltefosine in humans and low therapeutic effect suggested that resistance could rapidly develop. Furthermore, miltefosine high cost, teratogenic and severe gastrointestinal side effects, such as vomiting and nausea, observed in 60% of the patients treated, reduce its efficacy. Currently, among the drugs used in the treatment of VL infections, the first line of drugs are still pentavalent antimonials.

1.2. New tested drugs and promising targets for VL and ATL

In recent years, clinical trials of novel drugs and therapies for VL and ATL are being developed. Over the past decade, the focus has been the search for a more effective topical formulation for the treatment of CL and oral formulations to treat VL. Paramomycin (Figure 2) is an aminoglycosidic antibiotic that belongs to the neomycin family. There is evidence that the antimycotic azoles, ketoconazole, itraconazole and fluconazole (Figure 2) show activity against *Leishmania*. Clinical trials employing azoles against CL, MCL and VL have been carried out and their effectiveness were shown to be varied. An immunomodulatory drug, imiquimod (Figure 2) is currently being administered with antimony in CL treatment to increase leishmanicidal activity.

The rational chemotherapeutic approaches are focused on the metabolic differences between parasite and mammals. Some *Leishmania* enzymes are accepted as valid drug targets; among them are those involved with the parasite glycolytic pathway, the metabolism by trypanothione reductase, some cysteine proteases and dihydrofolate reductase.
The biosynthesis of polyamines is a metabolic pathway that has been successfully exploited for the development of antiparasitic drugs. The first enzyme in the pathway, ornithine decarboxylase (ODC) catalyzes the conversion of ornithine to putrescine. As it is a key enzyme in the pathway, its inhibition may provide a tool for anti-parasitic therapy. Leishmania and other trypanosomatids require the reduction of folates and pterines for proliferation, suggesting that the inhibition of these pathways may be an effective target for chemotherapy. The enzymes involved in the use of folate, such as dihydrofolate reductase (DHFR) and thymidylate synthase (TS), are important targets. A promising molecular target is trypanothione reductase, which is involved in the defense against oxidative damage in the parasite. The system of trypanothione in trypanosomatids is the only thiol redox system which protects the parasite, representing a promising source of metabolites and enzymes that have potential as molecular targets for anti-parasitic drugs. Finally, the structural differences between human and parasite topoisomerase enzymes set these molecular targets of interest for chemotherapeutic intervention, particularly topoisomerase II. This enzyme is required for kinetoplast replication and studies have shown that topoisomerase inhibitors have in vitro activity against Leishmania parasites.

2. Chagas Disease Biological Aspects

Chagas disease, also known as American trypanosomiasis or New World trypanosomiasis, is caused by the flagellate protozoan Trypanosoma cruzi. T. cruzi gains access to the human bloodstream through injured skin or mucous membranes with infected feces of triatomine bugs (Hemiptera: Reduviidae), also known as kissing bugs. Other forms of infection have been reported and are of epidemiological relevance, such as blood transfusion, congenital infection, organ donation or contaminated food. The disease was discovered by the Brazilian physician Carlos Chagas in 1909 when working in Lassance, in the state of Minas Gerais, to control the malaria outbreak that was hampering the construction of the Rio de Janeiro to central and northern Brazil railroad. Despite this seminal work and a continuous investigation of the parasite life cycle and physiology, only two compounds are in use for Chagas disease patient treatment.

The parasite has a complex life cycle that involves the invertebrate host, the triatomine bug, and the mammalian host, passing through several developmental stages as the T. cruzi cell migrates in the insect vector and the mammalian blood stream and intracellular digestive vacuole. Chagas disease infections in humans are characterized by three distinct and well-documented clinical phases. In the acute phase that lasts 4 to 8 weeks, most patients have mild, self-limited symptoms such as fever and edema at the infection point (chagoma), depending on the parasite load. At this stage T. cruzi cells can be easily detected in the bloodstream and parasitemia gradually decreases as the infection progresses. The fatality rate at this stage, due to complications of the clinical condition, is estimated to be in the range of 0.25 to 0.50%. The acute phase is succeeded by an indeterminate phase, generally asymptomatic and comprising a period of 10 to 20 years. Approximately 30% of the infected patients develop the chronic form of the disease, which may result into three different clinical manifestations: the cardiac, gastrointestinal forms or both simultaneously, the cardiodigestive form of Chagas disease. Of the patients that enter the chronic form, 60% die within 7 months to 2 years of the initial symptoms.

Due to its clinical cycle, the socioeconomic characteristics of the affected population, presence of infected insect vectors and absence of an appropriate chemotherapy protocol, Chagas disease is considered a major public health problem in many countries. It affects about 7-8 million people in a vast and populated region of the American continent that comprises the southern United States down to Patagonia, representing an endemic area of 21 countries, claiming approximately 14,000 deaths per year with another 100 million people at risk of contamination. Beyond the human live toll, it is estimated that a social and economic cost of 700,000 disability-adjusted life years (DALYs) per year are attributed to T. cruzi infection. Human infection of T. cruzi in South America (Chile) can be traced back 9,000 years, to the Chinchorro settlers, indicating a long period of adaptation to the human host, having a longer evolutionary history of adaptation to the mammalian parasitic lifestyle. This adaptation has lead to a robust human cycle that explains, in part, the present difficulty in developing a suitable chemotherapy.

2.1. Chagas disease chemotherapy: challenges, progress and limitations

The long history of Chagas disease chemotherapy had its beginning shortly after the description of the disease in 1909 with the employment of arsenical compounds (atoxyl), rosanilin dye (fuchsin), antimonials and mercury chloride (Figure 3), all of them without effective results. The discovery in the 1950s and 1960s of the nitrofuran-containing drugs led to several attempts for T. cruzi infection treatment, with controversial results and questionable clinical
Part of the failure in identifying a suitable drug was due to the lack of an appropriate methodology in the execution of clinical trials, preferential selection of acute instead of chronic cases and systematic use of symptom remission instead of parasitological diagnosis, to assess the success of the cure.

In subsequent works (Pan American Health Organization (PAHO)/WHO 1998) it was proposed that an ideal drug for the treatment of Chagas disease should fulfill several requirements: (i) The drug should show parasitological cure for both the acute and the chronic phase; (ii) it has to be effective in short treatment periods, preferentially in a single or few doses and without the need for hospitalization; (iii) be of low cost; (iv) result in no collateral or teratogenic side effects; and (v) the drug should not induce the selection of resistant strains of *T. cruzi*. This list of requirements has not been met by any compound tested so far. In the 1960s and 1970s two drugs were developed, that are still today the only options for Chagas disease treatment: nifurtimox (Lampit®, Bayer), 3-methyl-4-(5'-nitrofurfurylideneamino)tetrahydro-4H-1,4-thiazine-1,1-dioxide (Figure 3), and benznidazole (Rochagan®, Roche), N-benzyl-2-nitroimidazole acetamide (Figure 3). Some characteristics of these drugs are summarized in Table 2. The action of these drugs is directly affected by certain conditions, such as the duration of treatment, age and geographic distribution of patients, among others. Moreover, in adult patients in the chronic phase of infection, the best results were obtained in southern Brazil, Argentina and Chile, probably due to the type of *T. cruzi* strain predominanting in the region.

These compounds have shown low biochemical specificity in its action mode, contributing to the cytotoxic effects observed during treatment. The most common effects observed for nifurtimox are anorexia, weight loss, drowsiness, nausea, vomiting and intestinal cramps. The most common side effects of benznidazole can be separated into three classes: (i) hypersensitivity, such as dermatitis, with a rash that appears usually between the 7th and 10th days of treatment, periorbital or generalized edema, fever, lymphadenopathy, muscle and joint pain; (ii) bone marrow depression that includes neutropenia, agranulocytosis and thrombocytopenia; and (iii) peripheral neuropathy, represented by paresthesia and polyneuritis.

Drug discovery and development encompass a diverse number of strategies and a combination of traditional and modern methods that integrate specialties, such as biology and biochemistry, medicine and epidemiology, chemistry and pharmacology, among several others. The crucial step is, without doubt, the identification and validation of molecular targets in the parasite that are suitable for drug screening and design. The process is time-consuming and requires significant financial investment, usually available in large pharmaceutical companies. Coupled with a reduced financial return, it results in low commitment of the large pharmaceutical industries to parasitic diseases worldwide. This scenario renders Chagas disease the rank of an extremely neglected disease.

### 2.2. New tested drugs for Chagas disease

Benznidazol has been a commercially available drug in Brazil for several years, and more recently in Argentina, Chile and Uruguay. After the introduction of nifurtimox and benznidazol (Figure 3), few compounds were assayed in chagasic patients. Allopurinol (Zyloprim), 4-hydroxypyrazolo-(3,4-d)-pyrimidine HPP (Figure 4), is a hypoxanthine analog.
and an alternative substrate to hypoxanthine-guanine phosphoribosyltransferase (HGPRT), of the purine salvage pathway, leading to the formation of non-physiological nucleotides and interfering with RNA synthesis. Allopurinol has been tested with some promising results, but requires reevaluation of its efficacy in double blind randomized longitudinal studies.\textsuperscript{131-133} This drug has shown activity against \textit{Leishmania} and, subsequently, against \textit{T. cruzi}.\textsuperscript{137-139} In some cases it has shown low efficacy as a single therapy drug but in combination with other compounds it is a viable alternative.\textsuperscript{43,140}

\textit{T. cruzi} displays similarities to fungi in its sterol biosynthesis pathway indicating this pathway as a potential drug target. Ketoconazole (cis-(\textit{dl})-1-acetyl-4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-methyl)-1,3-ioxalan-4-yl]methoxy]-phenyl] piperazine) (Figure 2) shows \textit{in vitro} activity against \textit{T. cruzi} epimastigotes, with accumulation of metabolites of the sterol metabolism. Ketoconazole tested in acute phase experimental animals resulted in parasitological cure but was shown to be ineffective in the chronic phase.\textsuperscript{127}

Fluconazole (\(\alpha\)-(2,4-difluorophenyl)-\(\alpha\)-(1H-1,2,4-triazol-1-ylmethyl)-1H-1,2,4-triazol-1-ethanol) (5-6 mg kg\(^{-1}\) day\(^{-1}\) for 60 days) inhibits the cytochrome P450 enzyme, important for ergosterol in the cytoplasmic membrane, increased permeability. No significant side effects were observed.\textsuperscript{127, 134, 135}

Fluconazole has been assayed in \textit{T. cruzi} infected experimental animals, and their mechanism of action involves the interference on ergosterol synthesis leading to the development of the D(+) isomer of fluconazole that was shown to be promising.\textsuperscript{127, 135}

\begin{table}
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\begin{tabular}{|l|l|l|l|}
\hline
Drug & Mechanism of action & Outcome & Disadvantage & Reference \\
\hline
Arsenical & & & & \\
Rosanilin dye & & & & \\
Antimonials & Depends on the \textit{T. cruzi} strain & – & No effects & 121-123 \\
Mercury chloride & & & & \\
\hline
Nifurtimox & (3-Methyl-4-(5'-nitrofurfurylideneamino) & Induces oxidative stress in the parasite, activates reduction by a & Anorexia, weight loss, drowsiness, nausea, vomiting and intestinal cramps & Toxic side effects \\
(3,4-dihydro-4H-1,4-thiazine-1,1-dioxide) \& (15 mg kg\(^{-1}\) day\(^{-1}\) for 120 days, acute phase; 10 mg kg\(^{-1}\) day\(^{-1}\) for 30 or 120 days, chronic phase) & eukaryotic type I nitroreductase, inhibits parasite dehydrogenase activity and affects mitochondrial membrane potential & & 124, 127, 128 \\
& & & & \\
Benznidazole & \(N\)-Benzy1-2-nitroimidazole & Induces the formation of free radicals and electrophilic metabolites within the parasite; induces lesions in & Hypersensitivity, dermatitis, generalized edema, fever, lymphadenopathy, muscle and joint pain, neutropenia, agranulocytosis, thrombocytopenia, peripheral neuropathy & Effective at parasite eradication in the acute phase of infection but not in the prevalent chronic stage of the disease \\
acetamide & (5-10 mg kg\(^{-1}\) day\(^{-1}\) for 30 or 60 days) & the mitochondrial DNA & & 127, 129, 130 \\
\hline
Allopurinol & (4-Hydroxypyrazolo-(3,4-d)-pyrimidine HPP) & Alternative substrate to HGPRT,\(^\text{7}\) of the purine salvage pathway, leading to the formation of non-physiological nucleotides and interfering with RNA synthesis & Headache, nausea, weight loss, dark urine, jaundice, muscle weakness, vomiting, diarrhea & Ineffective during the acute phase \\
& (8-10 mg kg\(^{-1}\) for 60 days) & & & 131-134 \\
Fluconazole & ((\(\alpha\)-(2,4-difluorophenyl)-\(\alpha\)-(1H-1,2,4-triazol-1-ylmethyl)-1H-1,2,4-triazol-1-ethanol) & Inhibits the cytochrome P450 enzyme, important for ergosterol in the cytoplasmic membrane, increased permeability & No significant side effects & – \\
& (5-6 mg kg\(^{-1}\) day\(^{-1}\) for 60 days) & & & 127, 134, 135 \\
\hline
\end{tabular}
\caption{Drug discovery summary for the treatment of Chagas disease}
\end{table}

\begin{figure}
\centering
\includegraphics{Figure_4}
\caption{Chemical structures of allopurinol (1) and miconazole (2).}
\end{figure}
2.3. *Trypanosoma cruzi* promising targets

Developments in *T. cruzi* biochemistry and genomics allowed the identification of potentially novel chemotherapy targets.\textsuperscript{141-143} The increasing understanding of kinetoplastid biochemical pathways has allowed the development of new drugs and the identification of potential new targets. Among the various metabolic pathways that are being currently studied,\textsuperscript{144,145} some of relevance are the purine salvage pathway, polyamine and thiol metabolism, folate biosynthesis, DNA replication, glycolytic pathway and fatty acid biosynthetic pathways and sterol biosynthesis.

Sterol biosynthesis

Sterols are essential components of cell membranes. It has been shown that trypanosomatids incorporate cholesterol from complex culture medium containing either brain, heart or liver extracts and bovine serum or from the blood of infected animals. Ergosterol is the main sterol of *T. cruzi* by the sterol biosynthesis pathway, which thus makes this pathway such an attractive target for drug development.\textsuperscript{146}

Among representative inhibitors of ergosterol biosynthesis are the triazole posaconazole that inhibited epimastigote proliferation more efficiently than ketoconazole and the D(+) isomer of fluconazole.\textsuperscript{147} However, generation of resistant *T. cruzi* cells to azoles, such as flucanazole, ketoconazole, itraconazole (Figure 2) and miconazole (Figure 4), points to difficulties in the use of such compounds as chemotherapeutic agents.\textsuperscript{148}

Purine salvage pathway

Purine salvage pathway in trypanosomatids is attractive since these organisms are unable to synthesize purines de novo. Purine nucleotides are important as precursors of nucleic acids, and function as second messengers and modulators of enzyme activities. Therefore, trypanosomatids either are dependent of the host supply of purines or have to salvage purines.\textsuperscript{149} Enzymes from the purine salvage pathway have been detected in *T. cruzi* and *Leishmania* species, including adenine phosphoribosyl transferase (APRT), hypoxanthine-guanine-xanthine phosphoribosyl transferase (HGXPRT), adenosine kinase (AK) and nucleoside hydrolase (NH).\textsuperscript{150,151}

Several inhibitors of the salvage pathway enzymes were evaluated for antiparasitic activity, however, some authors considered parasitic growth inhibition induced by these inhibitors to be disappointing\textsuperscript{151} because the parasites can circumvent this inhibition through alternative metabolic pathways. In this way, some research groups have investigated subversive substrates as potential drugs.\textsuperscript{152} Although not directly intervening in the purine salvage pathway, the subversive substrates are metabolized by salvage enzymes being activated by a toxic product, e.g., allopurinol (Figure 4).\textsuperscript{139,151,153}

Thiol and polyamine metabolism

Enzymes involved in trypanothione metabolism are found exclusively in Kinetoplastida protozoa and do not have an equivalent in the mammalian host. They participate in protection of the organism against oxidative stress and redox homeostasis. In this pathway, trypanothione reductase, that catalyzes the reduction of trypanothione from its disulfide oxidized form, has been investigated as a target enzyme for generation of potential inhibitors against leishmaniasis and Chagas disease.\textsuperscript{152,154,155}

The enzymes ornithine decarboxylase (ODC) or S-adenosylmethionine decarboxylase (AdoMetDC), involved in spermidine synthesis, have become attractive targets for the development of new chemotherapies against trypanosomiasis and leishmaniasis as well.\textsuperscript{156}

Glycolytic pathway

The bloodstream form of *T. cruzi* relies on glycolysis for its ATP. The enzymes for the glycolytic pathway are, in its majority, compartmentalized in specialized organelles, the glycosomes.\textsuperscript{157} Due to the evolutionary distance between *T. cruzi* and humans, the parasite glycolytic enzymes have distinct properties from their mammalian homologues that can be exploited in the design of parasite-specific drugs. Inhibitors to phosphofructokinase (PFK) and pyruvate kinase (PyK) have shown potential as antitrypanosomal and antileishmanial drugs.\textsuperscript{158} Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibitors have been studied but in vivo effects have not been observed.\textsuperscript{159,160} Several classes of selective inhibitors have been designed as potential trypanocidal compounds.\textsuperscript{161,162} One class comprises substrate analogues,\textsuperscript{163} A second class mimics the transition state and high-energy intermediates of the enzymatic reaction.\textsuperscript{164,165}

A fascinating feature of these parasites that gives them the order name of Kinetoplastidae, is the presence of a concatenated mitochondrial DNA comprised of thousands of smaller DNA plasmid-like molecules called the minicircles and larger DNA circles, similar to the conventional mitochondrial DNA, called the maxicircles.\textsuperscript{166} This network of concatenated circles of DNA is replicated at each division cycle of the parasite, and requires the action of enzymes DNA topoisomerase I (EC 5.99.1.2) and II (EC 5.99.1.3). These enzymes have attracted great attention to the development of kinetoplast DNA
reduction of replication inhibitors as chemotherapeutic agents. Several commercially available drugs have shown a varied degree of inhibition to kinetoplastid topoisomerases. Anthracyclines, camptothecins, acridines and fluoroquinolones are well-known inhibitors, which showed promising results against blood trypomastigotes of T. cruzi.

3. Conclusions

At present, chemotherapy is the only viable route for treatment of protozoa infectious disease caused by kinetoplastid parasites (Leishmania, Trypanosoma), although exciting advances have been made toward the development of vaccines. However, the available therapies are far from satisfactory. Recent advances on genomics, with the mapping of several protozoa parasite genomes, transcriptomes and proteomes, together with a significant advance in standardization of target validation methods, lead compound testing protocols and awareness of parasite diversity are leading to a more complete definition of strategies for drug discovery. Advances in the area of parasite protein structural biology and the biochemical assessment of enzyme kinetic parameters, in silico techniques for compound docking and screening have created new tools that may lead to a future drug discovery. The in silico drug design approaches provide pivotal information to synthesize compounds that exhibit selectivity for specific targets. The interaction between compound and protein can be optimized by computer-based molecular docking. These approaches select compounds that are able to eliminate the parasite, but do not consider parasite in the host context. Thus, methodologies of phenotypic analysis, like high-content screening (HCS), should be used to integrate information about the parasite, the target and the host cells. It is important to combine several approaches to overcome some of the challenges of anti-trypanosomatids drug discovery.

In an effort to integrate information and create new opportunities for drug discovery and development, a special program within the WHO, the Tropical Disease Research (TDR), has developed a database (TDR targets database, https://www.tdrtargets.org). This database aims to facilitate the identification and prioritization of drugs and drug targets in neglected disease pathogens.

Nevertheless, all these advances are dependent on a coordinated effort between different laboratories in academic institutions, pharmaceutical industry and governmental agencies with a clear agenda of objectives, management of results, definition of protocols and interpretation of results to treat the “neglected” diseases of the “neglected” populations.

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