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# The Polyamine Pathway as a Potential Target for Leishmaniases Chemotherapy

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## Abstract

Considering the limitations of the current leishmaniases chemotherapy and the lack of effective vaccines, the identification of novel drugs and/or vaccine approaches for the leishmaniases treatment and control is urgently required. In fact, a rational strategy for the parasite control can be based on the identification of essential metabolic pathways of the parasite. One of the most important pathways is the polyamine biosynthesis. *Leishmania* is auxotrophic for many amino acids, such as L-arginine, a precursor of ornithine, putrescine, and spermidine. These metabolites are essential for parasite replication and establishment of infection in the mammalian host. In addition, *Leishmania* has a specific and complex machinery to uptake and metabolize exogenous sources of those molecules. In this chapter, we will focus on the main aspects of the polyamine pathway as a potential target for infection control aiming for new targets for *Leishmania* chemotherapy.

**Keywords:** amastigote, L-arginine metabolism, putrescine, ornithine, spermidine, spermine, amino acid permease 3, amino acid transport, nitric oxide, nitric oxide synthase, glycosome

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## 1. Introduction

Leishmaniases are diseases characterized by cutaneous, mucocutaneous, diffuse, or visceral clinical manifestations [1]. They are currently endemic in 98 countries and territories worldwide, with estimated 700,000 to 1 million new cases and 20,000–30,000 deaths occurring annually [2]. The incidence of this disease is mainly in underdeveloped countries within South East

Asia, East Africa, and Latin America; however, it is also endemic in several Mediterranean countries leading to highlight the importance of transmission in travelers [3]. *Leishmania*-HIV co-infection has emerged as an opportunistic infection and has been described as important clinical, diagnostic, and epidemiological implications [4]. The virus and the parasite compromise the immune response, leading to the replication of both and consequent progression of *Leishmania* infection [5].

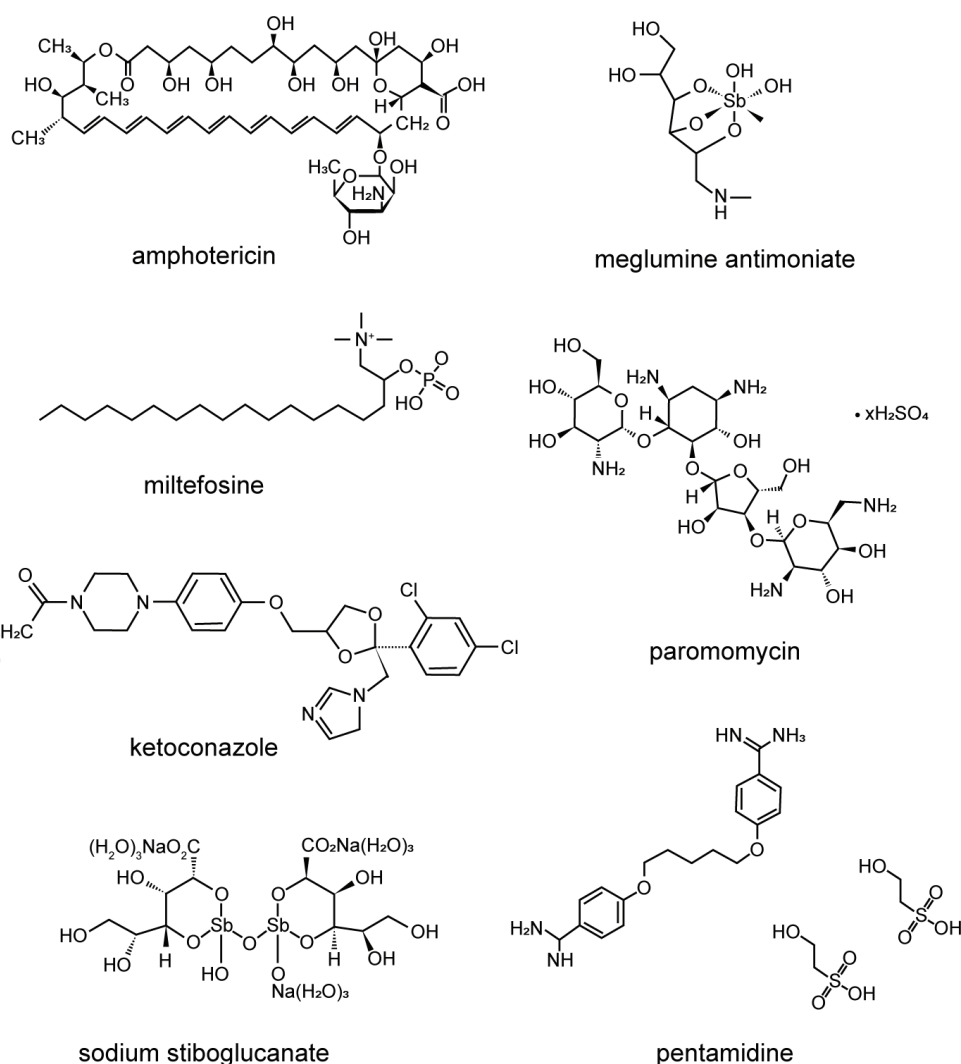
Leishmaniasis is caused by the protozoan parasites of *Leishmania* genus. The parasite presents two main morphological forms during its life cycle. The promastigote, an extracellular long and flagellated form, proliferates in the digestive tract of the invertebrate host, and the amastigote, an obligate intracellular form with a nonapparent flagellum, proliferates in the phagolysosome of the mammalian host macrophage [1, 6]. These two distinct host environments submit the parasite to a rapid adaptation in gene/protein expression, cellular signaling, metabolism, and morphology to survival during promastigote-to-amastigote differentiation [7–11]. In fact, the parasite can sense temperature, pH, and nutrient availability, controlling the amino acid and purine transport and osmoregulation to establish the infection [12].

The immune response in *Leishmania* infection is mediated by phagocytic cells such as neutrophils, macrophages, and dendritic cells. The monocyte recruitment and macrophage differentiation result in the recognition of the parasite, its phagocytosis, and consequent induction of inflammatory response with nitric oxide (NO) production through nitric oxide synthase 2 (NOS2) and reactive oxygen species production [13–16]. These actions coordinate the innate immune response and can promote the parasite killing, as showed for *L. amazonensis*, *L. major*, and *L. donovani* [17]. On the other hand, *Leishmania* is able to escape from these defense mechanisms leading to amastigote differentiation and proliferation in the macrophage phagolysosome [18]. Therefore, the antibodies have little or negligible effect in the infection. These coordinate mechanisms of evasion can be mediated by *Leishmania* polyamine pathway through induction of parasite-arginase (*L*-ARG) activity to produce polyamines [12, 19–22]. It is interesting to note that L-arginine is the common substrate for NOS2 and arginase 1 (ARG1). Both enzymes are competitively regulated by cytokines from T helper 1 (Th1), such as interferon gamma (IFN- $\gamma$ ), or T helper 2 (Th2), as interleukins IL-4, IL-13, and TGF- $\beta$ , inducing the macrophages M1 or M2 polarization, respectively [23–27]. M2 macrophages contribute to susceptibility in cutaneous leishmaniasis [28].

The preconized treatment for leishmaniasis is the use of antimonials, the same treatment used since its description by Gaspar Vianna in 1912 [6]. First, the recommendation was based on the use of trivalent antimonial; however, it was replaced with pentavalent antimonial, more efficient, and less toxic [29]. The pentavalent antimonial formulations are represented by meglumine antimoniate and sodium stibogluconate and still considered as the main line for leishmaniasis treatment today [30]. These compounds have side effects, the treatment is long, and they are administered through intramuscular injections or intravenous infusions, requiring the patient hospitalization [30]. The high toxicity of these compounds can be due to the high concentration used in the current treatment (about four times higher than 20 years ago) and to the acquired resistance by the parasite as a result of long exposure of the drug and inadequate dosages [30]. An alternative line of treatment can be based on the use of amphotericin and its liposomal derivatives; however, they also present side effects, which restrict its use

besides the high cost of the treatment [30]. Pentamidine has the mechanism of action based on the inhibition of polyamine synthesis [31, 32] and can also be used for unresponsive antimonial treatment [33]. Miltefosine, which is administrated as an oral drug, is a promisor alternative for leishmaniasis treatment [34] with high effective and tolerated rate in visceral leishmaniasis in India [35] and later also effective for cutaneous leishmaniasis [36–38]. However, miltefosine-unresponsive cases have been reported in regions outside India [36]. Paromomycin is another promising treatment for visceral leishmaniasis control in India, and it has been described as effective in monotherapy as well as in combination with other drugs [39]. Azole antifungal agents, like ketoconazole, have been also used in the leishmaniasis treatment for decades [40], and its mechanism of action is based on the inhibition of ergosterol biosynthesis [41].

The current leishmaniasis chemotherapy, as represented in **Figure 1**, is based on these seven compounds, which present different origins of discovery, unique structures, and distinct modes of action [42].



**Figure 1.** Chemical structure of the current antileishmanial drugs: amphotericin, meglumine antimoniate, miltefosine, paromomycin, ketoconazole, sodium stibogluconate, and pentamidine.

To date, there is no single effective treatment for leishmaniasis. In fact, the leishmaniasis treatment is complicated due to the complexity of the different species of *Leishmania* and their interaction with the host cells. The available therapies show high toxicity, low efficacy, long duration of treatment, high costs, and increased rate of resistant parasites. Then, there is an emergency and challenge in the development of new drugs for leishmaniasis chemotherapy. The emergency is due to the increased incidence of case reports, and the challenge is based on the leishmaniasis classification as a neglected tropical disease. The high incidence in underdeveloped regions of the world implicates a lack of interest in research development and minimum financial funding from pharmaceutical industries. There are many studies describing potential targets for vaccine approaches; however, no licensed vaccine is available for human treatment.

A rational strategy for the parasite control can be developed based on the identification of fundamental metabolic pathways of both the parasite and the host, such as polyamine biosynthesis. Polyamines are involved in chromatin structure and DNA replication. They interact with both DNA and RNA, promoting gene expression regulation and transport mechanisms [43].

In this review, we will point aspects of the current treatment, relevant targets and highlight the potential for polyamine pathway for leishmaniasis chemotherapy because it is essential for parasite replication and survival.

## 2. The current chemotherapy

The leishmaniasis chemotherapy was discovered in an empiric way, which means all drugs used are re-purposed from other therapeutic prescriptions. In fact, this scenario is far to be optimal for a disease whose incidence is increasing in the endemic areas.

Then, we will review the current leishmaniasis chemotherapy with description of the drugs and its mechanism of action.

### 2.1. Pentavalent antimonials

Pentavalent antimonials are represented by the formulations meglumine antimoniate and sodium stibogluconate (**Figure 1**) and consist in the most frequently used drug for leishmaniasis treatment because they are effective for both visceral and cutaneous leishmaniasis [30]. Both formulations present poor oral absorption, leading to an intramuscular or intravenous administration [44]. Besides its accumulation in the tissues, antimonials can cause severe cardiotoxicity, pancreatitis, and nephrotoxicity effects, requiring hospitalization and close monitoring of patients [45, 46]. In addition to these side effects, the long period of treatment leads to noncompliance and abandonment, favoring the emergence of resistant parasites and the drug efficacy varying from region to region compromising its use [33].

The antimonial mechanism of action involves the depletion of intracellular ATP due to interference in glycolysis and fatty acids  $\beta$  oxidation [47]. There are some studies evidencing that antimony kills the parasite by a process of apoptosis involving DNA fragmentation and externalization of phosphatidylserine on the membrane surface [48].

## 2.2. Pentamidine

Pentamidine is an aromatic diamidine (**Figure 1**) used mainly in the treatment of cutaneous leishmaniasis unresponsive to pentavalent antimonial treatment [33]. Most regimens are based on intramuscular injections or intravenous infusions per day for about 30 days [49]. However, due to its toxicity and rapidly emerging resistance, pentamidine was abandoned in India in 1990 and replaced by amphotericin B, as the recommended treatment [50]. In contrast, pentamidine is the first line of choice for treatment in French Guiana, where it is the only available drug [51].

The pentamidine mechanism of action is related to inhibition of the polyamine synthesis [31, 32], activity of *S*-adenosyl-L-methionine decarboxylase [52], the alteration of the membrane fluidity, lipid metabolism, mitochondrial activity [53], the calcium transport [54], disintegration of the kinetoplast and mitochondria, and collapse of the mitochondrial membrane [55]. Pentamidine binds to DNA essentially in AT-rich regions, such as the kDNA, affecting the transcription and replication process [53]. Additionally, *L. amazonensis* and *L. donovani* parasites treated with pentamidine showed decrease in arginine, ornithine, and putrescine pools, while the levels of spermidine remain intact [31]. In *L. donovani*, pentamidine is described as a competitive inhibitor in the arginine uptake [56] and a noncompetitive inhibitor of putrescine and spermidine transport in *L. infantum* [57], *L. donovani*, and *L. mexicana* [58]. In fact, pentamidine uses polyamine transporters to enter in the parasite leading to an altered polyamine uptake in pentamidine-resistant *Leishmania* [31, 59].

## 2.3. Amphotericin B

Amphotericin B (**Figure 1**) and its lipid formulation have been considered as the most striking advances for visceral leishmaniasis treatment [60, 61]. This antifungal antibiotic has also been considered as the first-line drug for treatment due to its high efficacy against antimonial-unresponsive cases [62]. Amphotericin B is administrated through intravenous infusion and can present side effects, such as nephrotoxicity and myocarditis, leading to close monitoring and hospitalization for 4–5 weeks [63]. The advent of liposome technology allows minimization of dose-limiting toxicity, providing highly effective and safe therapy. The ambisome formulation is probably the most efficient of all currently available drugs for leishmaniasis treatment, and it has been used as the first-line drug for treatment worldwide [64, 65].

The mechanism of action of amphotericin B is based on the sterols metabolism. It interferes in the ergosterol biosynthesis of the cell membrane of *Leishmania*, causing changes in the membrane permeability and leakage of intracellular components that damage the cell, triggering parasite killing [66].

## 2.4. Miltefosine

Miltefosine (**Figure 1**) was the first effective oral agent for visceral leishmaniasis treatment. This drug was originally used for cancer treatment, but it showed high efficacy for leishmaniasis unresponsive to antimonial treatment [67]. Since it has been described effective for leishmaniasis treatment, it has been used worldwide, however, with a variable rate of efficacy [37, 38, 68]. Drug-resistant cases have been reported, and increasing relapse rates can be due to the reflection of its long half-life in case of inadequate use [69].



Miltefosine interferes with cell membrane composition by inhibiting phospholipid metabolism with reduction of phosphatidylcholine content and enhancement of phosphatidylethanolamine content in the membrane of *L. donovani* [70]. Resistance to miltefosine is easily selected *in vitro* [71, 72]. The resistance mechanisms can be due to drug pressure inducing the mechanisms of regulation in *Leishmania* lipid metabolism by a defect in drug internalization mediated by miltefosine transporter machinery [69, 70, 72].

### 2.5. Paromomycin

Paromomycin is an aminoglycoside antibiotic (**Figure 1**) used to treat bacterial infections and requires metabolic energy from electron transport chain across plasmatic membrane [73, 74]. Paromomycin has shown high cure rate in leishmaniasis treatment in India [75]. When orally administered, paromomycin is poorly absorbed, limiting its use to intramuscular injections.

The mechanism of paromomycin action in *Leishmania* is not precisely known, but protein synthesis has been proposed as target, based on studies with bacteria. Other possible mechanisms had been proposed, including alteration of membrane fluidity and effects on the mitochondria membrane potential. The ribosomal complex, responsible for translating the genetic information from mRNA to protein, is the usual site of action for aminoglycoside antibiotics. Based on paromomycin-resistant *L. donovani*, the upregulation of ribosomal proteins was observed in the resistant parasite, suggesting that protein synthesis machinery is the site of action in *Leishmania* [76, 77]. Transcriptomic profile of paromomycin-resistant *L. donovani* shows decreased protein synthesis and degradation and the role in oxidative phosphorylation, glycosomal succinate fermentation, DNA synthesis and repair, and also alteration in the NO production during macrophage infection [78].

### 2.6. Ketoconazole

Ketoconazole is an oral antifungal drug (**Figure 1**) that inhibits ergosterol biosynthesis. Ergosterol is the major sterol in *Leishmania*, and it is a potential target of some drugs because it is absent in mammalian cells, in which cholesterol is the main sterol. The mechanism of action of ketoconazole causes the accumulation of methyl sterols due to changes in membrane permeability [41]. Ketoconazole treatment of murine macrophage infected with *L. mexicana* altered the levels of free sterols in amastigotes [41]. Oral ketoconazole treatment resulted in failure in the control of *L. braziliensis* cutaneous lesions or ulcers [79, 80]. On the other hand, some studies have shown efficacy in controlling *L. braziliensis* cutaneous lesions [40] and *L. amazonensis* murine infection *in vitro* and *in vivo* [81]. Ketoconazole associated with antimony presented toxicity to amastigote forms of *L. amazonensis* [82].

## 3. Biological targets for therapy

In the course of the *Leishmania* life cycle, environmental changes inside the invertebrate and mammalian host represent important external signals for gene expression regulation. These signals start with the starvation of nutrients, such as amino acids, signaling for metacyclogenesis during

the transformation from promastigote procyclic forms into promastigote metacyclic forms. In this step, procyclic forms are adhered to epithelia of insect midgut, and the starvation promotes their release and starts the differentiation to infective metacyclic forms accompanied with their migration to the insect proboscis. From this site, the metacyclic forms are regurgitated during a new blood meal [83]. The following signal is the temperature shift from the invertebrate host (25°C) to the mammalian host (37°C), representing a challenge for parasite survival and differentiation into amastigote forms. The heat-shock proteins are examples of gene activation that allows parasite survival in rapid temperature changes [10, 84, 85]. The pH change, from 7.0 to 5.5, is the last signal, due to the phagosome and lysosome fusion to form the phagolysosome inside the phagocytic cells, where the amastigotes replicate [9–11].

Despite these environmental changes, promastigotes and amastigotes have common metabolic features that distinguish them from their hosts and can be used as new antiparasitic targets. New potential drug targets have been described along the years, and we will describe here some of them, such as protein kinases, glycolate enzyme, purine pathway, and polyamine pathway. Additionally, besides considering the future of leishmaniasis treatment, encompassing new methodological approaches available today, the study of metabolic pathways allows the understanding of the biology and physiology of the parasite. Recent approaches, such as “omics”, have been provided new insights into fundamental pathways of the parasite and/or in the *Leishmania*-host interactions that can be explored as potential new targets.

### 3.1. Protein kinases

The protein kinases are involved in several essential biological processes, including metabolism, gene expression, cell proliferation, motility, differentiation, and death [86]. Protein kinases act on serine, threonine, tyrosine, or histidine residues of proteins leading to phosphorylation. Phosphorylation can modify the function of a protein in a variety of ways, such as protein activity, stabilization, or degradation. The localization within a particular compartment of a cell can initiate or disrupt its interaction with other proteins [87]. These kinases along with phosphatases play a major role in protein and enzyme regulation. *Leishmania*-activated kinases play an important role in parasite thermo-tolerance and virulence [88].

These enzymes have been explored as therapeutic target by pharmaceutical industry, with a focus on the discovery of non-ATP-competitive kinase inhibitors, directing these modulators to target sites that can regulate specific protein kinases [89, 90].

### 3.2. Glycolytic enzymes

The glycolytic enzymes of trypanosomes are attractive drug targets because the glycolysis is essential for energy requirements. The ATP synthesis through glycolytic enzymes starts with glucose to produce glycerol and pyruvate, maintaining the ATP and NAD balance of glycosome compartment. The glycolytic enzymes, localized in glycosome and cytosol, have emerged as drug targets in parasitic diseases [76, 91]. Targeting the glycosomal enzymes could alter the production of energy and NADH by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and its oxidation through mitochondrial glycerol-3-phosphate oxidase to shift the electrons [91].



The inhibition of energy production in *Leishmania* can be based on the use of adenosine analogs, blocking the binding of NAD<sup>+</sup> to GAPDH [92–95]. Some analogs have been described as inhibitor of *Leishmania* growth due the block of energy production.

### 3.3. Purine metabolism

Another interesting pathway to explore as new targets for leishmaniasis chemotherapy is the purine metabolism. *Leishmania* and other protozoa are unable to synthesize purine nucleotides *de novo* and must uptake them from the host [96]. The parasite uptake of preformed purine host is mediated by nucleoside transporters [97].

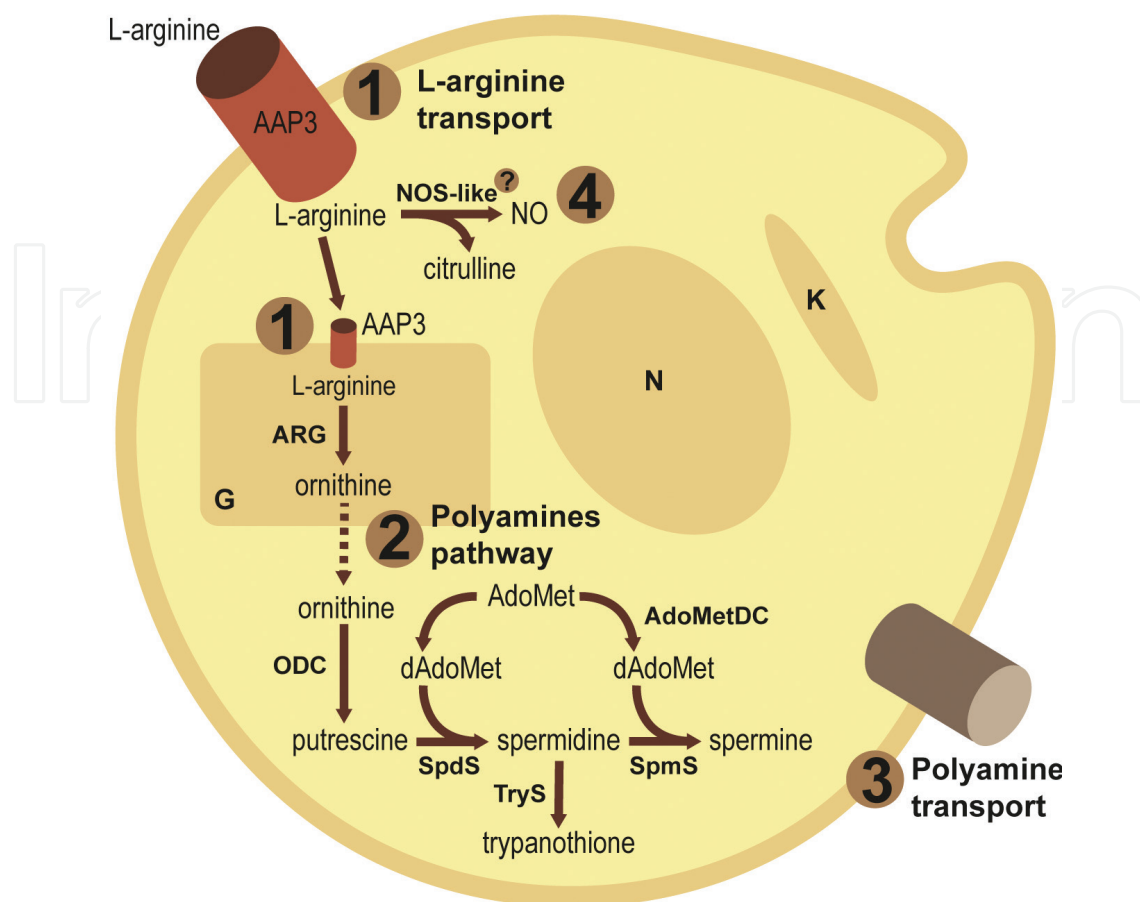
Purine nucleotides and their derivatives are precursors of vast cellular and metabolic processes, including energy production, cell signaling, synthesis of nucleic acids, modulation of enzymatic activities, and synthesis of co-enzymes [96, 97]. This unique characteristic may be the basis for susceptibility of *Leishmania* to purine analogs [97, 98]. Purine analogs have been described for use in the parasite control, such as tubercidin (TUB) [98–100]. TUB is effective against promastigotes from *L. amazonensis*, *L. braziliensis*, *L. infantum chagasi*, and *L. major* [98, 101]. The same antiparasitic efficacy is described for amastigotes from *L. amazonensis* when associated with a specific and selective inhibitor of nucleoside transport for mammalian cells, the nitrobenzylthioinosine (NMBPR) [98]. The selectivity of TUB-NBMPPR combined treatment for *Leishmania* produces highly selective toxicity against the parasite, inhibition of mammalian nucleoside transporter, supporting the hypothesis that these transporters are different between the parasite and its host [98].

In addition, based on tubercidin-resistant *L. major* parasites, the upregulation of the tubercidin-resistant protein, an endoplasmic reticulum protein is observed in these resistant line, suggesting that protein synthesis machinery is the site of action of TUB in *Leishmania* [99].

### 3.4. Polyamine pathway

The polyamine pathway is important for parasite replication and to the establishment of infection in the host [102]. Fundamental differences in this pathway are described between the parasite and its host, pointing to antileishmanial chemotherapy targets (**Figure 2**). Polyamines (putrescine, spermidine, and spermine) are essential substrates in all cells, including parasitic protozoa. Their intracellular concentration may be regulated at the level of their biosynthesis, interconversion, degradation, and transport [103]. In *Leishmania*, the hydrolysis of L-arginine by L-ARG to produce ornithine and urea is a crucial initial step for polyamines production [12, 102, 104–106] and for parasite growth and survival in promastigote and amastigote forms [107–110]. Thus, an inhibition of polyamine synthesis represents a promisor target for leishmaniasis chemotherapy (**Figure 2**).

The polyamines biosynthetic pathway is characterized by the decarboxylation of the amino acid ornithine to putrescine and catalyzed by ornithine decarboxylase (ODC), a key enzyme on this pathway. Putrescine is then converted into spermidine by the action of a spermidine synthase (SpdS). Finally, spermidine is used to form both spermine by spermine synthase (SpmS) and trypanothione through trypanothione synthase (TryS). Trypanothione is an important regulator of intracellular thiol redox balance [111, 112]. *Leishmania* also uses L-arginine to



**Figure 2.** Schematic representation of polyamine-related chemotherapeutic approaches for *Leishmania*, such as the (1) inhibition of amino acid permease 3 (AAP3) in both plasmatic membrane and glycosome, preventing the L-arginine uptake; (2) inhibition of polyamines synthesis by arginase (ARG), ornithine decarboxylase (ODC), S-adenosylmethionine decarboxylase (AdoMetDC), spermidine synthase (SpdS), spermine synthase (SpmS), and trypanothione synthase (TryS); (3) inhibition of polyamines, preventing the parasite replication; and (4) inhibition of the parasite nitric oxide synthase (NOS-like), preventing the nitric oxide (NO) production and amastigote differentiation and replication. (?) predicted but not confirmed cytosolic localization, (N) nucleus, (K) kinetoplast, and (G) glycosome.

produce NO and citrulline by enzyme NOS-like activity [113–115]. The controversial NO production by the parasite is still not completely understood because NO is the same molecule produced by host macrophages to promote the parasite's killing [105, 109, 116]. However, NO production has been related to metacyclogenesis signaling in promastigote forms and to amastigote replication of *L. amazonensis* in a dependent way to the L-arginine availability and L-ARG activity [7]. The participation of a NOS-like enzyme in this pathway was recently described based on *in silico* analysis of oxidoreductase family domains [7]. Besides this, previous metabolome evidences of NO and citrulline production also indicated the activity of the enzyme in *L. amazonensis* [115]. Although this enzyme is not completely characterized, NOS-like enzyme could also be a potential drug target (**Figure 2**), as an inhibitor of NO production decreasing amastigote differentiation and replication.

Once L-arginine is not synthesized *de novo*, the parasite developed molecular mechanisms to sense the amino acid availability and activity of enzymes involved in polyamines production [115–120]. L-ARG is an enzyme with regulatory roles, as the modulation of L-arginine

availability with consequently regulation of polyamine synthesis [121]. *L*-ARG presents a glycosomal import signal, the three amino acids SKL [122, 123]. Glycosomes are peroxisome-like organelles, essential to parasite survival, likely due to compartmentalization of key metabolic enzymes [124]. Glycosomes are not only involved in glycolysis but are also predicted to carry out gluconeogenesis, reaction of the hexose-monophosphate pathway, purine salvage and pyrimidine biosynthesis, fatty acids  $\beta$ -oxidation, fatty acids elongation, and biosynthesis of other lipids. In addition, they seem to have involved in oxidant stress protection [123]. Another role for the existence of glycosome in kinetoplastid organisms can be related with the importance of sequestering metabolic pathways into this compartmentalized organelle and then facilitated the parasite development because the turnover of pathways (or part of them) is more rapidly and efficiently upon induction when they are compartmentalized than when present in the cytosol as individual enzymes [124].

Besides the glycosomal signal, *L*-ARG was demonstrated in fact localized in the glycosome compartment of *L. mexicana* and *L. amazonensis* promastigotes [102, 104, 122]. In addition, this glycosomal localization is maintained in intracellular amastigotes in macrophage infections [102]. Interestingly, the glycosomal localization is crucial for its activity because the mislocalization of the enzyme reduced *in vitro* and *in vivo* infectivity [102]. RNA-seq data revealed that *L*-ARG expression is downregulated in *L. amazonensis* axenic amastigotes when compared to promastigotes [8]. In contrast, an upregulation is observed in *Leishmania* intracellular amastigotes from BALB/c macrophages infection [12]. Altogether, these data reinforce the existence of a differential modulation of the enzyme activity under different environment conditions [8].

*Leishmania* amino acid uptake can also be considered for new antileishmanial target (**Figure 2**). *L*-Arginine uptake in macrophages is mediated by cationic amino acids (CAT1, CAT2A, CAT2B, and CAT3) [125]. A reduction in CAT2B expression and *L*-arginine uptake by treatment with melatonin, hormone of dark signal to biological rhythm, impairs *in vitro* *L. amazonensis* infectivity in murine model [126]. In contrast, *Leishmania* has a complex and specific machinery to uptake this amino acid. *L*-Arginine uptake is mediated by amino acid permease 3 (AAP3) in *L. donovani* and *L. amazonensis* [116, 119, 120]. Furthermore, the AAP3 dual localization in the plasma membrane and in the glycosome from promastigotes and axenic amastigotes of *L. amazonensis* and *L. donovani* [102, 118, 119] can be an indicative that the inhibition of *L*-arginine trafficking through the plasma membrane and/or through the glycosome suggests a promisor target for leishmaniasis chemotherapy (**Figure 2**) [116, 119].

The drugs targeting enzymes involved in the polyamines production could reduce the parasite growth and survival. The 3-aminooxy-1-aminopropane (APA) and *L*- $\alpha$ -difluoromethylornithine (DFMO) are ODC inhibitors. APA is an isosteric analog of putrescine and inhibits the growth of *L. donovani* promastigotes and amastigotes [127]. However, DFMO presents a controversial data as a leishmanicidal compound. DFMO is described successful against African sleeping sickness [128], and it is also efficient in inhibition of *L. donovani*, *L. infantum*, and *L. guyanensis* infections but not for *L. major* and *L. mexicana* [129, 130]. Furthermore, other studies described DFMO inefficacy against *Leishmania* [131]. The basis for the selectivity toxicity of DFMO in the parasite is complex. The ODC from the parasite is no less susceptible to inhibition by DFMO than ODC from the host. However, many metabolic differences between parasites and

mammals have been identified, such as the inability of the parasite to obtain exogenous polyamines, the synthesis of trypanothione that could interfere in the susceptibility to DFMO [132] and its metabolic stability. ODC has a long half-life (more than 6 h), unlike the host protein (less than 30 min), which can be the basis for susceptibility of DFMO in the parasite [133].

A 5'-((Z)-4-amino-2-butenyl)methylamino)-5'-deoxyadenosine (MDL73811) can also be used as an inhibitor of adenosyl methionine decarboxylase (AdometDC), enzyme that forms decarboxylate S-adenosyl methionine. This molecule can be used with putrescine by SpdS to produce spermidine and with spermidine to produce spermine [58, 102, 121, 134–137], as described for *L. donovani* [137].

In addition, the importance of polyamine pathway has been described with the generation of *L. donovani*, *L. major*, *L. mexicana*, and *L. amazonensis* null mutants of essential enzymes involved in this pathway, such as ARG, ODC, AdoMetDC, or SpdS. Knockout parasites of these enzymes have been providing data of how these parasites synthesize polyamines and depend on supplementation of products of these enzymes for survival [7, 8, 102, 105, 106, 138]. The role of polyamine pathway in intracellular amastigotes is controversial; *in vitro* and *in vivo* infections with *L. amazonensis* arginase knockout parasites present lower infection index [102, 119]. In contrast, *in vivo* infection with *L. donovani* arginase knockout parasites reduces the parasite burden in the liver of mice but does not impact the parasite burden in the spleen [106]. Supplementation with putrescine recovered the levels of infectivity of *L. amazonensis* in murine macrophages with reduced levels of L-arginine transport [126]. A comparison of the polyamine pathway in these two different species reveals a variation in the polyamine pool within the phagolysosome, and consequently difference in the polyamine uptake by the parasite may contribute to differences in virulence [138].

### 3.5. “Omics”

The application of “omics” approaches in *Leishmania* research has been providing new insights into the biological processes that drive the replication and differentiation steps of the parasite. They can also provide insights into drug transport and metabolism. Furthermore, these approaches have been revealing as a fundamental tool for the biology, the discovery of new targets for chemotherapy, and the determination of drug-resistance mechanisms [139]. The progress of *Leishmania* spp. genome annotations has been providing a lot of information, including a review of previous genome annotations data and its misassembling, in an attempt to improve the current genome and gene annotations [8, 140, 141]. Since genomes are characterized by a high degree of synteny among the species, the genomic annotations can explain the specificity for tissues tropisms, differential immune responses, variations in drug susceptibility, gene content, and gene expression regulation, among the species.

*Leishmania* exhibit many unique features in their biology, and the elucidation of the molecular basis of them may lead to the development of new strategies for the control of the disease. *Leishmania* parasite genome is organized as large cluster of genes in the 5'–3' direction on the DNA strand. The polycistronic transcription occurs in an initiation site, forming a primary RNA transcript across the chromosome. The maturation of mRNA, as well as its abundance, occurs by post-transcriptional mechanisms [142, 143].



Based on that, the following approaches can be helpful for the future chemotherapy targets discovery. The genomic studies allow the identification of single-nucleotide polymorphisms (SNPs), copy number variations (CNVs), genomic rearrangements, and genomic annotations, using whole genome sequencing and *exome* techniques. The knowledge of biological targets can point to the selectivity of the drug and the use of known validated targets allowing a better understanding of their biological mechanisms. Gazanion et al. demonstrated by next-generation sequencing an unprecedented number of drug-resistance/target genes against all drugs currently used in leishmaniasis chemotherapy [139]. This screening method can be useful to discover the drug targets and to understand the resistance mechanisms [139].

The transcriptomic studies have been extensively used allowing the discovery of mRNA stability, mRNA processing, and gene expression regulation, through microarrays or RNA-seq techniques [7, 8, 119]. Previous studies revealed sequence elements that control the abundance of mRNAs by influencing their maturation and stability. These changes in transcripts abundance during the life cycle of the parasite may lead to the identification of essential genes and thus pointing them as potential candidates for vaccine or drug targets [144]. A previous study demonstrated that the differentiation of promastigotes to amastigotes from *L. amazonensis* leads to a modulation of genes involved in the polyamine biosynthesis [8]. Furthermore, the absence of arginase activity in promastigotes of *L. amazonensis* leads to a differential level of metabolites from this pathway: citrulline, L-arginine, and L-glutamate increase levels, whereas aspartate, proline, ornithine, and putrescine decrease levels [8]. These findings reveal the importance of L-ARG in parasite survival and differentiation and indicate the existence of a coordinate response in the absence of L-ARG activity in the polyamine pathway.

The proteomic studies allow the discovery of cellular components, protein expression, post-translation modification, and protein interaction, using quantitative proteomics by two-dimensional gel electrophoresis (2DE), liquid chromatography mass spectrometry (LC-MS), or stable isotope labeling by amino acids in cell culture (SILAC). Post-translation modifications are of particular interest in *Leishmania* because the parasite regulates gene expression at post-transcriptional and post-translational levels [143]. The metabolomic studies allow the discovery of metabolite signatures through MS or nuclear magnetic resonance spectroscopy (NMR) methodologies. This approach can be used to evaluate how specific metabolites respond under different environmental or physiological conditions, providing interesting data about the mode of action and resistance mechanisms of drugs in parasitic protozoa [145]. The metabolome fingerprints obtained with *L. amazonensis* in the absence of L-ARG activity and/or under amino acids starvation demonstrated how *Leishmania* is able to use an alternative route to provide substrates for the polyamine pathway [115]. In addition, metabolome fingerprints of *L. infantum* resistant to antimonials showed metabolite profile modification in *Leishmania* pathways, corresponding mainly to amino acids or their alternative metabolites in the polyamine pathways with the thiol-dependent redox metabolism [146].

#### 4. Concluding remarks

In the absence of effective vaccine and vector control, the eradication of leishmaniasis is mostly dependent on chemotherapy. Besides other vials, such as the protein kinases, glycolytic



enzymes, and purine metabolism, studies involving the polyamine pathway have been increasing over the years due to its consideration as a promisor target for leishmaniasis chemotherapy. The importance of this pathway for *Leishmania* replication is unquestionable, and the polyamine pathway exhibits significant differences compared to its host pathway. *Leishmania* amastigotes reside in the phagolysosome of host macrophage, but the ability to uptake polyamines may vary depending on the *Leishmania* species and/or the type of host macrophage. Then, the focus on the polyamine pathway as chemotherapeutic approaches, such as inhibition of polyamine transport, inhibition of L-arginine transport, inhibition of polyamine synthesis, inhibition of polyamine interconversion, or inhibition of NOS-like enzyme, may be considered in the future of leishmaniasis chemotherapy.

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