Chapter 7

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

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].

Leishmaniases are 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-γ), or T helper 2 (Th2), as interleukins IL-4, IL-13, and TGF-β, inducing the macrophages M1 or M2 polarization, respectively [23–27]. M2 macrophages contribute to susceptibility in cutaneous leishmaniasis [28].

The preconized treatment for leishmaniases 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 leishmaniases 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

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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 leishmaniases 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 leishmaniases treatment for decades [40], and its mechanism of action is based on the inhibition of ergosterol biosynthesis [41].

The current leishmaniases 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].

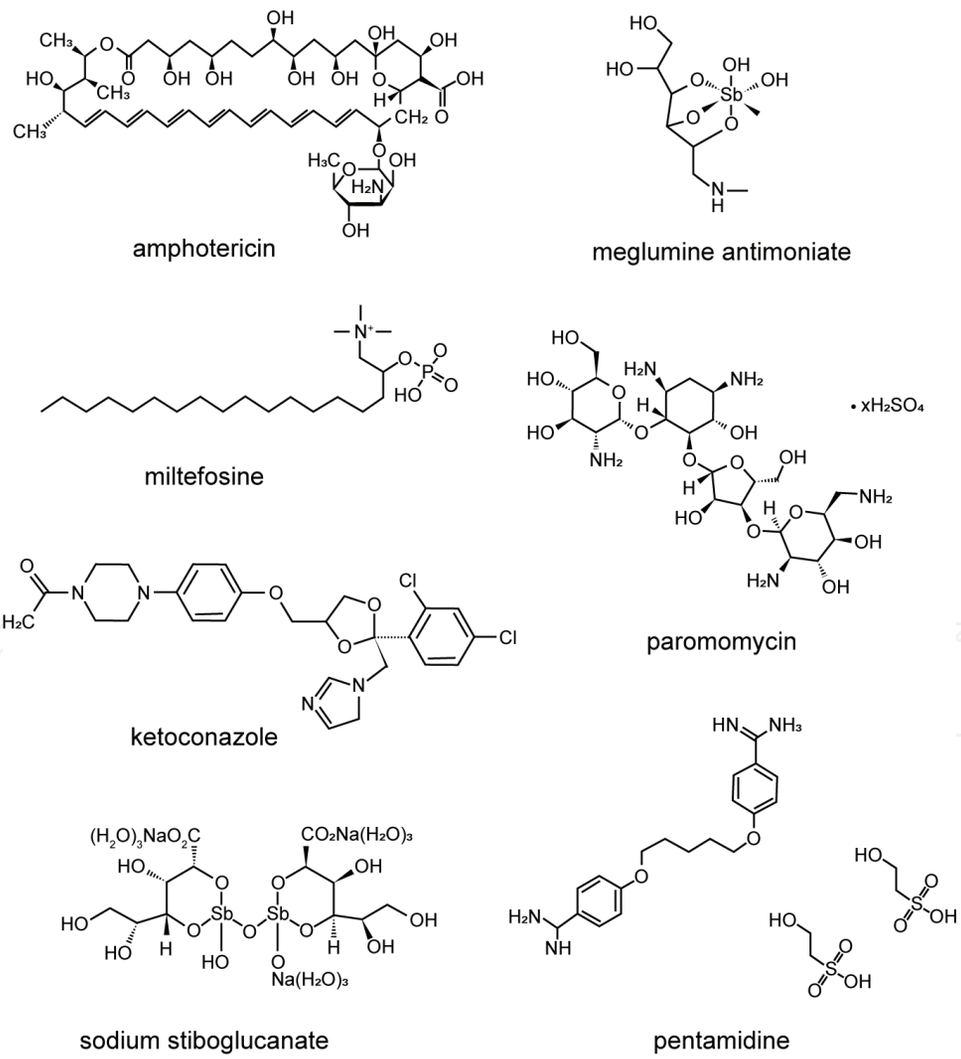

Figure 1. Chemical structure of the current antileishmanial drugs: amphotericin, meglumine antimoniate, miltefosine, paromomycin, ketoconazole, sodium stibogluconate, and pentamidine.

To date, there is no single effective treatment for leishmaniases. In fact, the leishmaniases 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 leishmaniases 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 leishmaniases chemotherapy because it is essential for parasite replication and survival.

2. The current chemotherapy

The leishmaniases 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 leishmaniases 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 leishmaniases treatment because they are effective for both visceral and cutaneous leishmaniases [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 β 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].

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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 leishmaniases 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 leishmaniases unresponsive to antimonial treatment [67]. Since it has been described effective for leishmaniases 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 leishmaniases 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

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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 leishmaniases 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 oxidization 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+ 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 leishmaniases 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-NBMPR 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 leishmaniases 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

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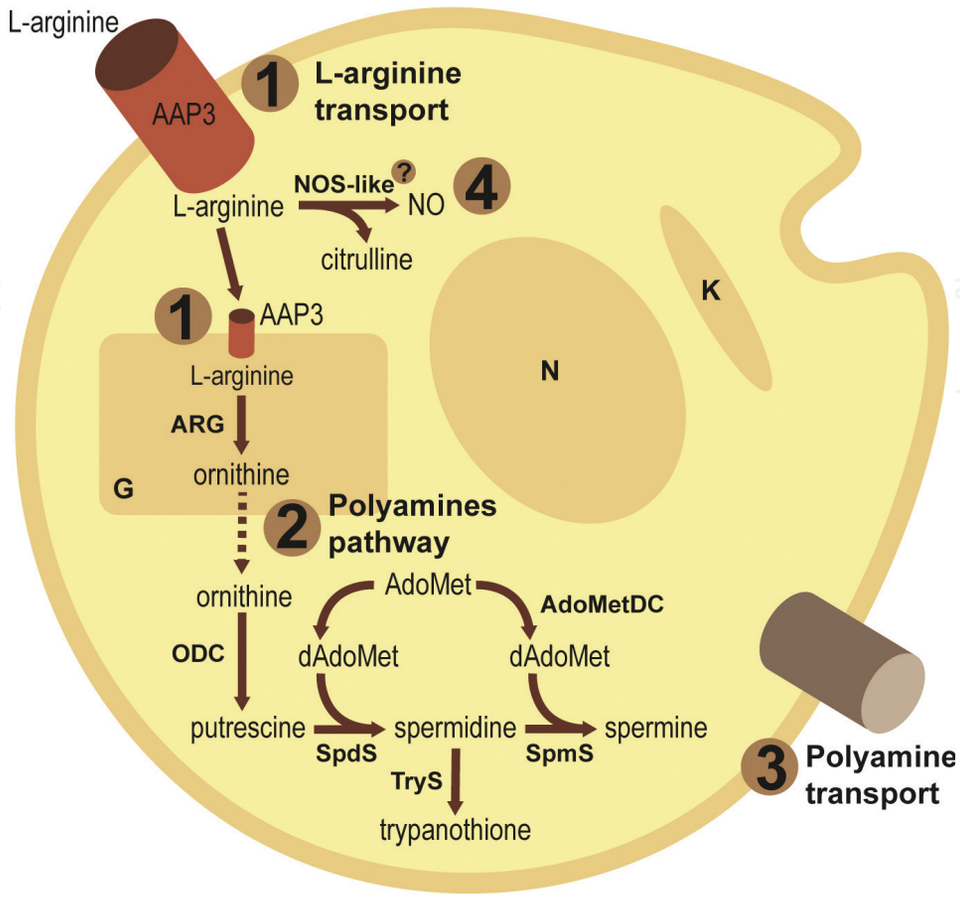

Figure 2. Schematic representation of polyamine-related chemotherapeutic approaches for *Leishmania*, such as the (1) inhibition of amino acid permease 3 (AAP3) in both plasmatic membrane and glycosome, preventing the L-arginine uptake; (2) inhibition of polyamines synthesis by arginase (ARG), ornithine 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 β-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 leishmaniases 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-α-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 leishmaniases 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 leishmaniases 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 leishmaniases 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 leishmaniases chemotherapy.

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References

[1] Ashford RW. The leishmaniases as emerging and reemerging zoonoses. International Journal for Parasitology. 2000;**30**(12-13):1269-1281. PubMed PMID: 11113254

[2] WHO. Leishmaniasis. 2017. [updated April 2017; cited 2017]. Available from: http://www.who.int/mediacentre/factsheets/fs375/en/

[3] Ehehalt U, Schunk M, Jensenius M, van Genderen PJ, Gkrania-Klotsas E, Chappuis F, et al. Leishmaniasis acquired by travellers to endemic regions in Europe: A EuroTravNet multi-centre study. Travel Medicine and Infectious Disease. 2014;**12**(2):167-172. DOI: 10.1016/j.tmaid.2013.12.003. PubMed PMID: 24388687

[4] Alvar J, Aparicio P, Aseffa A, Den Boer M, Cañavate C, Dedet JP, et al. The relationship between leishmaniasis and AIDS: The second 10 years. Clinical Microbiology Reviews. 2008;**21**(2):334-59, table of contents. DOI: 10.1128/CMR.00061-07. PubMed PMID: 18400800; PubMed Central PMCID: PMCPMC2292576

[5] Bernier R, Turco SJ, Olivier M, Tremblay M. Activation of human immunodeficiency virus type 1 in monocytoid cells by the protozoan parasite Leishmania donovani. Journal

of Virology. 1995;**69**(11):7282-5. PubMed PMID: 7474154; PubMed Central PMCID: PMCPMC189654

[6] Rey L. Parasitologia. Guanabara Koogan: Brazil; 2008. 888 p

[7] Acuna SM, Aoki JI, Laranjeira da Silva MF, Zampieri RA, Fernandes JC, Muxel SM, et al. Arginase expression modulates nitric oxide production in Leishmania (Leishmania) amazonensis. PLoS One. 2017;**12**(11):1-21. e018786. DOI: 10.1371/journal.pone.0187186

[8] Aoki JI, Muxel SM, Zampieri RA, Laranjeira-Silva MF, Muller KE, Nerland AH, et al. RNA-seq transcriptional profiling of Leishmania amazonensis reveals an arginase-dependent gene expression regulation. PLoS Neglected Tropical Diseases. 2017;**11**(10):e0006026). DOI: 10.1371/journal.pntd.0006026. PubMed PMID: 29077741

[9] Saar Y, Ransford A, Waldman E, Mazareb S, Amin-Spector S, Plumblee J, et al. Characterization of developmentally-regulated activities in axenic amastigotes of Leishmania donovani. Molecular and Biochemical Parasitology. 1998;**95**(1):9-20. PubMed PMID: 9763285

[10] Bates PA, Robertson CD, Tetley L, Coombs GH. Axenic cultivation and characterization of Leishmania mexicana amastigote-like forms. Parasitology. 1992;**105**(Pt 2):193-202. PubMed PMID: 1454417

[11] Zilberstein D, Shapira M. The role of pH and temperature in the development of Leishmania parasites. Annual Review of Microbiology. 1994;**48**:449-470. DOI: 10.1146/annurev.mi.48.100194.002313. PubMed PMID: 7826014

[12] Muxel SM, Laranjeira-Silva MF, Zampieri RA, Floeter-Winter LM. Leishmania (Leishmania) amazonensis induces macrophage miR-294 and miR-721 expression and modulates infection by targeting NOS2 and L-arginine metabolism. Scientific Reports. 2017;**7**:44141. DOI: 10.1038/srep44141. PubMed PMID: 28276497; PubMed Central PMCID: PMC 5343489

[13] Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(16):8841-8848. PubMed PMID: 10922044; PubMed Central PMCID: PMC34021

[14] Janeway CA Jr, Medzhitov R. Innate immune recognition. Annual Review of Immunology. 2002;**20**:197-216. DOI: 10.1146/annurev.immunol.20.083001.084359. PubMed PMID: 11861602

[15] Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007;**449**(7164):819-826. DOI: 10.1038/nature06246. PubMed PMID: 17943118

[16] Naderer T, McConville MJ. The Leishmania-macrophage interaction: A metabolic perspective. Cellular Microbiology. 2008;**10**(2):301-308. DOI: 10.1111/j.1462-5822.2007.01096.x. PubMed PMID: 18070117

[17] Matte C, Maion G, Mourad W, Olivier M. Leishmania donovani-induced macrophages cyclooxygenase-2 and prostaglandin E2 synthesis. Parasite Immunology. 2001;**23**(4):177-184. PubMed PMID: 11298294

[18] Gregory DJ, Olivier M. Subversion of host cell signalling by the protozoan parasite Leishmania. Parasitology. 2005;**130**(Suppl):S27-S35. DOI: 10.1017/S0031182005008139. PubMed PMID: 16281989

[19] Nasseri M, Modabber FZ. Generalized infection and lack of delayed hypersensitivity in BALB/c mice infected with Leishmania tropica major. Infection and Immunity. 1979;**26**(2):611-614. PubMed PMID: 546790; PubMed Central PMCID: PMC414661

[20] Bacellar O, D'Oliveira A Jr, Jeronimo S, Carvalho EM. IL-10 and IL-12 are the main regulatory cytokines in visceral leishmaniasis. Cytokine. 2000;**12**(8):1228-1231. DOI: 10.1006/cyto.2000.0694. PubMed PMID: 10930301

[21] Kane MM, Mosser DM. The role of IL-10 in promoting disease progression in leishmaniasis. Journal of Immunology. 2001;**166**(2):1141-1147. PubMed PMID: 11145695

[22] Yang Z, Mosser DM, Zhang X. Activation of the MAPK, ERK, following Leishmania amazonensis infection of macrophages. Journal of Immunology. 2007;**178**(2):1077-1085. PubMed PMID: 17202371; PubMed Central PMCID: PMC2643020

[23] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends in immunology. 2004;**25**(12):677-686. DOI: 10.1016/j.it.2004.09.015. PubMed PMID: 15530839

[24] Wang N, Liang H, Zen K. Molecular mechanisms that influence the macrophage m1-m2 polarization balance. Frontiers in Immunology. 2014;**5**:614. DOI: 10.3389/fimmu.2014.00614. PubMed PMID: 25506346; PubMed Central PMCID: PMC4246889

[25] Hrabak A, Bajor T, Temesi A, Meszaros G. The inhibitory effect of nitrite, a stable product of nitric oxide (NO) formation, on arginase. FEBS Letters. 1996;**390**(2):203-206. PubMed PMID: 8706860

[26] Boucher JL, Moali C, Tenu JP. Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization. Cellular and Molecular Life Sciences. 1999;**55**(8-9):1015-1028. PubMed PMID: 10484661

[27] Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: An immunologic functional perspective. Annual Review of Immunology. 2009;**27**:451-483. DOI: 10.1146/annurev.immunol.021908.132532. PubMed PMID: 19105661

[28] Holscher C, Arendse B, Schwegmann A, Myburgh E, Brombacher F. Impairment of alternative macrophage activation delays cutaneous leishmaniasis in nonhealing BALB/c mice. Journal of Immunology. 2006;**176**(2):1115-1121. PubMed PMID: 16394000

[29] Steck EA. The leishmaniases. Progress in Drug Research. 1974;**18**:289-351. PubMed PMID: 4453608

[30] Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. Lancet. 2005;**366**(9496):1561-1577. DOI: 10.1016/S0140-6736(05)67629-5. PubMed PMID: 16257344

[31] Basselin M, Badet-Denisot MA, Lawrence F, Robert-Gero M. Effects of pentamidine on polyamine level and biosynthesis in wild-type, pentamidine-treated, and pentamidine-resistant Leishmania. Experimental Parasitology. 1997;**85**(3):274-282. DOI: 10.1006/expr.1996.4131. PubMed PMID: 9085924

[32] Bachrach U, Brem S, Wertman SB, Schnur LF, Greenblatt CL. Leishmania spp.: Effect of inhibitors on growth and on polyamine and macromolecular syntheses. Experimental Parasitology. 1979;**48**(3):464-470. PubMed PMID: 510448

[33] Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clinical Microbiology Reviews. 2006;**19**(1):111-126. DOI: 10.1128/CMR.19.1.111-126.2006. PubMed PMID: 16418526; PubMed Central PMCID: PMCPMC1360270

[34] Croft SL, Coombs GH. Leishmaniasis—Current chemotherapy and recent advances in the search for novel drugs. Trends in Parasitology. 2003;**19**(11):502-508. PubMed PMID: 14580961

[35] Jha TK, Sundar S, Thakur CP, Bachmann P, Karbwang J, Fischer C, et al. Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. The New England Journal of Medicine. 1999;**341**(24):1795-1800. DOI: 10.1056/NEJM199912093412403. PubMed PMID: 10588964

[36] Soto J, Arana BA, Toledo J, Rizzo N, Vega JC, Diaz A, et al. Miltefosine for new world cutaneous leishmaniasis. Clinical Infectious Diseases. 2004;**38**(9):1266-1272. DOI: 10.1086/383321. PubMed PMID: 15127339

[37] Soto J, Berman J. Treatment of new world cutaneous leishmaniasis with miltefosine. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006;**100**(Suppl 1):S34-S40. DOI: 10.1016/j.trstmh.2006.02.022. PubMed PMID: 16930649

[38] Soto J, Toledo JT. Oral miltefosine to treat new world cutaneous leishmaniasis. The Lancet Infectious Diseases. 2007;**7**(1):7. DOI: 10.1016/S1473-3099(06)70665-X. PubMed PMID: 17182338

[39] Sinha PK, Jha TK, Thakur CP, Nath D, Mukherjee S, Aditya AK, et al. Phase 4 pharmacovigilance trial of paromomycin injection for the treatment of visceral leishmaniasis in India. Journal of Tropical Medicine. 2011;**2011**:645203. DOI: 10.1155/2011/645203. PubMed PMID: 22174722; PubMed Central PMCID: PMCPMC3235903

[40] Saenz RE, Paz H, Berman JD. Efficacy of ketoconazole against Leishmania braziliensis panamensis cutaneous leishmaniasis. The American Journal of Medicine. 1990;**89**(2):147-155. PubMed PMID: 2166429

[41] Berman JD, Holz GG Jr, Beach DH. Effects of ketoconazole on growth and sterol biosynthesis of Leishmania mexicana promastigotes in culture. Molecular and Biochemical Parasitology. 1984;**12**(1):1-13. PubMed PMID: 6087138

[42] Nagle AS, Khare S, Kumar AB, Supek F, Buchynskyy A, Mathison CJ, et al. Recent developments in drug discovery for leishmaniasis and human African trypanosomiasis. Chemical Reviews. 2014;**114**(22):11305-11347. DOI: 10.1021/cr500365f. PubMed PMID: 25365529; PubMed Central PMCID: PMCPMC4633805

[43] Müller S, Coombs GH, Walter RD. Targeting polyamines of parasitic protozoa in chemotherapy. Trends in Parasitology. 2001;**17**(5):242-249. PubMed PMID: 11323309

[44] Chulay JD, Fleckenstein L, Smith DH. Pharmacokinetics of antimony during treatment of visceral leishmaniasis with sodium stibogluconate or meglumine antimoniate. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1988;**82**(1):69-72. PubMed PMID: 2845611

[45] Olliaro PL, Bryceson AD. Practical progress and new drugs for changing patterns of leishmaniasis. Parasitology Today. 1993;**9**(9):323-328. PubMed PMID: 15463794

[46] Berman JD. Human leishmaniasis: Clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clinical Infectious Diseases. 1997;**24**(4):684-703. PubMed PMID: 9145744

[47] Berman JD, Waddell D, Hanson BD. Biochemical mechanisms of the antileishmanial activity of sodium stibogluconate. Antimicrobial Agents and Chemotherapy. 1985;**27**(6):916-920. PubMed PMID: 2411217

[48] Sereno D, Holzmuller P, Mangot I, Cuny G, Ouaissi A, Lemesre JL. Antimonial-mediated DNA fragmentation in Leishmania infantum amastigotes. Antimicrobial Agents and Chemotherapy. 2001;**45**(7):2064-2069. DOI: 10.1128/AAC.45.7.2064-2069.2001. PubMed PMID: 11408224; PubMed Central PMCID: PMCPMC90601

[49] Thakur CP, Kumar M, Pandey AK. Evaluation of efficacy of longer durations of therapy of fresh cases of kala-azar with sodium stibogluconate. The Indian Journal of Medical Research. 1991;**93**:103-110. PubMed PMID: 1649790

[50] Sundar S. Drug resistance in Indian visceral leishmaniasis. Tropical Medicine & International Health. 2001;**6**(11):849-854. PubMed PMID: 11703838

[51] Roussel M, Nacher M, Frémont G, Rotureau B, Clyti E, Sainte-Marie D, et al. 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 Parasitology. 2006;**100**(4):307-314. DOI: 10.1179/136485906X105561. PubMed PMID: 16762111

[52] Bitonti AJ, Dumont JA, McCann PP. Characterization of Trypanosoma brucei brucei S-adenosyl-L-methionine decarboxylase and its inhibition by Berenil, pentamidine and methylglyoxal bis(guanylhydrazone). The Biochemical Journal. 1986;**237**(3):685-689. PubMed PMID: 3800910; PubMed Central PMCID: PMCPMC1147045

[53] Basselin M, Robert-Gero M. Alterations in membrane fluidity, lipid metabolism, mitochondrial activity, and lipophosphoglycan expression in pentamidine-resistant Leishmania. Parasitology Research. 1998;**84**(1):78-83. PubMed PMID: 9491432

[54] Vercesi AE, Docampo R. Ca^{2+} transport by digitonin-permeabilized Leishmania donovani. Effects of Ca^{2+}, pentamidine and WR-6026 on mitochondrial membrane potential in situ. Biochemical Journal. 1992;**284**(Pt 2):463-467. PubMed PMID: 1376113; PubMed Central PMCID: PMCPMC1132661

[55] Croft SL, Brazil RP. Effect of pentamidine isethionate on the ultrastructure and morphology of Leishmania mexicana amazonensis in vitro. Annals of Tropical Medicine and Parasitology. 1982;**76**(1):37-43. PubMed PMID: 7082077

[56] Kandpal M, Fouce RB, Pal A, Guru PY, Tekwani BL. Kinetics and molecular characteristics of arginine transport by Leishmania donovani promastigotes. Molecular and Biochemical Parasitology. 1995;**71**(2):193-201. PubMed PMID: 7477101

[57] Reguera R, Balaña Fouce R, Cubria JC, Alvarez Bujidos ML, Ordoñez D. Putrescine uptake inhibition by aromatic diamidines in Leishmania infantum promastigotes. Biochemical Pharmacology. 1994;**47**(10):1859-1866. PubMed PMID: 8204103

[58] Basselin M, Coombs GH, Barrett MP. Putrescine and spermidine transport in Leishmania. Molecular and Biochemical Parasitology. 2000;**109**(1):37-46. PubMed PMID: 10924755

[59] Basselin M, Denise H, Coombs GH, Barrett MP. Resistance to pentamidine in Leishmania mexicana involves exclusion of the drug from the mitochondrion. Antimicrobial Agents and Chemotherapy. 2002;**46**(12):3731-3738. PubMed PMID: 12435669; PubMed Central PMCID: PMCPMC132791

[60] Olliaro PL, Guerin PJ, Gerstl S, Haaskjold AA, Rottingen JA, Sundar S. Treatment options for visceral leishmaniasis: A systematic review of clinical studies done in India, 1980-2004. The Lancet Infectious Diseases. 2005;**5**(12):763-774. DOI: 10.1016/S1473-3099(05)70296-6. PubMed PMID: 16310148

[61] Thakur CP, Sinha GP, Pandey AK, Barat D, Sinha PK. Amphotericin B in resistant kala-azar in Bihar. National Medical Journal of India. 1993;**6**(2):57-60. PubMed PMID: 8477209

[62] Sundar S, Goyal AK, More DK, Singh MK, Murray HW. Treatment of antimony-unresponsive Indian visceral leishmaniasis with ultra-short courses of amphotericin-B-lipid complex. Annals of Tropical Medicine and Parasitology. 1998;**92**(7):755-764. PubMed PMID: 9924533

[63] Thakur CP, Kumar P, Kumar N, Singh GN, Singh AK, Narain S. A randomized comparison of classical mode of administration of amphotericin B with its newer modes of administration in kala-azar. The Journal of the Association of Physicians of India. 1998;**46**(9):779-783. PubMed PMID: 11229246

[64] Berman JD, Badaro R, Thakur CP, Wasunna KM, Behbehani K, Davidson R, et al. Efficacy and safety of liposomal amphotericin B (AmBisome) for visceral leishmaniasis in endemic developing countries. Bulletin of the World Health Organization. 1998;**76**(1): 25-32. PubMed PMID: 9615494; PubMed Central PMCID: PMCPMC2305623

[65] Thakur CP. A single high dose treatment of kala-azar with Ambisome (amphotericin B lipid complex): A pilot study. International Journal of Antimicrobial Agents. 2001;**17**(1):67-70. PubMed PMID: 11137652

[66] Ramos H, Valdivieso E, Gamargo M, Dagger F, Cohen BE. Amphotericin B kills unicellular leishmanias by forming aqueous pores permeable to small cations and anions. The Journal of Membrane Biology. 1996;**152**(1):65-75. PubMed PMID: 8660406

[67] Sundar S, Olliaro PL. Miltefosine in the treatment of leishmaniasis: Clinical evidence for informed clinical risk management. Therapeutics and Clinical Risk Management. 2007;**3**(5):733-40. PubMed PMID: 18472998; PubMed Central PMCID: PMCPMC2376078

[68] Sundar S, Singh A, Rai M, Prajapati VK, Singh AK, Ostyn B, et al. Efficacy of miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. Clinical Infectious Diseases. 2012;**55**(4):543-550. DOI: 10.1093/cid/cis474. PubMed PMID: 22573856

[69] Pérez-Victoria FJ, Sánchez-Cañete MP, Seifert K, Croft SL, Sundar S, Castanys S, et al. Mechanisms of experimental resistance of Leishmania to miltefosine: Implications for clinical use. Drug Resistance Updates. 2006;**9**(1-2):26-39. DOI: 10.1016/j.drup.2006.04.001. PubMed PMID: 16814199

[70] Rakotomanga M, Blanc S, Gaudin K, Chaminade P, Loiseau PM. Miltefosine affects lipid metabolism in Leishmania donovani promastigotes. Antimicrobial Agents and Chemotherapy. 2007;**51**(4):1425-1430. DOI: 10.1128/AAC.01123-06. PubMed PMID: 17242145; PubMed Central PMCID: PMCPMC1855451

[71] Coelho AC, Boisvert S, Mukherjee A, Leprohon P, Corbeil J, Ouellette M. Multiple mutations in heterogeneous miltefosine-resistant Leishmania major population as determined by whole genome sequencing. PLoS Neglected Tropical Diseases. 2012;**6**(2):e1512. DOI: 10.1371/journal.pntd.0001512. PubMed PMID: 22348164; PubMed Central PMCID: PMCPMC3279362

[72] Coelho AC, Trinconi CT, Costa CH, Uliana SR. In vitro and in vivo miltefosine susceptibility of a Leishmania amazonensis isolate from a patient with diffuse cutaneous leishmaniasis. PLoS Neglected Tropical Diseases. 2014;**8**(7):e2999. DOI: 10.1371/journal.pntd.0002999. PubMed PMID: 25033218; PubMed Central PMCID: PMCPMC4102453

[73] Bryan LE, Kwan S. Roles of ribosomal binding, membrane potential, and electron transport in bacterial uptake of streptomycin and gentamicin. Antimicrobial Agents and Chemotherapy. 1983;**23**(6):835-845. PubMed PMID: 6351731; PubMed Central PMCID: PMC184978

[74] Jhingran A, Chawla B, Saxena S, Barrett MP, Madhubala R. Paromomycin: Uptake and resistance in Leishmania donovani. Molecular and Biochemical Parasitology. 2009;**164**(2):111-117. DOI: 10.1016/j.molbiopara.2008.12.007. PubMed PMID: 19146886; PubMed Central PMCID: PMC3039421

[75] Sundar S, Jha TK, Thakur CP, Sinha PK, Bhattacharya SK. Injectable paromomycin for visceral leishmaniasis in India. The New England Journal of Medicine. 2007;**356**(25):2571-2581. DOI: 10.1056/NEJMoa066536. PubMed PMID: 17582067

[76] Chawla B, Madhubala R. Drug targets in Leishmania. Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology. 2010;**34**(1):1-13. DOI: 10.1007/s12639-010-0006-3. PubMed PMID: 21526026; PubMed Central PMCID: PMC3081701

[77] Chawla B, Jhingran A, Panigrahi A, Stuart KD, Madhubala R. Paromomycin affects translation and vesicle-mediated trafficking as revealed by proteomics of paromomycin-susceptible-resistant Leishmania donovani. PLoS One. 2011;**6**(10):e26660. DOI: 10.1371/journal.pone.0026660. PubMed PMID: 22046323; PubMed Central PMCID: PMCPMC3203147

[78] Verma A, Bhandari V, Deep DK, Sundar S, Dujardin JC, Singh R, et al. Transcriptome profiling identifies genes/pathways associated with experimental resistance to paromomycin in Leishmania donovani. International Journal for Parasitology Drugs and Drug Resistance. 2017;**7**(3):370-377. DOI: 10.1016/j.ijpddr.2017.10.004. PubMed PMID: 29035735; PubMed Central PMCID: PMC5645162

[79] Dedet JP, Jamet P, Esterre P, Ghipponi PM, Genin C, Lalande G. Failure to cure Leishmania braziliensis guyanensis cutaneous leishmaniasis with oral ketoconazole. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1986;**80**(1):176. PubMed PMID: 3726992

[80] Dan M, Verner E, El-On J, Zuckerman F, Michaeli D. Failure of oral ketoconazole to cure cutaneous ulcers caused by Leishmania braziliensis. Cutis. 1986;**38**(3):198-199. PubMed PMID: 3769557

[81] Pirson P, Leclef B, Trouet A. Activity of ketoconazole derivatives against Leishmania mexicana amazonensis within mouse peritoneal macrophages. Annals of Tropical Medicine and Parasitology. 1990;**84**(2):133-139. PubMed PMID: 2383093

[82] Nunes DCO, Bispo-da-Silva LB, Napolitano DR, Costa MS, Figueira M, Rodrigues RS, et al. In vitro additive interaction between ketoconazole and antimony against intramacrophage Leishmania (Leishmania) amazonensis amastigotes. PLoS One. 2017;**12**(6):e0180530. DOI: 10.1371/journal.pone.0180530. PubMed PMID: 28662149; PubMed Central PMCID: PMC5491259

[83] Bates PA, Tetley L. Leishmania mexicana: Induction of metacyclogenesis by cultivation of promastigotes at acidic pH. Experimental Parasitology. 1993;**76**(4):412-423. DOI: 10.1006/expr.1993.1050. PubMed PMID: 8513879

[84] Krobitsch S, Brandau S, Hoyer C, Schmetz C, Hubel A, Clos J. Leishmania donovani heat shock protein 100. Characterization and function in amastigote stage differentiation. Journal of Biological Chemistry. 1998;**273**(11):6488-6494. PubMed PMID: 9497383

[85] Hombach A, Ommen G, MacDonald A, Clos J. A small heat shock protein is essential for thermotolerance and intracellular survival of Leishmania donovani. Journal of Cell Science. 2014;**127**(Pt 21):4762-4773. DOI: 10.1242/jcs.157297. PubMed PMID: 25179594; PubMed Central PMCID: PMC4215717

[86] Naula C, Parsons M, Mottram JC. Protein kinases as drug targets in trypanosomes and Leishmania. Biochimica et Biophysica Acta. 2005;**1754**(1-2):151-159. DOI: 10.1016/j.bbapap.2005.08.018. PubMed PMID: 16198642; PubMed Central PMCID: PMCPMC1452262

[87] Hanks SK, Quinn AM. Protein kinase catalytic domain sequence database: Identification of conserved features of primary structure and classification of family members. Methods in Enzymology. 1991;**200**:38-62. PubMed PMID: 1956325

[88] Cardenas D, Carter PM, Nation CS, Pizarro JC, Guidry J, Aiyar A, et al. LACK, a RACK1 ortholog, facilitates cytochrome c oxidase subunit expression to promote Leishmania major fitness. Molecular Microbiology. 2015;**96**(1):95-109. DOI: 10.1111/mmi.12924. PubMed PMID: 25582232

[89] Sanderson L, Yardley V, Croft SL. Activity of anti-cancer protein kinase inhibitors against Leishmania spp. The Journal of Antimicrobial Chemotherapy. 2014;**69**(7):1888-1891. DOI: 10.1093/jac/dku069. PubMed PMID: 24668412

[90] Tirado-Duarte D, Marín-Villa M, Ochoa R, Blandón-Fuentes G, Soares MJ, Robledo SM, et al. The Akt-like kinase of Leishmania panamensis: As a new molecular target for drug discovery. Acta Tropica. 2018;**177**:171-178. DOI: 10.1016/j.actatropica.2017.10.008. PubMed PMID: 29037519

[91] Verlinde CL, Hannaert V, Blonski C, Willson M, Perie JJ, Fothergill-Gilmore LA, et al. Glycolysis as a target for the design of new anti-trypanosome drugs. Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy. 2001;**4**(1):50-65. DOI: 10.1054/drup.2000.0177. PubMed PMID: 11512153

[92] Kim H, Feil IK, Verlinde CL, Petra PH, Hol WG. Crystal structure of glycosomal glyceraldehyde-3-phosphate dehydrogenase from Leishmania mexicana: Implications for structure-based drug design and a new position for the inorganic phosphate binding site. Biochemistry. 1995;**34**(46):14975-14986. PubMed PMID: 7578111

[93] Aronov AM, Suresh S, Buckner FS, Van Voorhis WC, Verlinde CL, Opperdoes FR, et al. Structure-based design of submicromolar, biologically active inhibitors of trypanosomatid glyceraldehyde-3-phosphate dehydrogenase. Proceedings of the National Academy of Sciences of the United States of America. 1999;**96**(8):4273-4278. PubMed PMID: 10200252; PubMed Central PMCID: PMC16322

[94] Bressi JC, Verlinde CL, Aronov AM, Shaw ML, Shin SS, Nguyen LN, et al. Adenosine analogues as selective inhibitors of glyceraldehyde-3-phosphate dehydrogenase of Trypanosomatidae via structure-based drug design. Journal of Medicinal Chemistry. 2001;**44**(13):2080-2093. PubMed PMID: 11405646; PubMed Central PMCID: PMC2957370

[95] Suresh S, Bressi JC, Kennedy KJ, Verlinde CL, Gelb MH, Hol WG. Conformational changes in Leishmania mexicana glyceraldehyde-3-phosphate dehydrogenase induced by designed inhibitors. Journal of Molecular Biology. 2001;**309**(2):423-435. DOI: 10.1006/jmbi.2001.4588. PubMed PMID: 11371162

[96] Hammond DJ, Gutteridge WE. Purine and pyrimidine metabolism in the Trypanoso matidae. Molecular and Biochemical Parasitology. 1984;**13**(3):243-261. PubMed PMID: 6396514

[97] Landfear SM, Ullman B, Carter NS, Sanchez MA. Nucleoside and nucleobase transporters in parasitic protozoa. Eukaryotic Cell. 2004;**3**(2):245-254. PubMed PMID: 15075255; PubMed Central PMCID: PMCPMC387651

[98] Aoki JI, Yamashiro-Kanashiro EH, Ramos DCC, Cotrim PC. Efficacy of the tubercidin antileishmania action associated with an inhibitor of the nucleoside transport. Parasitology Research. 2009;**104**(2):223-228. DOI: 10.1007/s00436-008-1177-z. PubMed PMID: WOS: 000262652600004

[99] Aoki JI, Coelho AC, Muxel SM, Zampieri RA, Sanchez EM, Nerland AH, et al. Characterization of a novel endoplasmic reticulum protein involved in Tubercidin resistance in Leishmania major. PLoS Neglected Tropical Diseases. 2016;**10**(9):e0004972. DOI: 10.1371/journal.pntd.0004972. PubMed PMID: 27606425; PubMed Central PMCID: PMCPMC5015992

[100] el Kouni MH. Potential chemotherapeutic targets in the purine metabolism of parasites. Pharmacology & Therapeutics. 2003;**99**(3):283-309. PubMed PMID: 12951162

[101] Baer HP, Serignese V, Ogbunude PO, Dzimiri M. Nucleoside transporters in Leishmania major: Diversity in adenosine transporter expression or function in different strains. The American Journal of Tropical Medicine and Hygiene. 1992;**47**(1):87-91. PubMed PMID: 1636887

[102] da Silva MF, Zampieri RA, Muxel SM, Beverley SM, Floeter-Winter LM. Leishmania amazonensis arginase compartmentalization in the glycosome is important for parasite infectivity. PLoS One. 2012;**7**(3):e34022. DOI: 10.1371/journal.pone.0034022. PubMed PMID: 22479507; PubMed Central PMCID: PMC3316525

[103] Vieira LL, Cabantchik ZI. Amino acid uptake and intracellular accumulation in Leishmania major promastigotes are largely determined by an H(+)-pump generated membrane potential. Molecular and Biochemical Parasitology. 1995;**75**(1):15-23. PubMed PMID: 8720171

[104] Roberts SC, Tancer MJ, Polinsky MR, Gibson KM, Heby O, Ullman B. Arginase plays a pivotal role in polyamine precursor metabolism in Leishmania. Characterization of gene deletion mutants. Journal of Biological Chemistry. 2004;**279**(22):23668-23678. DOI: 10.1074/jbc.M402042200. PubMed PMID: 15023992

[105] Laranjeira-Silva MF, Floeter-Winter LM. Arginase in *Leishmania*. In: Andre LS Santos MHB, d'Avila-Levy CM, Kneipp LF, Sodre CL, editors. Proteins and Proteomics of Leishmania and Trypanossoma. Subcellular Biochemistry. Vol. 67. Springer: Springer; 2014. pp. 103-118

[106] Boitz JM, Gilroy CA, Olenyik TD, Paradis D, Perdeh J, Dearman K, et al. Arginase is essential for survival of Leishmania donovani promastigotes but not intracellular amastigotes. Infection and Immunity. 2017;**85**(1). DOI: 10.1128/IAI.00554-16. PubMed PMID: 27795357

[107] Camargo EP, Coelho JA, Moraes G, Figueiredo EN. Trypanosoma spp., Leishmania spp. and Leptomonas spp.: Enzymes of ornithine-arginine metabolism. Experimental Parasitology. 1978;**46**(2):141-144. PubMed PMID: 569593

[108] Mukhopadhyay R, Madhubala R. Effects of bis(benzyl)polyamine analogs on Leishmania donovani promastigotes. Experimental Parasitology. 1995;**81**(1):39-46. DOI: 10.1006/expr.1995.1090. PubMed PMID: 7628565

[109] Wanasen N, Soong L. L-arginine metabolism and its impact on host immunity against Leishmania infection. Immunologic Research. 2008;**41**(1):15-25. DOI: 10.1007/s12026-007-8012-y. PubMed PMID: 18040886; PubMed Central PMCID: PMC2639710

[110] Wanasen N, MacLeod CL, Ellies LG, Soong L. L-arginine and cationic amino acid transporter 2B regulate growth and survival of Leishmania amazonensis amastigotes in macrophages. Infection and Immunity. 2007;**75**(6):2802-2810. DOI: 10.1128/IAI.00026-07. PubMed PMID: 17387163; PubMed Central PMCID: PMC1932894

[111] Oza SL, Shaw MP, Wyllie S, Fairlamb AH. Trypanothione biosynthesis in Leishmania major. Molecular and Biochemical Parasitology. 2005;**139**(1):107-116. DOI: 10.1016/j.molbiopara.2004.10.004. PubMed PMID: 15610825

[112] Krauth-Siegel RL, Meiering SK, Schmidt H. The parasite-specific trypanothione metabolism of trypanosoma and leishmania. Biological Chemistry. 2003;**384**(4):539-549. DOI: 10.1515/BC.2003.062. PubMed PMID: 12751784

[113] Genestra M, Souza WJ, Guedes-Silva D, Machado GM, Cysne-Finkelstein L, Bezerra RJ, et al. Nitric oxide biosynthesis by Leishmania amazonensis promastigotes containing a high percentage of metacyclic forms. Archives of Microbiology. 2006;**185**(5):348-354. DOI: 10.1007/s00203-006-0105-9. PubMed PMID: 16575586

[114] Genestra M, Guedes-Silva D, Souza WJ, Cysne-Finkelstein L, Soares-Bezerra RJ, Monteiro FP, et al. Nitric oxide synthase (NOS) characterization in Leishmania amazonensis axenic amastigotes. Archives of Medical Research. 2006;**37**(3):328-333. DOI: 10.1016/j.arcmed.2005.07.011. PubMed PMID: 16513480

[115] Castilho-Martins EA, Canuto GA, Muxel SM, da Silva MF, Floeter-Winter LM, Del Aguila C, et al. Capillary electrophoresis reveals polyamine metabolism modulation in Leishmania (Leishmania) amazonensis wild type and arginase knockout mutants under arginine starvation. Electrophoresis. 2015. DOI: 10.1002/elps.201500114. PubMed PMID: 26202519

[116] Darlyuk I, Goldman A, Roberts SC, Ullman B, Rentsch D, Zilberstein D. Arginine homeostasis and transport in the human pathogen Leishmania donovani. The Journal of Biological Chemistry. 2009;**284**(30):19800-19807. DOI: 10.1074/jbc.M901066200. PubMed PMID: 19439418; PubMed Central PMCID: PMC2740405

[117] Landfear SM. Nutrient transport and pathogenesis in selected parasitic protozoa. Eukaryotic Cell. 2011;**10**(4):483-493. DOI: 10.1128/EC.00287-10. PubMed PMID: 21216940; PubMed Central PMCID: PMC3127635

[118] Goldman-Pinkovich A, Balno C, Strasser R, Zeituni-Molad M, Bendelak K, Rentsch D, et al. An arginine deprivation response pathway is induced in Leishmania during macrophage invasion. PLoS Pathogens. 2016;**12**(4):e1005494. DOI: 10.1371/journal.ppat.1005494. PubMed PMID: 27043018; PubMed Central PMCID: PMC4846328

[119] Aoki JI, Muxel SM, Zampieri RA, Acuna SM, Fernandes JCR, Vanderlinde RH, et al. L-arginine availability and arginase activity: Characterization of amino acid permease 3 in Leishmania amazonensis. PLoS Neglected Tropical Diseases. 2017;**11**(10):e0006025. DOI: 10.1371/journal.pntd.0006025. PubMed PMID: 29073150

[120] Castilho-Martins EA, Laranjeira da Silva MF, dos Santos MG, Muxel SM, Floeter-Winter LM. Axenic Leishmania amazonensis promastigotes sense both the external and internal arginine pool distinctly regulating the two transporter-coding genes. PLoS One. 2011;**6**(11):e27818. DOI: 10.1371/journal.pone.0027818. PubMed PMID: 22114701; PubMed Central PMCID: PMC3218042

[121] Wu G, Morris SM Jr. Arginine metabolism: Nitric oxide and beyond. The Biochemical Journal. 1998;**336**(Pt1):1-17. PubMed PMID: 9806879; PubMed Central PMCID: PMC 1219836

[122] da Silva ER, da Silva MF, Fischer H, Mortara RA, Mayer MG, Framesqui K, et al. Biochemical and biophysical properties of a highly active recombinant arginase from Leishmania (Leishmania) amazonensis and subcellular localization of native enzyme. Molecular and Biochemical Parasitology. 2008;**159**(2):104-111. DOI: 10.1016/j.molbiopara.2008.02.011. PubMed PMID: 18400316

[123] Opperdoes FR, Szikora JP. In silico prediction of the glycosomal enzymes of Leishmania major and trypanosomes. Molecular and Biochemical Parasitology. 2006;**147**(2):193-206. DOI: 10.1016/j.molbiopara.2006.02.010. PubMed PMID: 16546274

[124] Gualdrón-López M, Brennand A, Hannaert V, Quiñones W, Cáceres AJ, Bringaud F, et al. When, how and why glycolysis became compartmentalised in the Kinetoplastea. A new look at an ancient organelle. International Journal for Parasitology. 2012;**42**(1): 1-20. DOI: 10.1016/j.ijpara.2011.10.007. PubMed PMID: 22142562

[125] Closs EI, Simon A, Vekony N, Rotmann A. Plasma membrane transporters for arginine. The Journal of Nutrition. 2004;**134**(10 Suppl):2752S-2759S; discussion 65S-67S. PubMed PMID: 15465780

[126] Laranjeira-Silva MF, Zampieri RA, Muxel SM, Floeter-Winter LM, Markus RP. Melatonin attenuates Leishmania (L.) amazonensis infection by modulating arginine metabolism. Journal of Pineal Research. 2015. DOI: 10.1111/jpi.12279. PubMed PMID: 26383232

[127] Singh S, Mukherjee A, Khomutov AR, Persson L, Heby O, Chatterjee M, et al. Antileishmanial effect of 3-aminooxy-1-aminopropane is due to polyamine depletion. Antimicrobial Agents and Chemotherapy. 2007;**51**(2):528-534. DOI: 10.1128/AAC.01055-06. PubMed PMID: 17101681; PubMed Central PMCID: PMC1797741

[128] Van Nieuwenhove S, Schechter PJ, Declercq J, Boné G, Burke J, Sjoerdsma A. Treatment of gambiense sleeping sickness in the Sudan with oral DFMO (DL-alpha-difluoromethylornithine), an inhibitor of ornithine decarboxylase; first field trial. Transactions of the Royal Society of Tropical Medicine and Hygiene 1985;**79**(5):692-698. PubMed PMID: 3938090

[129] Gradoni L, Iorio MA, Gramiccia M, Orsini S. In vivo effect of eflornithine (DFMO) and some related compounds on Leishmania infantum preliminary communication. Farmaco. 1989;**44**(12):1157-1166. PubMed PMID: 2517472

[130] Keithly JS. FAH. Inhibition of Leishmania Species by α-Difluoromethylornithine. In: Hart DT, editor. Leishmaniasis. NATO ASI Series (Series A: Life Sciences). Boston, MA: Springer; 1989

[131] Kaur K, Emmett K, McCann PP, Sjoerdsma A, Ullman B. Effects of DL-alpha-difluoromethylornithine on Leishmania donovani promastigotes. The Journal of Protozoology. 1986;**33**(4):518-521. PubMed PMID: 3098971

[132] Fairlamb AH, Blackburn P, Ulrich P, Chait BT, Cerami A. Trypanothione: A novel bis(glutathionyl)spermidine cofactor for glutathione reductase in trypanosomatids. Science. 1985;**227**(4693):1485-1487. PubMed PMID: 3883489

[133] Carrillo C, Cejas S, Cortés M, Ceriani C, Huber A, González NS, et al. Sensitivity of trypanosomatid protozoa to DFMO and metabolic turnover of ornithine decarboxylase. Biochemical and Biophysical Research Communications. 2000;**279**(2):663-668. DOI: 10.1006/bbrc.2000.3996. PubMed PMID: 11118342

[134] da Silva ER, Castilho TM, Pioker FC, Tomich de Paula Silva CH, Floeter-Winter LM. Genomic organisation and transcription characterisation of the gene encoding Leishmania (Leishmania) amazonensis arginase and its protein structure prediction. International Journal for Parasitology 2002;**32**(6):727-737. PubMed PMID: 12062491

[135] Gilroy C, Olenyik T, Roberts SC, Ullman B. Spermidine synthase is required for virulence of Leishmania donovani. Infection and Immunity. 2011;**79**(7):2764-2769. DOI: 10.1128/IAI.00073-11. PubMed PMID: 21536795; PubMed Central PMCID: PMC3191959

[136] Roberts SC, Jiang Y, Jardim A, Carter NS, Heby O, Ullman B. Genetic analysis of spermidine synthase from Leishmania donovani. Molecular and Biochemical Parasitology. 2001;**115**(2):217-226. PubMed PMID: 11420108

[137] Roberts SC, Scott J, Gasteier JE, Jiang Y, Brooks B, Jardim A, et al. S-adenosylmethionine decarboxylase from Leishmania donovani. Molecular, genetic, and biochemical characterization of null mutants and overproducers. Journal of Biological Chemistry. 2002;**277**(8):5902-5909. DOI: 10.1074/jbc.M110118200. PubMed PMID: 11734561

[138] Roberts S, Ullman B. Parasite polyamines as pharmaceutical targets. Current Pharmaceutical Design. 2017;**23**(23):3325-3341. DOI: 10.2174/1381612823666170601101644. PubMed PMID: 28571553

[139] Gazanion É, Fernández-Prada C, Papadopoulou B, Leprohon P, Ouellette M. Cos-Seq for high-throughput identification of drug target and resistance mechanisms in the protozoan parasite Leishmania. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**(21):E3012-E3021. DOI: 10.1073/pnas.1520693113. PubMed PMID: 27162331; PubMed Central PMCID: PMCPMC4889358

[140] Fiebig M, Kelly S, Gluenz E. Comparative life cycle transcriptomics revises Leishmania mexicana genome annotation and links a chromosome duplication with parasitism of vertebrates. PLoS Pathogens. 2015;**11**(10):e1005186. DOI: 10.1371/journal.ppat.1005186. PubMed PMID: 26452044; PubMed Central PMCID: PMC4599935

[141] Alonso G, Rastrojo A, Lopez-Perez S, Requena JM, Aguado B. Resequencing and assembly of seven complex loci to improve the Leishmania major (Friedlin strain) reference genome. Parasites & Vectors. 2016;**9**:74. DOI: 10.1186/s13071-016-1329-4. PubMed PMID: 26857920; PubMed Central PMCID: PMC4746890

[142] Stiles JK, Hicock PI, Shah PH, Meade JC. Genomic organization, transcription, splicing and gene regulation in Leishmania. Annals of Tropical Medicine and Parasitology. 1999;**93**(8):781-807. PubMed PMID: 10715672

[143] Clayton CE. Life without transcriptional control? From fly to man and back again. EMBO Journal. 2002;**21**(8):1881-8. DOI: 10.1093/emboj/21.8.1881. PubMed PMID: 11953307; PubMed Central PMCID: PMCPMC125970

[144] Holzer TR, McMaster WR, Forney JD. Expression profiling by whole-genome interspecies microarray hybridization reveals differential gene expression in procyclic promastigotes, lesion-derived amastigotes, and axenic amastigotes in Leishmania mexicana. Molecular and Biochemical Parasitology. 2006;**146**(2):198-218. DOI: 10.1016/j.molbiopara.2005.12.009. PubMed PMID: 16430978

[145] Vincent IM, Barrett MP. Metabolomic-based strategies for anti-parasite drug discovery. Journal of Biomolecular Screening. 2015;**20**(1):44-55. DOI: 10.1177/1087057114551519. PubMed PMID: 25281738

[146] Canuto GA, Castilho-Martins EA, Tavares M, López-Gonzálvez A, Rivas L, Barbas C. CE-ESI-MS metabolic fingerprinting of Leishmania resistance to antimony treatment. Electrophoresis. 2012;**33**(12):1901-1910. DOI: 10.1002/elps.201200007. PubMed PMID: 22740478