Major therapeutic targets in trypanosomatids

S. O. Silva, C. V. Nakamura, T. Ueda-Nakamura, D. Lazarin-Bidóia and V. C. Desoti

Universidade Estadual de Maringá, Maringá, Paraná, Brazil

Trypanosomatids are protozoan parasites that have some peculiar characteristics in biological terms, such as the presence of a single flagellum and kinetoplast (i.e., a specialized organelle that concentrates mitochondrial DNA in the parasite) and alternating forms in vectors and hosts with distinct modes of morphology, biochemistry, and exhaust. Among the diseases caused by trypanosomatids are leishmaniasis and Chagas' disease, both considered serious public health problems that affect millions of people worldwide. Researchers have prioritized the study of chemotherapeutic agents that act on specific and essential targets in trypanosomatids. These targets include energy metabolism, the trypanothione reductase system, topoisomerases enzymes, arginase, superoxide dismutase, cysteine protease, and the biosynthesis of sterol, microtubules, and polyamines.

Keywords targets of action; trypanosomatids

1. Trypanosomatids

1.1. *Trypanosoma Genus*

Trypanosomatids are protozoans that cause many serious diseases in mammals, such as Chagas' disease caused by *Trypanosoma cruzi*, sleeping sickness caused by *Trypanosoma brucei*, and leishmaniasis caused by different species of *Leishmania* [1]. Although these protozoa are eukaryotic cells, they have some metabolic pathways and organelles that can be distinguished from mammalian cells, with unique cell structures and processes that can be targeted in these trypanosomatids [2].

Taxonomically, trypanosomatids are classified within the order Kinetoplastida. This order covers unicellular and flagellated protozoan parasites that exhibit a unique structure, the kinetoplast. The kinetoplast is a specialized mitochondrial region that contains mitochondrial DNA (kinetoplast DNA [kDNA]) [3] that is rich in molecules with double-stranded circles, minicircles, and maxicircles that are concatenated into a network. Furthermore, they exhibit unique mitochondria that are distinct from mammalian mitochondria [4], making this organelle a target for chemotherapeutic agents [5].

The Trypanosomatidae family comprises monoxenic parasites that present a single invertebrate host during their life cycle and heteroxenic parasites that alternate their life cycle between two hosts, a vertebrate and an invertebrate [6]. Monoxenic trypanosomatids are not considered pathogenic in mammals [7]. However, these protozoa may act as opportunistic pathogens. Heteroxenic trypanosomatids present a complex life cycle, exhibiting morphological, structural, and biochemical alterations, of which the genus *Trypanosoma* and *Leishmania* stand out [8].

This chapter focuses on the main features of the genus *Trypanosoma* and *Leishmania* and the targets of action of chemotherapeutic agents.

1.1.1. *Trypanosoma cruzi*

Trypanosoma cruzi is the etiologic agent of Chagas' disease or American trypanosomiasis. It is an obligatory intracellular parasite that circulates between an invertebrate host (Triatomine insects) and a vertebrate host. It presents a complex life cycle that involves at least three evolutionary forms: epimastigotes, trypomastigotes, and amastigotes. Replicative epimastigotes are not infective and are found in the intestines of the insect vector. When these forms adhere to the terminal portions of the insect intestine, they differentiate into metacyclic, non-replicative, and infective trypomastigotes. Upon blood feeding, metacyclic trypomastigotes are eliminated with the feces and urine of the insect vector and can penetrate through mucous and conjunctiva in vertebrate hosts to invade various cells types. Inside these cells, the trypomastigote form differentiates into an amastigote that reproduces by binary fission. After successive rounds of replication, amastigotes differentiate into sanguine trypomastigotes, which are released from the infected cell [9].

1.1.2. *Trypanosoma brucei*

Trypanosoma brucei is the etiologic agent of sleeping sickness or human African trypanosomiasis [10]. This disease is transmitted by the tsetse fly (genus *Glossina*). The epimastigote and trypomastigote forms are also found during its life cycle. The fly ingests infective trypomastigotes that differentiate into procyclic forms and then epimastigote forms that multiply in the salivary gland and differentiate into metacyclic trypomastigotes, which are responsible for infection in the vertebrate host. When the fly bites the vertebrate host, it injects metacyclic trypomastigotes that differentiate into

blood trypomastigotes that multiply in the bloodstream and can reach the central nervous system. Once inside the vertebrate, the trypomastigotes escape from the host's immune system by continuously replacing major parasite antigens on the plasma membrane [11].

1.2. *Leishmania Genus*

Leishmaniasis is caused by different protozoan species of the genus *Leishmania*. It is transmitted to vertebrate hosts by female insects that belong to the genus *Lutzomyia* [12] and *Phlebotomus* [13]. During the repast, sanguine promastigotes are released in the saliva of the infected gnat. Defense cells then phagocytose the promastigotes, which then differentiate into amastigotes. These, in turn, multiply within parasitophorous vacuoles to disrupt macrophages, mainly affecting the lymph nodes, liver, spleen, and bone marrow (i.e., organs that are rich in cells of the mononuclear phagocyte system) [14,15].

Leishmaniasis has several clinical presentations that can affect the skin, mucous membranes, and viscera. For example, the species *L. braziliensis* and *L. amazonensis* are responsible for cutaneous and mucocutaneous American Tegumentary Leishmaniasis, and the species *L. chagasi* and *L. donovani* are responsible for visceral leishmaniasis [15-16].

2. Major Targets of Chemotherapeutic Agents

2.1. Energy metabolism

The energy metabolism of trypanosomatids differs between different species and can be completely different between the various stages of the life cycle of the same species [17]. These variations may be associated with differences in the availability of nutrients [18]. The energy metabolism of Trypanosomatidae family members is highly dependent on glycolysis for adenosine triphosphate (ATP) production. Moreover, they have several glycolytic enzymes with specific characteristics that make the energy metabolism of these protozoa a potential target for new chemotherapeutic agents [19].

Studies have investigated various enzymes of the glycolytic pathway of trypanosomatids, including fructose-1, 6-diphosphate aldolase, phosphoglycerate kinase, pyruvate kinase, triosephosphate isomerase, glycerol-3-phosphate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase. The latter enzyme has attracted great interest among researchers. Glyceraldehyde-3-phosphate dehydrogenase is responsible for catalyzing the oxidative phosphorylation of D-glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate in the presence of NAD^+ and inorganic phosphate [19].

In trypanosomatids, glycolysis exhibits characteristics that are different from other eukaryotes. Studies have shown that energy metabolism in trypanosomatids can be divided into four modes, with the exception of bloodstream forms of *T. brucei* [17]. This parasite produces energy exclusively by glycolysis [20] because it possesses poorly developed mitochondria. The first mode is characterized by the production of pyruvate from glucose. In the second mode, the main end-product of glucose breakdown is acetate rather than pyruvate. In the third mode, the parasites produce succinate from glucose. In the fourth mode, the parasites use amino acids in addition to glucose [17].

The first mode is used by bloodstream forms of *T. brucei* that use glycolysis to produce pyruvate to synthesize ATP. Compared with the metabolic capacities of other trypanosomatids, the members of this category have less complex and therefore less flexible energy metabolism. In the second mode, some bloodstream forms also rely solely on glucose for the production of ATP. However, they do not produce pyruvate. Instead, they form acetate as the main end-product. In the third mode, some bloodstream forms exclusively use glucose for the production of ATP. Occurring between these end-products are acetate and succinate. This requires an additional electron acceptor for the reoxidation of NADH because acetate production is larger than succinate production. The fourth mode involves trypanosomatids with the most complex metabolic capacities, including the forms found in mammalian and insect hosts. These protozoa do not only depend on glucose. They all have complex mitochondrial metabolism and can degrade both amino acids and glucose. They use a cytochrome-containing respiratory chain and oxidative phosphorylation for the generation of ATP [17].

2.2. Trypanothione system

Trypanosomatids lack the major disulfide reductases glutathione reductase and thioredoxin reductase. These protozoa have a unique thiol-redox system, trypanothione, which is based on the low-molecular-weight molecules N^1,N^8-bis-glutathionylspermidine, dihydrotrypanothione ($T[SH]_2$), and trypanothione reductase (TryR). TryR maintains $T(SH)_2$ in the reduced form. This system is capable of sustaining many cellular functions that are mediated by thiol-dependent (redox) processes. It is involved in cell proliferation, antioxidant defense, the removal of toxic compounds, and iron-sulfur metabolism. However, this system has a limited capacity to cope with oxidative stress compared with mammalian cells. Thus, the trypanothione system is also considered an attractive target for new chemotherapeutic agents [21].

The trypanothione system is formed by a cascade of reactions in which trypanothione disulfide (TS_2) is initially reduced to $T(SH)_2$ by TryR, which is NADPH-dependent. Subsequently, tryparedoxin (TXN) reduces hydroperoxides with spending $T(SH)_2$ in the presence of tryparedoxin peroxidase (TXNPx; Fig. 1) [22].

$T(SH)_2$ is a low-molecular-weight thiol that consists of two molecules of spermidine-conjugated glutathione (GSH) [23,24]. The synthesis of $T(SH)_2$ is catalyzed by the ATP-dependent enzyme trypanothione synthetase (TryS). In this system, GSH, the major antioxidant compound of mammalian cells, appears to play a secondary role compared with trypanothione $T(SH)_2$, although as a monothiol it can participate in the modulation of activity, the protection of certain protein subsets, or elimination of xeno- and endobiotics [24].

NADPH in the detoxification process is generated from the enzymes glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) via pentose. G6PD catalyzes the oxidation of glucose-6-phosphate (G6P) to 6-phosphogluconate, with the concomitant production of NADPH [25].

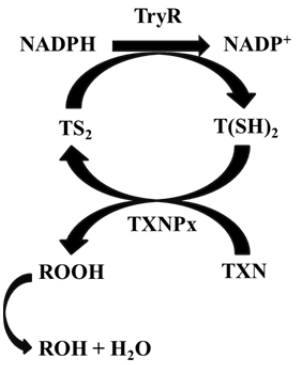

Fig. 1 Scheme of trypanothione-dependent hydroperoxide metabolism in trypanosomatids. Trypanothione disulfide (TS_2) is reduced to dihydrotrypanothione ($T(SH)_2$) by NADPH-dependent trypanothione reductase (TryR). Tryparedoxin (TXN) reduces hydroperoxide (ROOH) to the corresponding alcohol (ROH) at the expense of $T(SH)_2$, but only in the presence of the tryparedoxin peroxidase (TXNPx).

2.3. Topoisomerases enzymes

Topoisomerases are enzymes that participate in many cellular processes, such as replication, transcription, and recombination. These enzymes act by single-strand breaks (type I) or double-strand breaks (type II) in DNA [26]. In addition to its role in DNA metabolism, these enzymes are also involved in the organization and replication of kinetoplast DNA in trypanosomatids and may be an important target for the chemotherapy of diseases caused by trypanosomatids.

The kinetoplast concentrates approximately 30% of the DNA in trypanosomatids. This DNA is formed by two types of molecules: minicircles and maxicircles [27]. Approximately 10,000 minicircles and 50 maxicircles form a network of kDNA [28]. Maxicircles encode ribosomal RNA and mitochondrial proteins. Minicircles are involved in the formation of small RNA guides that control the editing process of mRNA from maxicircles. Minicircles from determined species are heterogeneous with regard to their nucleotide sequence, although the size of these circles in kDNA is equal. Despite the heterogeneity, trypanosomatid minicircles have at least one conserved location that corresponds to the origin of replication. Maxicircles are more homogeneous with regard to DNA sequences, presenting a greater number of conserved regions [29].

The replication of kDNA is restricted to the S phase of the cell cycle, in which the minicircles are covalently linked, decatenated, and released from the center of the kDNA network [27]. When released, the minicircles move up to one of the two protein complexes located on opposite sides of the periphery of the kinetoplast where the topoisomerases are needed for kDNA replication.

In these protozoa, topoisomerases play an important role in the replication of kDNA. This enzyme enables the minicircle kDNA network to multiply as free molecules, catalyzing the segregation of new minicircles and reconnecting them to the DNA network [30]. In addition to catalytic activity, topoisomerases II play a structural role in linking maxicircles to the membrane of mitochondria and movement of maxicircles during the replicative phase.

2.4. Arginase

Arginase is an enzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea in the final step of the urea cycle [27]. One of the products of this pathway, L-ornithine, is a precursor in the synthesis of polyamines. There are two arginase isoenzymes: the cytosolic enzyme arginase I and mitochondrial enzyme arginase II [31]. These enzymes act negatively in the regulation of the levels of nitric oxide (NO) produced by nitric oxide synthase (NOS) through the consumption of arginine (Fig. 2). Consequently, a reduction of NO production by macrophages is observed, impairing

the microbicidal response of these cells. The production of NO occurs through two stages: a monooxygenase stage that produces the intermediate Nω-hydroxy-L-arginine and further hydrolysis that generates L-citrulline and NO [31].

Arginase is an attractive target for new chemotherapeutic agents because its inhibition leads to a decrease in the capacity of trypanosomatids to establish infection in macrophages, in addition to affecting the biosynthesis of polyamines that are essential to the growth and differentiation of these protozoa [32].

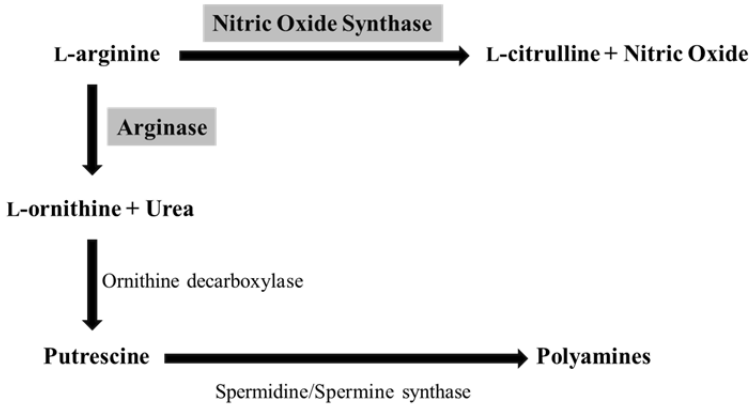

Fig. 2 Scheme the competition between the enzymes oxide nitric synthase and arginase by L-arginine. Arginase catalyzes the hydrolysis of L-arginine to L-ornithine and urea. L-ornithine is precursor in the synthesis of polyamines. Furthermore, the nitric oxide synthase produces nitric oxide and L-citrulline by consumption of L-arginine.

2.5. Superoxide dismutase

Superoxide dismutase (SOD) is a metalloenzyme that constitutes the first line of defense against damage caused by superoxide anions in eukaryotes and prokaryotes [33,34]. This enzyme catalyzes the removal of superoxide radicals to molecular oxygen and hydrogen peroxide [35] through alternating cellular oxidoreduction reactions of metals present in the SOD active site [36]. The inhibition of SOD in trypanosomatids leads to oxidative damage through the accumulation of superoxide radicals that can cause the death of the parasites, making these enzymes another potential chemotherapeutic target [27].

Three classes of SOD have been described, based on prosthetic groups (i.e., iron, manganese, and copper-zinc). They are found in different locations in the cell, including the cytosol, other cell organelles, and cell secretions [33]. Trypanosomatids have only iron-SOD, which differs from the vertebrate host both structurally and with regard to their specific inhibitors [37,38].

2.6. Cysteine protease

Cysteine proteases play numerous indispensable roles in the biology of protozoa. They are involved in nutrition, enzyme activation, immunoevasion, virulence, and tissue and cellular invasion [39]. Blocking these enzymes has been an important target for chemotherapeutic agents [40] because they are essential to the life cycle and pathogenicity of trypanosomatids. This functional diversity is attributable to their unique nucleophilicity, adaptability, and stability in different biological environments [39].

Cysteine proteases can be distributed into groups, based on their structural and functional similarities. The first cysteine protease discovery was papain. Since then, many proteases with sequences in common with papain have been referred to as “papain-like” [41]. In trypanosomes, the main cysteine proteases of the papain family are cruzipain (*T. cruzi*) [42,43] and rhodesain (*T. brucei*) [44]. They are responsible for the majority of proteolytic activity, acting on the survival of the parasite in the infected cell, infection, and differentiation processes. The levels of these enzymes vary according to the evolving forms and strains of the parasite and are more abundantly expressed in the multiplicative forms. All cysteine proteinase inhibitors act through steric blockade of substrate access in the enzymatic catalytic center [45].

2.7. Sterol biosynthesis

One of the distinct characteristics of trypanosomatids in mammals is sterol metabolism that, similar to fungi, synthesizes ergosterol instead of cholesterol [46]. Ergosterol in trypanosomatids is involved in cytokinesis, cell growth, and cell membrane integrity. For this reason, this metabolic pathway has been intensively studied as a potential chemotherapeutic target in trypanosomatids [47].

Sterols comprise a lipid class found in cellular membranes and are essential for their normal structure and function [48]. In mammalian cells, cholesterol is the main sterol found in various membranes [46] and is directly related to the regulation of fluidity and plasma membrane permeability. Trypanosomatids produce ergosterol and other 24-methyl

sterols that are directly involved in cell viability and the regulation of membrane enzymes. Among the enzymes involved in this metabolism are 3-hydroxy-3-metilglutaryl coenzyme A reductase, farnesyl pyrophosphate synthase, squalene synthase, squalene epoxidase, lanosterol synthase, 14α-demethylase, and sterol 24-methyltransferase [49].

Cholesterol and ergosterol differ in a few minor ways. Cholesterol has only one double bond in the B ring and has a fully saturated side chain without a methyl group at C24. The presence of the 3β-OH grouping and absence of methyl groups at C4 and C14 are required for cell growth in both sterols. However, the presence of two double bonds in the B ring of the steroid nucleus, presence of a β methyl at position 24, and double bond at C22 in the side chain in the ergosterol molecule are essential for the growth of trypanosomatids (Fig. 3).

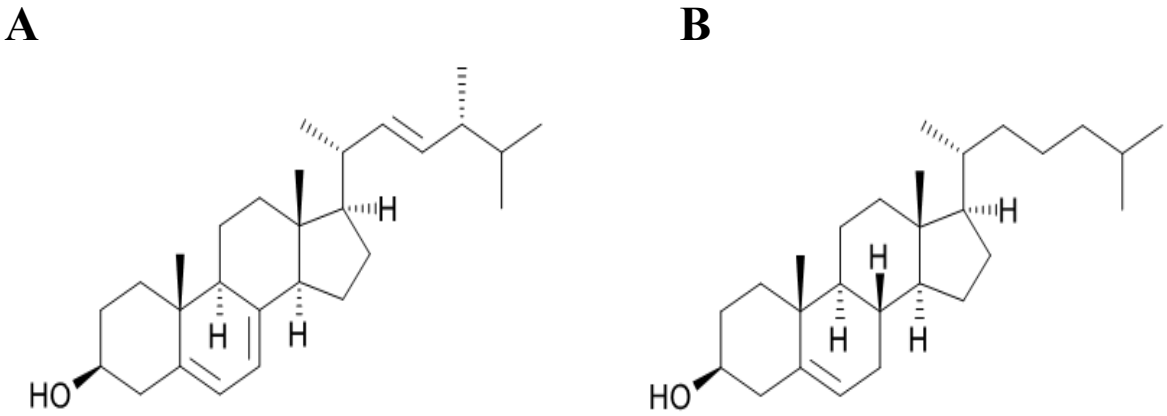

Fig. 3 Chemical structure of ergosterol (A) and cholesterol (B).

2.8. Microtubules biosynthesis

Microtubules are polymers constructed from tubulin heterodimers. Tubulin can be divided into α-tubulin and β-tubulin [50,51], which can bind to form protofilaments. Several protofilaments form a microtubule. Microtubules are cylindrical structures that can rapidly disassociate and rearrange to create essential components of eukaryotic cells, such as flagella [52]. The main function of microtubules is to provide structural support for the maintenance of cell shape and arrangement of internal organelles. Thus, microtubule disruption and stabilization may be another potential chemotherapeutic target in trypanosomatids.

In trypanosomatids, microtubules are one of most important components of the cytoskeleton of the cell. These protozoa have structurally approximately 18% more tubulin compared with mammals. In addition to α- and β-tubulin, trypanosomatids have γ-tubulin, which plays a fundamental role in the formation of microtubules and function of flagella, suggesting a possible molecular target [53,54].

2.9. Polyamines biosynthesis

Polyamines are low-molecular-weight aliphatic amines that are essential to the growth and differentiation of trypanosomatids. Furthermore, these amines function as a substrate for trypanothione reductase in the synthesis of trypanothione. The disruption of the usual functions of polyamines has been one strategy in the search for new chemotherapeutic agents [55].

Among the main polyamines are spermidine and spermine (Fig. 4), which act on DNA packaging. The enzymes involved in polyamine biosynthesis include ornithine decarboxylase (ODC) and *S*-adenosy-L-methionine decarboxylase (AdoMetDC) [56]. In *T. brucei*, the enzymes ODC and AdoMetDC have a long half-life, and spermidine synthase (SpdS) is perennial. *T. brucei* conjugates spermidine and GSH using two enzymes, glutationilespermidine synthase (GSS) and TryS, to form trypanothione. In *Leishmania*, the enzymes involved in the synthesis of polyamines and trypanothione are similar to *T. brucei*, but *Leishmania* possesses one polyamine transport system. *T. cruzi* does not have the enzyme ODC, but AdoMetDC and aminopropyltransferases are present. TryS is an enzyme with polyamine substrate specificity, catalyzing the production of trypanothione and analogs. *T. cruzi* depend on efficient putrescine uptake and exhibit a high-affinity transporter [57] (Fig. 5).

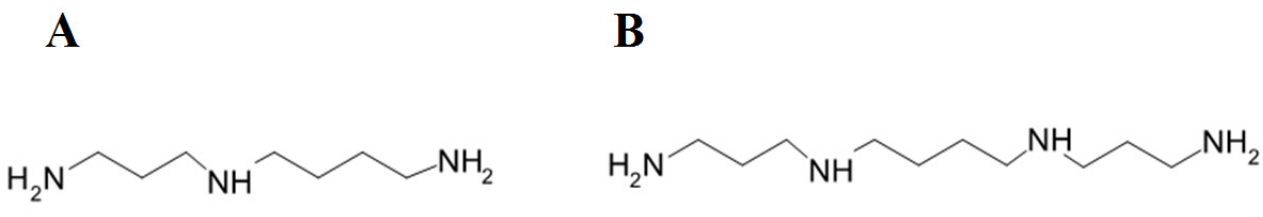

Fig. 4 Chemical structure of spermidine (A) and spermine (B).

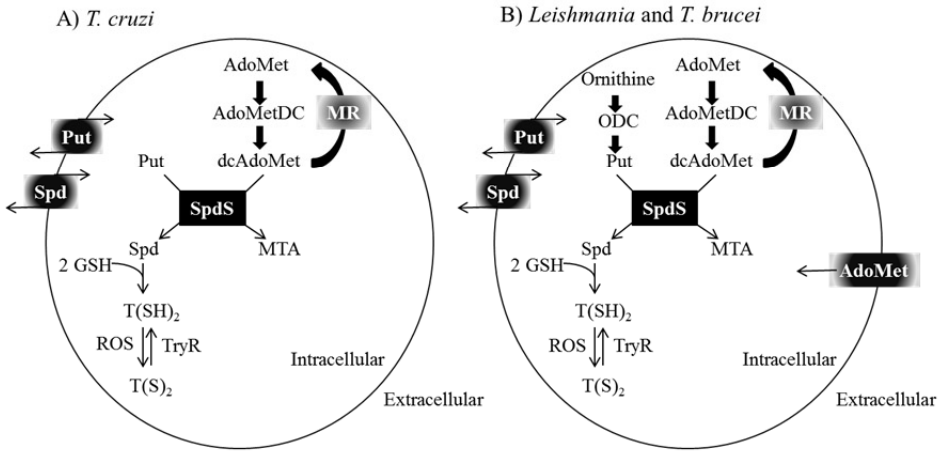

Fig. 5 Scheme of polyamine metabolism in (A) *T. cruzi* and (B) in *Leishmania* and *T. brucei*. ODC appears to be absent from *T. cruzi* (A). Put synthesis occurs in *T. brucei* and *Leishmania*, and both possess a functional ODC (B). Therefore, *T. cruzi* is dependent of Put from the host, which is obtained by efficient transport systems for Put and Spd. Different transporters for Put and Spd have been observed in distinct stages of *Leishmania*, whereas *T. brucei* need efficient transport systems for polyamines. Abbreviations: AdoMet, *S*-adenosy-L-methionine; AdoMetDC, *S*-adenosy-L-methionine decarboxylase; dcAdoMet, decarboxylated *S*-adenosy-L-methionine; MR, met recycling pathway; ODC, ornithine decarboxylase; Put, putrescine; SpdS, spermidine synthase; Spd, spermidine; MTA, methylthioadenosine; GSH, glutathione; ROS, reactive oxygen species; $T(SH)_2$, dihydrotrypanothione; TS_2, trypanothione disulfide; TryR, trypanothione reductase.

Acknowledgements The support by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Capacitação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Financiadora de Estudos e Projetos (FINEP), Programa de Núcleos de Excelência (PRONEX/Fundação Araucária) is gratefully acknowledged.

References

[1] Tonelli RR, Colli W, Alves M.J.M. Selection of binding targets in parasites using phage-display and aptamer libraries *in vivo* and *in vitro*. *Frontiers in Immunology*. 2013;3:1-16.

[2] Barrett MP, Burchmore RJS, Stich A, Lazzari JO, Frasch AO, Cazzulo JJ, Krishna S. The trypanosomiases. *The Lacent.* 2003;362:1469-1480.

[3] Nguewa PA, Fuertes MA, Valladares B, Alonso C, Pérez JM. Programmed cell death in trypanosomatids: away to maximize their biological fitness? *TRENDS in Parasitology*. 2004;20:375-380.

[4] Torri AF, Carpenter LR, Englund PT. Kinetoplast DNA Replication. *DNA Replication in Eukaryoric Cells.*1996: 1029-1042.

[5] Menna-Barreto RF., Gonçalves RSL, Costa EM, Silva RSF, Pinto AV, Oliveira MF, Castro, SL. The activity on *Trypanosoma cruzi* of novel synthetic naphthoquinones is mediated by mitochondrial dysfunction. *Free Radical Biology & Medicine*. 2009;47:644–653.

[6] Barcisnki MA. Apoptosis in Trypanosomatids: Evolutionary and phylogenetic considerations. *Genetics and Molecular Biology*. 1998;21:1.

[7] Nascimento MTC, Garcia MCF, Silva KP, Silva LHP, Atella GC, Motta MCM, Saraiva EM. Interaction of the monoxenic trypanosomatid *Blastocrithidia culicis* with the *Aedes aegypti* salivary gland. *Acta Tropica*. 2010;113:269-278.

[8] Hammarton TC, Monnerat S, Mottram JC. Cytokinesis in trypanosomatids. *Current Opinion in Microbiology*. 2007;10:520-527.

[9] Silva Júnior EN, Souza MCBV, Fernandes MC, Menna-Barreto RFS, Pinto MCRF, Lopes FA, Simone CA, Andrade, CKZ, Pinto AV, Ferreira VF, Castro SL. Synthesis and anti-*Trypanosoma cruzi* activity of derivatives from nor-lapachones and lapachones. *Bioorganic & Medicinal Chemistry*. 2008;16:5030–5038.

[10] Garcia-Salcedo J, Pérez-Morga D, Gijón P, Dilbeck V, Pays V, Nolan DP. A differential role for actin during the life cycle of *Trypanosoma brucei*. *The EMBO Journal.* 2004;23:780–789
[11] Vickerman K. Developmental cycles and biology of pathogenic trypanosomes. *British Medical Bulletin*. 1985;41: 105–114.
[12] Bray DP, Alves GB, Dorval ME, Brazil RP, Hamilton JGC. Synthetic sex pheromone attracts the leishmaniasis vector *Lutzomyia longipalpis* to experimental chicken sheds treated with insecticide. *Parasites & Vectors*. 2010;3:1-11.
[13] Helhazar M, Leitão J, Duarte A, Tavares L, Fonseca IP. Natural infection of synathropic rodent species *Mus musculus* and *Rattus norvegicus* by *Leishmania infantum* in Sesimbra and Sintra – Portugal. *Parasites & Vectors*. 2013;6:1-6.
[14] Ghosh M, Bandyopadhyay S. Interaction of *Leishmania* parasites with dendritic cells and its functional consequences. *Immunobiology.* 2004;209:173–177.
[15] Glycoconjugates in New World species of *Leishmania*: Polymorphisms in lipophosphoglycan and glycoinositolphospholipids and interaction with hosts. *Biochimica et Biophysica Acta*. 2012;1820:1354–1365.
[16] Kaye P, Scott P. Leishmaniasis: complexity at the host–pathogen interface. *Nature Reviews*. 2011;9:604-615.
[17] Tielens AGM, Hellemond JJ. Surprising variety in energy metabolism within Trypanosomatidae. *TRENDS in Parasitology*. 2009;25:482-490.
[18] Ginger ML. Niche metabolism in parasitic protozoa. *Philosophical Transactions of the Royal Society B.* 2006;361:101-118.
[19] Verlinde CLMJ, Hannaert V, Blonski C, Willson M, Périé JJ, Fothergill-Gilmore LA, Opperdoes FR, Gelb MH, Hol WGJ, Michels PAM. Glycolysis as a target for the design of new anti-trypanosome drugs. *Drug Resistance Updates*. 2001;4-50-65.
[20] Hellemond JJ, Bakker BM, Tielens AGM. Energy Metabolism and Its Compartmentation in *Trypanosoma brucei*. *Advances in Microbial Physiology*. 2005;50:199-226.
[21] Manta B, Comini M, Medeiros A, Hugo M, Trujillo M, Radi R. Trypanothione: A unique bis-glutathionyl derivative in trypanosomatids. *Biochimica et Biophysica Acta*. 2013;1830:3199–3216.
[22] Turrens JF. Oxidative stress and antioxidant defenses: a target for the treatment of diseases caused by parasitic protozoa. *Molecular Aspects of Medicine*. 2004;25:211–220.
[23] Tomás AM, Castro H. Redox Metabolism in Mitochondria of Trypanosomatids. *Antioxidants & Redox Signaling*. 2012;00:1-12.
[24] Krauth-Siegel RL, Comini MA. Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. *Biochimica et Biophysica Acta.* 2008;1780:1236–1248.
[25] Igoillo-Esteve M, Cazzulo JJ. The glucose-6-phosphate dehydrogenase from *Trypanosoma cruzi*: Its role in the defense of the parasite against oxidative stress. *Molecular & Biochemical Parasitology*. 2006;149:170–181.
[26] Champoux JJ. DNa topoisomerases: structure, function, and mechanism. *Annual Review of Biochemistry.* 2001;70:369-413.
[27] Melos JLR, Echevarria A. Sistemas enzimáticos de Tripanossomatídeos como potenciais alvos quimioterápicos. *Revista Virtual de Química*. 2012;4:374-392.
[28] Shapiro TA, Englund PT. The structure and replication of kinetoplast DNA. *Annual Review of Microbiology.* 1995;49:117-143.
[29] Simpson L, Thiemann OH, Savill NJ, Alfonzo JD, Maslov DA. Evolution of RNA editing in trypanosome mitochondria. *PNAS.* 2000;97:6986-6993.
[30] Shapiro TA. Mitochondrial topoisomerase II activity is essential for kinetoplast DNA minicircle segregation. *Molecular and Cellular Biology*. 1994;14:3660-3667.
[31] Bogdan C. Nitric oxide and the immune response. *Nature Immunology*. 2001;2:907-916.
[32] Balaña-Fouce R, Calvo-Álvarez E, Álvarez-Velilla R, Prada CF, Pérez-Pertejo Y, Reguera RM. Role of trypanosomatid's arginase in polyamine biosynthesis and pathogenesis. *Molecular & Biochemical Parasitology*. 2012;181:85-93.
[33] Villágran ME, Marín C, Rodríguez-Gonzalez I, Diego JÁ, Sánchez-Moreno M. Use of an iron superoxide dismutase excreted by *Trypanosoma cruzi* in the diagnosis of Chagas disease: seroprevalence in rural zones of the state of Queretaro, Mexico. *The American Society of Tropical Medicine and Hygiene*. 2005;73:510–516.
[34] Fridovich I. The primary defense against the damage that can be caused by O_2^-, and by its reactive progeny, is the SODs. *The Journal of Biological Chemistry*. 1997;272: 18515–18517.
[35] Ahmed H, Schott EJ, Gauthier JD, Vasta GR, Superoxide dismutase from the oyster parasite *Perkinsus marinus*: purification, biochemical characterization, and development of a plate microassay for activity. *Analytical Biochemistry.* 2003;318:132–141.
[36] Ludwig ML, Metzger AL, Pattridge KA, Stallings WC. Manganese superoxide dismutase from *Thermus thermophilus*. A structural model refined at 1.8 A resolution. *Journal of Molecular Biology.* 1991;*219*:335-358.
[37] Le Trant N, Meshnick DR, Kitchener K, Eaton JW, Cerami A. Iron-containing superoxide dismutase from Crithidia fasciculata. Purification, characterization, and similarity to Leishmanial and trypanosomal enzymes. The *Journal of Biological Chemistry.* 1983;*258:*125-130.
[38] Temperton NJ, Wilkinson SR, Meyer DJ, Kelly JM. Overexpression of superoxide dismutase in *Trypanosoma cruzi* results in increased sensitivity to the trypanocidal agents gentian violet and benznidazole. *Molecular and Biochemical Parasitology*. 1998;96:167–176.
[39] Sajid M, McKerrow JH. Cysteine proteases of parasitic organisms. *Molecular and Biochemical Parasitology*. 2002;120:1–21.
[40] Mckerrow JH. Development of cysteine protease inhibitors as chemotherapy for parasitic diseases: insights on safety, target validation, and mechanism of action. *International Journal for Parasitology.* 1999;29:833–837.
[41] Turk D, Guncar G, Podobnik M, Turk B. Revised definition of substrate binding sites of papain-like cysteine proteases. *Biological Chemistry*. 1998;379:137–147.
[42] Stoka V, Nycander M, Lenarcic B, Labriola C, Cazzulo JJ, Bjork I, Turk V. Inhibition of cruzipain, the major cysteine proteinase of the protozoan parasite, *Trypanosoma cruzi,* by proteinase inhibitors of the cystatin superfamily. *FEBS Letters.*1995;370:101-104.
[43] Gea S, Guiñazu N, Pellegrini A, Carrera Silva EA, Giordanengo L, Cano R, Aoki MP. Cruzipain, a major *Trypanosoma cruzi* cystein protease in the host-parasite interplay. *Inmunología.* 2006;25:225-238.
[44] Caffrey CR, Hansell E, Lucas KD, Brinen LS, Hernandez AA, Cheng J, Gwaltney SL, Roush WR, Stierhof YD, Bogyo M, Steverding D, McKerrow JH. Active site mapping, biochemical properties and subcellular localization of rhodesain, the major cysteine protease of *Trypanosoma brucei rhodesiense*. *Molecular & Biochemical Parasitology*. 2001;118:61-73.

[45] Rzychon M, Chmiel D, Stec-Niemczyk J. Modes of inhibition of cysteine proteases. *Acta Biochimica Polonica*. 2004;51:861-873.
[46] Song Z, Nes WD. Sterol biosynthesis inhibitors: potential for transition state analogs and mechanism-based inactivators targeted at sterol methyltransferase. *Lipids.* 2007;42: 15-33.
[47] Urbina JA. Lipid biosynthesis pathways as chemotherapeutic targets in kinetoplastid parasites. *Parasitology.* 1997;114:S91-S99.
[48] Demel, RA, De Kruyff B. The function of sterols in membranes. *Biochimica et Biophysica Acta*. 1976;457:109-132.
[49] De Souza W, Rodrigues JCF. Sterol biosynthesis pathway as target for anti-trypanosomatid drugs. *Interdisciplinary Perspectives on Infectious Diseases.* 2009;2009:1-19.
[50] Downing KH, Nogales E. Tubulin and microtubule structure. *Current Opinion in Cell Biology*. 1998;10:16-22.
[51] Downing KH, Nogales E. Tubulin structure: insights into microtubule properties and functions. *Current Opinion in Structural Biology*. 1998;8:785-791.
[52] Morrissette NS, Mitra A, Sept D, Sibley D. Dinitroanilines bind α-tubulin to disrupt microtubules. Molecular *Biology of the Cell*. 2004;15:1960–1968.
[53] Armson A, Kamau SW, Grimm F, Reynolidon JA, Best WN, Macdonald LM, Thompson RCA. A comparison of the effects of a benzimidazole and the dinitroanilines against Leishmania infantum. *Acta Tropica*. 1999;*73*:303-311.
[54] Libusová L, Sulimenko T, Sulimenko V, Hozák O, Draber P. Gamma-tubulin in *Leishmania*: cell cycle-dependent changes in subcellular localization and heterogeneity of its isoforms. *Experimental Cell Research*. 2004;*295:*375-386.
[55] Müller S, Coombs GH, Walter RD. Targeting polyamines of parasitic protozoa in chemotherapy. *TRENDS in Parasitology*. 2001;17:242-249.
[56] Brun R, Buhler Y, Sanmaier U, Kaminsky R, Bachi CJ, Rattendi D, Lane S, Croft S, Snowdon D, Yardley V, Caravatti G, Frei J, Stanek J, Mett H. *In vitro* trypanocidal activities of new S-adenosylmethionine decarboxylase inhibitors. *Antimicrobial Agents and Chemotherapy*. 1996;40:1442-1447.
[57] Heby O, Persson L, Rentala M. Targeting the polyamine biosynthetic enzymes: a promising approach to therapy of African sleeping sickness, Chagas' disease, and leishmaniasis. *Amino Acids*. 2007;33:359-366.

