Metabolite Transporters in Trypanosomatid Parasites: Promising Therapeutic Targets But… How to Deal with Them?

C.A. Pereira[1], M. Sayé[1], C. Wrenger[2] and M.R. Miranda*,[1]

[1]*Laboratorio de Biología Molecular de Trypanosoma cruzi (LBMTC), Instituto de Investigaciones Médicas 'Alfredo Lanari', Universidad de Buenos Aires and CONICET, Buenos Aires, Argentina;* [2]*Unit for Drug Discovery, Department of Parasitology, Institute of Biomedical Science, University of São Paulo, São Paulo-SP, Brazil*

Abstract: Infections caused by protozoan parasites are one of the most important public health problems in developing countries. One approach to design new drugs for these parasitic diseases relies on metabolic and molecular features which are ideally absent in mammalian hosts. Out of them, nutrient transporters play an important role since they were subjected to millions of years of adaptation to parasitism, in which this protozoan replaced many biosynthetic routes for transport systems. Here we address the current knowledge of trypanosomatids transport systems and the molecules related to such processes, including a description of permeases involved in drug uptake, and also those responsible of drug resistance. The latter process produces, in many cases, the treatment failure due to the loss of the transporter function, as is the case of eflornithine, as well as by increasing the extrusion of drugs, in which usually ABC-type transporters are involved. All these aspects and the perspectives on this topic are briefly updated in this review.

Keywords: Transporters, permeases, protozoan parasites, drug resistance, *Trypanosoma*, *Leishmania.*

1. INTRODUCTION

Protozoan parasites from the family Trypanosomatidae include important pathogens of plants, cattle and humans. They belong to the taxonomical order Kinetoplastida, characterized by the presence of a unique mitochondrial DNA structure known as kinetoplast [1]. *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* spp. are parasites from this group that produce human infection diseases which are major causes of mortality [2]. Taken together, these parasites affect approximately 25 million people in the endemic areas all over the world, with an estimated population in risk of acquiring the infection of more than 350 million people [2].

The control of these infections remains to be solved and represents a serious problem. There are currently no vaccines and the use of drugs is the principal way of intervention. Chemotherapy mainly depends on the developmental stage pathogen or nature of the infection and on the success of the treatment. Four drugs have been licensed so far to treat African trypanosomiasis, the aromatic diamidine, pentamidine; the polyanionic compound, suramin; the arsenic-based drug, melarsoprol; and the ornithine analogue, eflornithine. Nifurtimox, a nitrofuran, is also recommended in some cases [3]. For the treatment of Leishmaniases pentavalent antimony (such as sodium stibogluconate and meglumine antimoniate), amphotericin B, pentamidine, miltefosine, sitamaquine and the aminoglycoside antibiotic paromomycin are employed [4]. In the case of American trypanosomiasis, only two drugs are clinically approved, nifurtimox and the nitroimidazole benznidazole (Table **1**) [5]. The majority of these chemotherapies were introduced more than 40 years ago and present several problems that include toxicity, drug efficacy, delivery and resistance emergency. Despite the vast efforts of numerous investigators, there are still no adequate therapies and consequently an urgent need to develop new chemotherapeutic or prophylactic treatments for these diseases. In this sense, some requirements should be kept in mind for new drug design; targets should be essential molecules and should be absent from mammalian hosts.

Trypanosomatid organisms have an extended metabolic plasticity; they switch to different environments along its complex life cycle from the insect vector to the mammalian blood and host cell cytoplasm. The parasite must express nutrient uptake systems that compensate for these environment alterations and may employ regulatory mechanisms to alter the quality and quantity of uptake according to the nutrient availability.

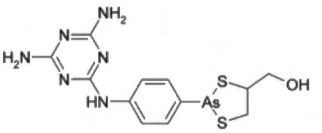
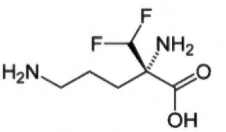
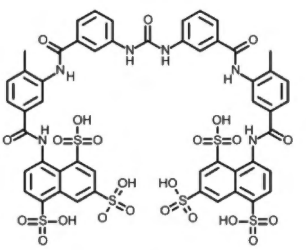
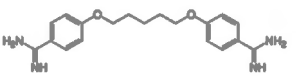
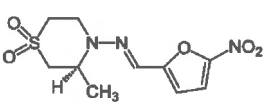
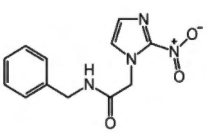
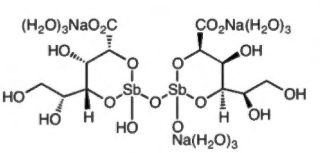
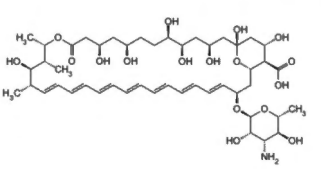
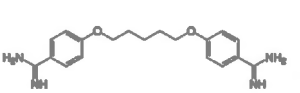
Transport systems comprise an essential feature of every living cell: they allow the entry of all essential nutrients into the cell and its compartments and regulate the intracellular concentrations of metabolites [6]. The capacity of a parasite to import critical nutrients is essential considering that after millions of years of adaptation to parasitism, it replaced biosynthetic routes for transport systems. These metabolic differences between trypanosomatids and their hosts point out transporters as suitable targets for drug design [7].

In the past applying molecular genetic approaches as well as the completion of genome projects have allowed the identification and functional characterization of a variety of transporters and their genes in these organisms which broadened our understanding about metabolite uptake systems in these parasites. Major attention was drawn on hexose-, purine- and amino acid- permeases which have been studied in well-established experimental systems.

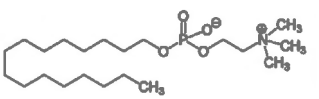
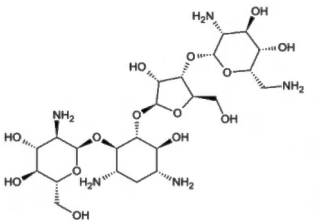
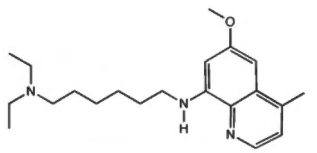
*Address correspondence to this author at the IDIM, Combatientes de Malvinas 3150, (1427) Bs. As, Argentina; Tel: 5411-4514-8701; Fax: 5411-4523-8947; E-mail: mirandamariana@gmail.com

0929-8673/14 $58.00+.00

Table 1. Drugs Currently Used Against Diseases Caused by Trypanosomatid Parasites

Parasite	Drug	Molecule	Treatment	Action Mechanism	Entry[a]
T. brucei	Melarsoprol		Late stage of both Human African Trypanosomiasis	Not fully understood. Inhibits glycolytic enzymes and trypanothione reductase	P2/TbAT1 – AQP2
	Eflornithine		Late stage of Human African Trypanosomiasis produced by *T. b. gambiense*	Irreversible inhibitor of ornithine decarboxylase	TbAAT6
	Suramin		Early stage of Human African Trypanosomiasis produced by *T. b. rhodesiense*	Not fully understood. Inhibits glycolytic enzymes	Receptor-mediated endocytosis
	Pentamidine		Early stage of Human African Trypanosomiasis produced by *T. b. gambiense*	Not fully understood. Competes with polyamines for nucleic acid binding. Interferes with DNA replication and transcription at mitochondrial level	P2/TbAT1 – AQP2 – HAPT – LAPT
T. cruzi	Nifurtimox		Acute phase of Chagas Disease	Produces highly toxic reduced oxygen metabolites	?
	Benznidazole		Acute Phase of Chagas Disease	Covalent modification of macromolecules by nitro reduction intermediates	TcHT1
Leishmania spp.	Sodium stibogluconate		Visceral and Cutaneous Leishmaniasis	Not fully understood. Inhibit estrypanothione reductase, glycolysis and metabolic pathways. Produces DNA fragmentation	LmAQP1
	Amphotericin B		Visceral and Cutaneous Leishmaniasis	Interacts with ergosterol of *Leishmania* spp and cholesterol of host macrophages, thus inhibiting binding to host cells	?
	Pentamidine		Visceral Leishmaniasis	Not fully understood. Competes with polyamines for nucleic acid binding. Interferes with DNA replication and transcription at mitochondrial level	LmPOT1

(Table 1) contd….

Parasite	Drug	Molecule	Treatment	Action Mechanism	Entry[a]
	Miltefosine		Visceral Leishmaniasis	Not fully understood. Induces apoptosis-like death	Lipid complex LdMT/Ld Ros3
	Paromomycin		Visceral and Cutaneous Leishmaniasis	Not fully understood. Inhibits protein synthesis	?
	Sitamaquine		Visceral Leishmaniasis	Involves inhibition of the respiratory chain complex II, which in turn triggers oxidative stress and finally leads to an apoptosis-like death	Electrostatic interaction with anionic phospholipids of the plasma membrane

[a] P2/TbAT1: *T. brucei* Adenosine Transporter (9); AQP2: *T. brucei* Aquaglyceroporine 2 (10); TbAAT6: *T. brucei* Neutral Amino Acid Transporter (15); Receptor-mediated endocytosis (47); HAPT: High Affinity Pentamidine Transporter (48); LAPT: Low Affinity Pentamidine Transporter (48); TcHT1; *T. cruzi* Hexose Transporter (13); LmAQP1: *L. major* Aquaglyceroporine (11); LmPOT1: *L. major* Polyamine Transporter (44); LdMT/LdRos3: L. donovani Miltefosine Transporter, and its subunit Ros3 (49); and electrostatic interactions with anionic phospholipids of the plasma membrane (50).

2. INVOLVEMENT OF TRANSPORTERS IN DRUG UPTAKE

Trypanosomes bear a number of transporters at the cell surface, many of them in the "flagellar pocket". This structure represents an invagination of the pellicular membrane where endocytosis and exocytosis take place, and it is also responsible for nutrient uptake. Since many transporters are located in the flagellar pocket, therapeutic drugs can also enter cells through this region. Currently, there is an improved understanding of drug uptake by parasites due to new studies on the mechanisms of drug resistance in trypanosomes, as is explained in the next section. Early in the 50´s, arsenical-diamidine resistant strain was reported in *T. brucei* [8]. Such cross-resistance is explained by the findings that the melamine-based arsenical melarsoprol and the diamidine pentamidine are imported into trypanosomes by the same transporter whose physiological substrates are adenine and adenosine, both of which compete with melarsoprol for uptake and protect trypanosomes from lysis [9]. Moreover, other transporters, including the aquaporine AQP2, were associated to the same resistant phenotype. Aquaporins facilitate the transport of water and small neutral solutes across membranes in organisms and are involved in osmoregulation. Recent evidence suggests that this transporter also participates in the import of both drugs [10].

In *L. major*, the aquaporin LmAQP1 mediates the uptake of trivalent antimony, the active metabolite of sodium stibogluconate. Overexpression of the LmAQP1 gene induces increased sensitivity to antimony- and arsenic-containing compounds, and the deletion of one copy of this gene confers a decreased sensitivity to these drugs [11].

Hexose transporters have been tested to be inhibited by different D-glucose and D-fructose analogues (bromoacetamide-containing analogues). However, these compounds seem to block the *T. brucei* transporter being not translocated into the cells by permeases [12]. Additionally, *T. cruzi* glucose uptake is participating in benznidazole resistance since recent studies demonstrated that this transport activity dramatically decreases in benznidazole resistant strains obtained *in vitro*. These results suggest, that TcHT1 might be involved in this drug uptake [13].

Finally, new trypanocidal drugs such as furamidine and its derivatives, recently evaluated in clinical trials for the treatment of African trypanosomiasis, are actively accumulated by *T. brucei,* being the aminopurine TbAT1 (P2) permease the main transport route [14].

3. TRANSPORTERS, DRUG RESISTANCE AND DEVELOPMENT OF NEW DRUGS

As was previously introduced, the understanding in drug uptake by parasites took place once resistant phenotypes were observed. Thus, some examples explained above are also reviewed in this section. One of the first molecular identification of a transporter associated with drug resistance in trypanosomes was in 1993 by Carter *et al.* [9], when they

discovered that melarsoprol-resistant trypanosomes lack the P2 adenosine transporter, suggesting that resistance to these arsenicals is due to loss of uptake. The trypanocidal effect of melarsoprol could be abolished by adenine, adenosine and dipyridamole, which all compete for the uptake by an adenosine transporter. *T. brucei* has two high-affinity adenosine transport systems; the P1 type, which also transports inosine; and the P2 type, which is capable to transport adenine and melarsoprol.

Eflornithine, an ornithine analogue, is used as a first line treatment for human African trypanosomiasis. Eflornithine resistance was studied by *in vitro* and *in vivo* analyzes [15]. Interestingly, neither the target of the drug, ornithine decarboxylase, nor the intermediates of the polyamine biosynthetic pathway were altered in resistant cells. However the intracellular concentration of eflornithine was shown to be diminished in resistant lines. An amino acid transporter gene, TbAAT6 (Tb927.8.5450), was found to be deleted in the resistant parasites. Additionally, RNAi silencing of such gene was sufficient to facilitate resistance [15]. These results led easily to the identification of resistant parasites in the field by PCR tests, enabling more appropriate treatment. Strikingly, the orthologs of TbAAT6 in *Leishmania* spp. and *T. cruzi* do not transport ornithine but proline/alanine or proline respectively [16-18].

ATP-binding cassette (ABC) transporters constitute the biggest family of membrane proteins involved in drug resistance and other biological activities. Drug resistance represents a major problem in different treatments. In the specific case of Malaria, recent identifications highlighted the importance of mutations in transporter molecules for being major contributors to drug resistance in the human malaria parasite *Plasmodium falciparum*. Three transporters are of particular importance in drug resistance, the "chloroquine resistance transporter" (PfCRT) [19], the "multi-drug resistance transporter 1" (PfMDR1) [20], and the "multi-drug resistance-associated protein" (PfMRP) [21, 22]. In a similar way, in trypanosomatids multidrug resistance is related to the P-glycoprotein (Pgp), an energy dependent efflux pump that extrudes drugs and metabolites across the membrane. The genes TcPGP1 and TcPGP2 have been described in *T. cruzi*, although the function of these genes has not been fully elucidated. *In vitro* induced cell lines resistant to thiosemicarbazones and benznidazole have a higher Pgp activity, and Pgp inhibitors could abolish the resistance suggesting that multidrug resistance is a consequence of an increased drug efflux [23]. These results are particularly relevant since benznidazole is the main drug in therapy of Chagas' disease.

As occurs in *T. cruzi*, resistance of *Leishmania* spp. to therapeutic drugs increases in developing countries and in many instances it is due to over-expressed ABC efflux pumps. Cell lines resistant to the drug camptothecin (CPT) are characterized by an overexpression of an ABC transporter called ABCG6 [24]. Transfection and overexpression of LdABCG6 in wild type parasites, visualized the transporter primarily in the plasma membrane and the flagellar pocket region, where most of the permeases are also located [25, 26]. Similar to other organisms, overexpression of LdABCG6 confers CPT resistance to the parasites mediated by rapid drug efflux.

In this paragraph we address not only a variety of transporters that might be effective candidates for drug targeting, but also other functions of permeases such as transporting modified drugs as well as novel high-throughput drug screening methods.

Nucleoside transporters have been used to deliver toxic agents to trypanosomes; in addition, glucose and amino acid transporters have been evaluated to carry modified drugs into the cell cytoplasm. While there are a variety of metabolite permeases that can be studied as potential therapeutic targets, in this paragraph we will focus on three main groups composed by transporters of nucleoside, amino acids and polyamines.

One clear example of the transport of modified drugs is the case of the melarsoprol and pentamidine that can enter via an aminopurine transporter. However, other toxic compounds have also been designed to enter via this route [27]. Recently it has been reported that addition of melamine-based P2-targeting motifs to three different compound classes fluoroquinolones, difluoromethylornithine and artesunate derivatives, improved the trypanocidal activity through increased selective uptake [28].

The first multigenic family of amino acid transporter from *T. cruzi* (TcAAAP) was identified by Bouvier *et al.* [16]. A couple of years ago, few members of this family have been characterized in trypanosomatids, including an arginine, lysine and proline/alanine permeases [25, 26, 29, 30]. One interesting feature of this permease family is the absence of orthologs in mammalian genomes emphasizing that these molecules could serve as putative targets for novel trypanocidal drugs [16, 18, 31].

In *T. cruzi*, arginine is an essential amino acid and a key substrate for several metabolic pathways which is scavenged from the host via different transport systems or by intracellular proteolysis [32-34]. Arginine participates in the cell energy management through an arginine kinase, which is absent in mammalian tissues [33, 35]. Recently it has been reported an arginine permease (TcAAP3) which activity regulates the downstream processes of the arginine metabolism, such as arginine kinase activity. This regulation mechanism involves the ATP homeostasis since an increase in arginine transport dramatically decreases the intracellular ATP level thus affecting the parasites' viability [26]. These evidences suggest that modulating the TcAAP3 activity by a drug, would affect the survival of *T. cruzi*.

On the other hand, it is well established that L-proline has several roles in the biology of trypanosomatids. In *T. cruzi*, this amino acid is involved in energy metabolism, differentiation processes and resistance to osmotic stress [36, 37]. Recently, the use of the proline analogue L-thiazolidine-4-carboxylic acid (T4C) was analyzed. It was demonstrated that T4C significantly diminished parasite survival in combination with nutrient starvation and oxidative stress conditions. These data suggest that T4C could be used in combination with compounds that produce oxidative stress an interesting therapeutic drug [38].

In trypanosomes, polyamines are involved in crucial cellular processes including the synthesis of the antioxidant trypanothione (bis-glutathionylspermidine) which has been

found exclusively in trypanosomatids [39]. Polyamines could be obtained by the *de novo* synthesis from ornithine, arginine, or transported from extracellular sources. In contrast to other protozoa, *T. cruzi* is the only trypanosomatid which is auxotroph for polyamines because of its inability to synthesize putrescine due to the lack of both, arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) [40, 41]. Therefore, the intracellular availability of polyamines in *T. cruzi* depends exclusively on transport processes. Because the essentiality and differences in the metabolic pathway in comparison to the host, polyamine transport and metabolism constitute an appropriate target for chemotherapeutic strategies against parasitic diseases [42], since blocking ornithine decarboxylase activity by eflornithine is a validated treatment for African trypanosomiasis [43]. Recently, a high-affinity polyamine permease from *L. major* was cloned and functionally characterized [44]. This was the first report of a eukaryotic polyamine permease at the molecular level. Once the *L. major* POT1 was published we were able to identify a *T. cruzi* polyamine permease [45] which has previously been described as a member of the TcAAAP family [16]. Despite the intensive use as antiparasitic drug little is known about the effect of pentamidine in *T. cruzi*. Recent studies demonstrated that pentamidine blocks a polyamine transporter present in *L. major* [44]; consequently, it might also block these transporters in *T. cruzi*.

New antimetabolites have been tested as trypanocidal drugs. The accumulation of such compounds inside the cell strongly depends on nutrient transporters [27]. Some examples of them are: acivicin, 6-diazo-5-oxo-L-norleucine, azaserine (glutamine analogues), tryptophan benzyl ester, dihydroxitryptamine (tryptophan analogues), buthioninesulfoximine (methionine analogue), and hydroxydopamine (tyrosine analogue). In addition to a wide variety of analogues, new high-throughput techniques have been applied to discover new drug targets. Recently, five different drugs used for the treatment of African trypanosomiasis were assayed in a genome-wide RNA Interference Target sequencing (RIT-seq) screening in *T. brucei*. Obtained results reveal that transporters and other unrelated mechanisms facilitate the trypanocidal drug activity. In terms of drug uptake, RIT-seq profiling identified a bloodstream stage-specific invariant surface glycoprotein (ISG75) family which mediates suramin uptake and aquaglyceroporins involved in pentamidine and melarsoprol cross-resistance. These results provide unprecedented molecular insights into the mode of action of antitrypanosomal drugs [46].

4. PERSPECTIVES

Although there are many therapies against trypanosomiases, none of them is totally satisfactory. Some drugs have limited efficacy, resistance is an increasing problem, dosage regimes can be complex and drug administration requires medical supervision. Thus the development of new drugs is urgently required. One of the limitations towards the discovery of new transporter-targeted drugs is the lack of an efficient high throughput screening method. Due to the lipophilic nature of the trans membrane domains of the permeases they are hardly to be recombinantly expressed and subsequently employed in drug screening assays. Therefore automated screening techniques should be developed in the future deploying more complex systems such as transporters incorporated in liposomes or high throughput drug selection of auxotrophic yeast complemented with the target permease. In addition, membrane transporters can be used for selected targeting of trypanocidal compounds into the parasites. The potency of various well-known trypanocidal drugs can be enhanced by chemical modifications mimicking the natural substrate and thereby increasing uptake capacity [28].

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 2010-0685 and 2011-0263), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT PICT 2008-1209, 2010-0289 and 2012-0559), and Fundación Bunge y Born (grant 2012). CAP and MRM are members of the career of scientific investigator. MS is a research fellow from CONICET. CW is supported by the grants 2009/54325-2 and 2010/20647-0 of the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

REFERENCES

[1] Simpson, A.G.; Stevensm J.R.; Lukes, J. The evolution and diversity of kinetoplastid flagellates. *Trends Parasitol.,* **2006**, *22*(4), 168-174.

[2] Barrett, M.P.; Burchmore, R.J.; Stich, A.; Lazzari, J.O.; Frasch, A.C.; Cazzulo, J.J.; Krishna, S. The trypanosomiases. *Lancet*, **2003**, *1*, *362*(9394), 1469-1480.

[3] Fairlamb, A.H. Chemotherapy of human African trypanosomiasis: current and future prospects. *Trends Parasitol.*, **2003**, *19*(11), 488-494.

[4] Singh, N.; Kumar, M.; Singh, R.K. Leishmaniasis: current status of available drugs and new potential drug targets. *Asian Pac. J. Trop. Med.*, **2012**, *5*(6), 485-497.

[5] Urbina, J.A. Specific chemotherapy of Chagas disease: relevance, current limitations and new approaches. *Acta Trop.*, **2010**, *115*(1-2), 55-68.

[6] Busch, W.; Saier, M.H. Jr. The transporter classification (TC) system, **2002**. *Crit. Rev. Biochem. Mol. Biol.,* **2002**, *37*(5), 287-337.

[7] Pereira, C.A.; Carrillo, C.; Miranda, M.R.; Bouvier, L.A.; Canepa, G.E. *Trypanosoma cruzi*: transport of essential metabolites acquired from the host. *Medicina (B Aires).*, **2008**, *68*(5), 398-404.

[8] Rollo, I.M.; Williamson, J. Acquired resistance to 'Melarsen', tryparsamide and amidines in pathogenic trypanosomes after treatment with 'Melarsen' alone. *Nature,* **1951**, *167*(4239), 147-148.

[9] Carter, N.S.; Fairlamb, A.H. Arsenical-resistant trypanosomes lack an unusual adenosine transporter. *Nature,* **1993**, *361*(6408), 173-176.

[10] Baker, N.; Glover, L.; Munday, J.C.; Aguinaga Andres, D.; Barrett, M.P.; de Koning, H.P.; Horn, D. Aquaglyceroporin 2 controls susceptibility to melarsoprol and pentamidine in African trypanosomes. *Proc. Natl. Acad. Sci. U S A.,* **2012**, *109*(27), 10996-11001.

[11] Gourbal, B.; Sonuc, N.; Bhattacharjee, H.; Legare, D.; Sundar, S.; Ouellette, M.; Rosen, B.P.; Mukhopadhyay, R. Drug uptake and modulation of drug resistance in Leishmania by an aquaglyceroporin. *J. Biol. Chem.,* **2004**, *279*(30), 31010-31017.

[12] Azema, L.; Claustre, S.; Alric, I.; Blonski, C.; Willson, M.; Perie, J.; Baltz, T.; Tetaud, E.; Bringaud, F.; Cottem, D.; Opperdoes, F.R.; Barrett, M.P. Interaction of substituted hexose analogues with the *Trypanosoma brucei* hexose transporter. *Biochem. Pharmacol.,* **2004**, *67*(3), 459-467.

[13] dos Santos, P.F.; Ruiz, J.C.; Soares, R.P.; Moreira, D.S.; Rezende, A.M.; Folador, E.L.; Oliveira, G.; Romanha, A.J.; Murta, S.M. Molecular characterization of the hexose transporter gene in benznida-

zole resistant and susceptible populations of *Trypanosoma cruzi*. *Parasit. Vectors*, **2012**, *5*, 161.

[14] Ward, C.P.; Wong, P.E.; Burchmore, R.J.; de Koning, H.P.; Barrett, M.P. Trypanocidal furamidine analogues: influence of pyridine nitrogens on trypanocidal activity, transport kinetics, and resistance patterns. *Antimicrob. Agents Chemother.*, **2011**, *55*(5), 2352-2361.

[15] Vincent, I.M.; Creek, D.; Watson, D.G.; Kamleh, M.A.; Woods, D.J.; Wong, P.E.; Burchmore, R.J.; Barrett, M.P. A molecular mechanism for eflornithine resistance in African trypanosomes. *PLoS Pathog.*, **2010**, *6*(11), e1001204.

[16] Bouvier, L.A.; Silber, A.M.; Galvao Lopes, C.; Canepa, G.E.; Miranda, M.R.; Tonelli, R.R.; Colli, W.; Alves, M.J.; Pereira, C.A. Post genomic analysis of permeases from the amino acid/auxin family in protozoan parasites. *Biochem. Biophys. Res. Commun.*, **2004**, *321*(3), 547-556.

[17] Jackson, A.P. Origins of amino acid transporter loci in trypanosomatid parasites. *BMC Evol. Biol.*, **2007**, *7*, 26.

[18] Akerman, M.; Shaked-Mishan, P.; Mazareb, S.; Volpin, H.; Zilberstein, D. Novel motifs in amino acid permease genes from Leishmania. *Biochem. Biophys. Res. Commun.*, **2004**, *325*(1), 353-366.

[19] Sidhu, A.B.; Verdier-Pinard, D.; Fidock, D.A. Chloroquine resistance in Plasmodium falciparum malaria parasites conferred by pfcrt mutations. *Science*, **2002**, *298*(5591), 210-213.

[20] Foote, S.J.; Thompson, J.K.; Cowman, A.F.; Kemp, D.J. Amplification of the multidrug resistance gene in some chloroquine-resistant isolates of P. falciparum. *Cell*, **1989**, *57*(6), 921-930.

[21] Sanchez, C.P.; Dave, A.; Stein, W.D.; Lanzer, M. Transporters as mediators of drug resistance in Plasmodium falciparum. *Int. J. Parasitol.*, **2010**, *40*(10), 1109-1118.

[22] Wilson, C.M.; Serrano, A.E.; Wasley, A.; Bogenschutz, M.P.; Shankar, A.H.; Wirth, D.F. Amplification of a gene related to mammalian mdr genes in drug-resistant Plasmodium falciparum. *Science*, **1989**, *244*(4909), 1184-1186.

[23] Campos, M.C.; Castro-Pinto, D.B.; Ribeiro, G.A.; Berredo-Pinho, M.M.; Gomes, L.H.; da Silva Bellieny, M.S.; Goulart, C.M.; Echevarria, A.; Leon, L.L. P-glycoprotein efflux pump plays an important role in *Trypanosoma cruzi* drug resistance. *Parasitol. Res.*, **2013**, *112*(6), 2341-2351.

[24] BoseDasgupta, S.; Ganguly, A.; Roy, A.; Mukherjee, T.; Majumder, H.K. A novel ATP-binding cassette transporter, ABCG6 is involved in chemoresistance of Leishmania. *Mol. Biochem. Parasitol.*, **2008**, *158*(2), 176-188.

[25] Inbar, E.; Canepa, G.E.; Carrillo, C.; Glaser, F.; Suter Grotemeyer, M.; Rentsch, D.; Zilberstein, D.; Pereira, C.A. Lysine transporters in human trypanosomatid pathogens. *Amino Acids.*, **2012**, *42*(1), 347-360.

[26] Miranda, M.R.; Saye, M.; Bouvier, L.A.; Camara Mde, L.; Montserrat, J.; Pereira, C.A. Cationic amino acid uptake constitutes a metabolic regulation mechanism and occurs in the flagellar pocket of *Trypanosoma cruzi*. *PLoS One*, **2012**, *7*(2), e32760.

[27] Barrett, M.P.; Gilbert, I.H. Targeting of toxic compounds to the trypanosome's interior. *Adv. Parasitol.*, **2006**, *63*, 125-183.

[28] Chollet, C.; Baliani, A.; Wong, P.E.; Barrett, M.P.; Gilbert, I.H. Targeted delivery of compounds to *Trypanosoma brucei* using the melamine motif. *Bioorg. Med. Chem.*, **2009**, *17*(6), 2512-2523.

[29] Shaked-Mishan, P.; Suter-Grotemeyer, M.; Yoel-Almagor, T.; Holland, N.; Zilberstein, D.; Rentsch, D. A novel high-affinity arginine transporter from the human parasitic protozoan Leishmania donovani. *Mol. Microbiol.*, **2006**, *60*(1), 30-38.

[30] Carrillo, C.; Canepa, G.E.; Giacometti, A.; Bouvier, L.A.; Miranda, M.R.; de los Milagros Camara, M.; Pereira, C.A. *Trypanosoma cruzi* amino acid transporter TcAAAP411 mediates arginine uptake in yeasts. *FEMS Microbiol Lett.*, **2010**, *306*(2), 97-102.

[31] Silber, A.M.; Colli, W.; Ulrich, H.; Alves, M.J.; Pereira, C.A. Amino acid metabolic routes in *Trypanosoma cruzi*: possible therapeutic targets against Chagas' disease. *Curr. Drug Targets Infect. Disord.*, **2005**, *5*(1), 53-64.

[32] Pereira, C.A.; Alonso, G.D.; Paveto, M.C.; Flawia, M.M.; Torres, H.N. L-arginine uptake and L-phosphoarginine synthesis in *Trypanosoma cruzi*. *J. Eukaryot. Microbiol.*, **1999**, *46*(6), 566-570.

[33] Pereira, C.A.; Alonso, G.D.; Paveto, M.C.; Iribarren, A.; Cabanas, M.L.; Torres, H.N.; Flawiá, M.M. *Trypanosoma cruzi* arginine kinase characterization and cloning. A novel energetic pathway in protozoan parasites. *J. Biol. Chem.*, **2000**, *275*(2), 1495-1501.

[34] Canepa, G.E.; Silber, A.M.; Bouvier, L.A.; Pereira, C.A. Biochemical characterization of a low-affinity arginine permease from the parasite *Trypanosoma cruzi*. *FEMS Microbiol. Lett.*, **2004**, *236*(1), 79-84.

[35] Alonso, G.D.; Pereira, C.A.; Remedi, M.S.; Paveto, M.C.; Cochella, L.; Ivaldi, M.S.; Gerez de Burgos, N.M.; Torres, H.N.; Flawiá, M.M. Arginine kinase of the flagellate protozoa *Trypanosoma cruzi*. Regulation of its expression and catalytic activity. *FEBS Lett.*, **2001**, *498*(1), 22-25.

[36] Martins, R.M.; Covarrubias, C.; Rojas, R.G.; Silber, A.M.; Yoshida, N. Use of L-proline and ATP production by *Trypanosoma cruzi* metacyclic forms as requirements for host cell invasion. *Infect. Immun.*, **2009**, *77*(7), 3023-3032.

[37] Tonelli, R.R.; Silber, A.M.; Almeida-de-Faria, M.; Hirata, I.Y.; Colli, W.; Alves, M.J. L-proline is essential for the intracellular differentiation of *Trypanosoma cruzi*. *Cell Microbiol.*, **2004**, *6*(8), 733-741.

[38] Magdaleno, A.; Ahn, I.Y.; Paes, L.S.; Silber, A.M. Actions of a proline analogue, L-thiazolidine-4-carboxylic acid (T4C), on *Trypanosoma cruzi*. *PLoS One*, **2009**, *4*(2), e4534.

[39] Fairlamb, A.H.; Blackburn, P.; Ulrich, P.; Chait, B.T.; Cerami, A. Trypanothione: a novel bis(glutathionyl)spermidine cofactor for glutathione reductase in trypanosomatids. *Science*, **1985**, *227*(4693), 1485-1487.

[40] Carrillo, C.; Cejas, S.; Huber, A.; Gonzalez, N.S.; Algranati, I.D. Lack of arginine decarboxylase in *Trypanosoma cruzi* epimastigotes. *J. Eukaryot. Microbiol.*, **2003**, *50*(5), 312-316.

[41] Carrillo, C.; Cejas, S.; Gonzalez, N.S.; Algranati, I.D. *Trypanosoma cruzi* epimastigotes lack ornithine decarboxylase but can express a foreign gene encoding this enzyme. *FEBS Lett.*, **1999**, *454*(3), 192-196.

[42] Algranati, I.D. Polyamine metabolism in *Trypanosoma cruzi*: studies on the expression and regulation of heterologous genes involved in polyamine biosynthesis. *Amino Acids*, **2010**, *38*(2), 645-651.

[43] Bitonti, A.J.; Bacchi, C.J.; McCann, P.P.; Sjoerdsma, A. Catalytic irreversible inhibition of *Trypanosoma brucei* brucei ornithine decarboxylase by substrate and product analogs and their effects on murine trypanosomiasis. *Biochem. Pharmacol.*, **1985**, *34*(10), 1773-1777.

[44] Hasne, M.P.; Ullman, B. Identification and characterization of a polyamine permease from the protozoan parasite Leishmania major. *J. Biol. Chem.*, **2005**, *280*(15), 15188-15194.

[45] Carrillo, C.; Canepa, G.E.; Algranati, I.D.; Pereira, C.A. Molecular and functional characterization of a spermidine transporter (TcPAT12) from *Trypanosoma cruzi*. *Biochem. Biophys. Res. Commun.*, **2006**, *344*(3), 936-940.

[46] Alsford, S.; Eckert, S.; Baker, N.; Glover, L.; Sanchez-Flores, A.; Leung, K.F.; Turner, D.J.; Field, M.C.; Berriman, M.; Horn, D. High-throughput decoding of antitrypanosomal drug efficacy and resistance. *Nature*, **2012**, *482*(7384), 232-236.

[47] Coppens, I.; Opperdoes, F.R.; Courtoy, P.J.; Baudhuin, P. Receptor-mediated endocytosis in the bloodstream form of *Trypanosoma brucei*. *J. Protozool.*, **1987**, *34*(4), 465-473.

[48] De Koning, H.P. Uptake of pentamidine in *Trypanosoma brucei* brucei is mediated by three distinct transporters: implications for cross-resistance with arsenicals. *Mol. Pharmacol.*, **2001**, *59*(3), 586-592.

[49] Perez-Victoria, F.J.; Gamarro, F.; Ouellette, M.; Castanys, S. Functional cloning of the miltefosine transporter. A novel P-type phospholipid translocase from Leishmania involved in drug resistance. *J. Biol. Chem.*, **2003**, *278*(50), 49965-49971.

[50] Duenas-Romero, A.M.; Loiseau, P.M.; Saint-Pierre-Chazalet, M. Interaction of sitamaquine with membrane lipids of Leishmania donovani promastigotes. *Biochim. Biophys. Acta*, **2007**, *1768*(2), 246-252.