Antimony transport mechanisms in resistant leishmania parasites

Frédéric Frézard · Rubens Monte-Neto · Priscila G. Reis

Received: 26 July 2013 / Accepted: 5 December 2013 / Published online: 25 January 2014
© International Union for Pure and Applied Biophysics (IUPAB) and Springer-Verlag Berlin Heidelberg 2014

Abstract Antimonial compounds have been used for more than a century in the treatment of the parasitic disease leishmaniasis. Although pentavalent antimonials are still first-line drugs in several developing countries, this class of drugs is no longer recommended in the Indian sub-continent because of the emergence of drug resistance. The precise mechanisms involved in the resistance of leishmania parasites to antimony are still subject to debate. It is now well documented that drug resistance in leishmania parasites is a multifactorial phenomenon involving multiple genes whose expression pattern synergistically leads to the resistance phenotype. The reduction of intracellular antimony accumulation is a frequent change observed in resistant leishmania cells; however, no comprehensive transport model has been presented so far to explain this change and its contribution to *Leishmania* resistance. The present review firstly covers the actual knowledge on the metabolism of antimonial drugs, the mechanisms of their transmembrane transport and intracellular processing in *Leishmania*. It further describes both the functional and molecular changes associated with Sb resistance in this organism. Possible transport models based on the actual knowledge are then presented, as well as their functional implications. Biophysical and pharmacological strategies are finally proposed for the precise identification of the transport pathways.

Keywords Antimony · Resistance · Leishmania · Transport · ABC transporters · AQP1

Abbreviations

T(SH) ₂	trypanothione
GSH	glutathione
AQP1	aquaglyceroporin 1
MRPA	multidrug resistance-associated protein A
MRP	multidrug resistance-associated protein
MDR	multidrug resistance
MAPK	mitogen-activated protein kinase
TryR	trypanothione reductase
ODC	ornithine decarboxylase
γ-GCS	γ-glutamylcysteine synthetase
HBTF	terbinafine-associated encoding gene
PRT1	pterine reductase
APX	ascorbate-dependent peroxidase
G6PDH	glucose-6-phosphate dehydrogenase
NT4	nucleobase transporter

Introduction

The most significant clinical use of antimonial compounds during the last century is certainly that in the treatment of leishmaniasis. Leishmaniasis are infective parasitic diseases, which are endemic in 98 countries, reaching up to 1.2 million new cases per year, affecting mainly poor and marginalized populations with 90 % of cases in six countries: India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia (Alvar et al. 2012). The clinical manifestations of the complex of diseases can involve the skin, with local (cutaneous), diffuse (diffuse cutaneous) or disfiguring lesions (mucocutaneous), or the viscera (liver, spleen and bone marrow) leading to death if untreated. It is caused by parasitic protozoa belonging to the

Special Issue Advances in Biophysics in Latin America

F. Frézard (✉) · P. G. Reis

Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Av. Antônio Carlos 6627, Pampulha, 31270-901 Belo Horizonte, Minas Gerais, Brazil
e-mail: frezardf@gmail.com

R. Monte-Neto

Centre de Recherche en Infectiologie du Centre Hospitalier de l'Université Laval, 2705, Boulevard Laurier, RC-709, G1V 4G2 Québec, QC, Canada

genus *Leishmania*, transmitted to humans via the bite of sandflies. Wild and domesticated animals, and humans themselves, can act as a reservoir of infection. The leishmania parasite is found as a motile flagellated promastigote in the sandfly gut, it differentiates into an aflagellar amastigote when internalized by host macrophages, and resides in the acidic environment of secondary lysosomes.

At the beginning of the last century, Gaspar Vianna, pioneer researcher in the treatment of leishmaniasis, reported the efficacy of trivalent antimonial drug tartar emetic for treatment of mucocutaneous leishmaniasis (Vianna 1912). Later, the clinical use of this compound was interrupted because of their severe side effects.

From the decade of the 1940s, the less toxic, pentavalent antimony (Sb(V)) complexes, including meglumine antimoniate and sodium stibogluconate, were introduced in the therapeutics of leishmaniasis. Even though pentavalent antimonials are still the first-line drugs against all forms of leishmaniasis in several countries, their use in the clinical setting shows several limitations (Berman 1997). These compounds have to be given parenterally, daily, for at least 3 weeks (typically, 20 mg of Sb/kg/day for 20–30 days). Antimony therapy is often accompanied by local pain during intramuscular injections and by systemic side effects, requiring very careful medical supervision.

Widespread resistance to antimonials has emerged in India, but not in Africa or South America. Therefore, antimonials are no longer recommended for treatment of leishmaniasis in the Indian sub-continent (Guerin et al. 2002; Matlashewski et al. 2011). A hypothesis to explain the emergence of resistance in this specific region of India refers to the role of arsenic in drinking water (Perry et al. 2011). Since 2003, the Government of India implemented several strategies to eradicate leishmaniasis as part of the *kala-azar* elimination program (Dhillon et al. 2008) including the use of amphotericin B and oral drug miltefosine to treat visceral leishmaniasis (Matlashewski et al. 2011). However, increased failure rate and resistant clinical isolates against these drugs have recently been found (Sundar et al. 2012; Das et al. 2013; Purkait et al. 2012). To subvert this scenario, drug combination has emerged as a useful strategy for leishmaniasis chemotherapy (Sundar et al. 2011; Meheus et al. 2010; Sundar et al. 2008); however, drug-combined resistant parasites were already selected in vitro (Garcia-Hernandez et al. 2012), putting in doubt the efficacy of long-term exposition to drug combinations.

Studies concerning experimental resistance to antimony in *Leishmania* indicate that several mechanisms may occur concurrently in the same parasite and that different mechanisms may operate in field isolates compared to laboratory-selected resistant leishmania lines (Croft et al. 2006; Decuypere et al. 2012; Ouellette et al. 2004). On the other hand, some features observed in in vitro studies have also been demonstrated in field isolates (Mukherjee et al. 2007; Decuypere et al. 2005;

Kumar et al. 2012; Kazemi-Rad et al. 2013; Rai et al. 2013; Berg et al. 2013)

The overexpression of the ABC transporter multidrug resistance-associated protein A (MRPA), identified in intracellular vesicular membranes, is a common change observed in both field isolates and laboratory-selected leishmania strains resistant to antimony (Dey et al. 1994; Legare et al. 2001; Mukherjee et al. 2007; Decuypere et al. 2005; Rai et al. 2013). Furthermore, resistance to antimony in *Leishmania* usually involves a reduction in its intracellular accumulation (Brochu et al. 2003). However, attempts to characterize the transport pathways in resistant strains overexpressing the MRPA transporter showed apparently conflicting results, with either increased efflux (Dey et al. 1994) or decreased influx of trivalent antimony Sb(III) (Callahan et al. 1994). In this context, no comprehensive transport model has been presented so far to explain the reduced drug accumulation and its participation in *Leishmania* resistance.

In order to address the possible transport mechanisms that may account for the reduced intracellular accumulation of antimony in resistant strains, the present review first covers the actual knowledge on the metabolism of antimonial drugs, the mechanisms of transmembrane transport and intracellular processing of antimonial drugs in leishmania cells and both functional and molecular changes associated with Sb transport in resistant strains. The final part describes possible transport models based on the present knowledge and their functional implications. Biophysical and pharmacological strategies are also proposed for the precise identification of the transport pathways.

Metabolism of antimonial drugs, mechanisms of transmembrane transport and intracellular processing in *Leishmania*

Figure 1 summarizes the actual knowledge on the metabolism of antimonial drugs, the transport pathways identified for antimony and its intracellular processing in *Leishmania*.

Reduction of Sb(V) or release of Sb(III)

The metabolism and mechanism of action of pentavalent antimonials against leishmania parasites are not fully understood (Frezard et al. 2009). It is generally assumed that Sb(V) behaves as a prodrug that is reduced within the organism into the more toxic and active trivalent form. This model is supported by several reports that Sb(V) is reduced into Sb(III) in the vertebrate host and that reduction could also happen in the parasite (Goodwin and Page 1943; Shaked-Mishan et al. 2001; Ferreira et al. 2003; Wyllie and Fairlamb 2006; Hansen et al. 2011). However, the exact microenvironment where the reduction occurs is still an open question.

Fig. 1 Illustration of main pharmacological properties of antimonial drugs, including metabolism, transport pathways and intracellular processing in *Leishmania*. $T(SH)_2$, trypanothione; GSH, glutathione; AQP1, aquaglyceroporin 1; MRPA, multidrug resistance-associated protein A; TryR, trypanothione reductase; CCCCH-Zn, Zinc-finger protein with CCCCH domain

Leishmania promastigote forms are insensitive to $Sb(V)$, with exception of the non-pathogenic to humans *L. tarentolae* (Brochu et al. 2003). On the other hand, axenically cultured amastigote-like parasites are sensitive to pentavalent antimonials, suggesting the occurrence of intracellular $Sb(V)$ reduction in this life-cycle stage (Serenio et al. 1998). However, in the latter case, thiol availability from the culture medium, and low pH as well as high temperature may also be determinant factors in $Sb(V)$ to $Sb(III)$ reduction and consequently high sensitivity to $Sb(V)$ (Frezard et al. 2001). Carrio et al. (2000) found that, by maintaining the pH and fetal calf serum concentration, $Sb(V)$ activity was similar in promastigotes, intracellular amastigotes and axenic amastigote-like of *L. infantum* (Carrio et al. 2000). Not only environmental factors but also stage specific development is required to reach $Sb(V)$ sensitivity in axenically cultured amastigotes of *L. donovani* (Ephros et al. 1999). Furthermore, differential $Sb(V)$ sensitivity among different species may vary according to cellular intrinsic metabolism, membrane composition or intracellular thiol levels. The higher sensitivity of intramacrophagic amastigotes to $Sb(V)$ compared to axenic amastigote-like favors the hypothesis of host-associated $Sb(V)$ to $Sb(III)$ reduction (Serenio et al. 1998).

Thiols have been reported as potential reducing agents in this conversion (Frezard et al. 2001; Yan et al. 2003). Reduction of $Sb(V)$ to $Sb(III)$ was found to occur spontaneously in the presence of the following thiols: (1) glutathione (GSH), which is the main thiol in the cytosol of mammalian cells; (2) cysteine (Cys) and cysteinyl-glycine (Cys-Gly)

found predominantly within lysosomes; and (3) the bis(glutathione)-spermidine conjugate, trypanothione ($T(SH)_2$), which is the main thiol within the parasite (Fairlamb and Cerami 1992).

The observations that Cys, Cys-Gly and $T(SH)_2$ are more effective reducing agents than GSH and that this reaction is favored an acidic pH (Ferreira et al. 2003) led to the hypothesis that $Sb(V)$ may be reduced *in vivo* by $T(SH)_2$ within leishmania parasites and by Cys or Cys-Gly within the acidic compartments of mammalian cells. Both promastigotes and intracellular amastigotes maintain intracellular pH values close to neutral (~ 7) even in presence of extracellular acidic pH (~ 4 – 5) (Glaser et al. 1988). This observation reinforces the hypothesis that thiol-mediated $Sb(V)$ to $Sb(III)$ reduction takes place preferentially in the host cell.

Other studies have suggested the participation of a parasite-specific enzymes in the process of reduction of $Sb(V)$ to $Sb(III)$ (Denton et al. 2004; Zhou et al. 2004), and also as one possible explanation for the unique $Sb(V)$ sensitivity observed in promastigote forms of *L. tarentolae* (Brochu et al. 2003). However, these parasite-specific enzymes are expressed in both life-cycle stages and therefore it is difficult to see how this would account for the differential toxicity of pentavalent antimony against promastigotes and amastigotes.

Recently, the pentavalent antimonial drug meglumine antimoniate was found to contain up to 30 % of $Sb(III)$ indicating that the mode of action of this drug could be mediated by this residual amount of $Sb(III)$ (Salaun and Frezard 2013). Additionally, the availability of $Sb(III)$ increased at reduced pH values, suggesting that this drug may

act as molecular carrier releasing the active $\text{Sb}(\text{OH})_3$ form specifically in the acidic intracellular compartment where leishmania parasite resides (Salaun and Frezard 2013).

Route of antimony entry

It is now well established that $\text{Sb}(\text{III})$ enters leishmania cells primarily through an aquaglyceroporin named AQP1 (Gourbal et al. 2004).

The $\text{Sb}(\text{III})$ transport in *Leishmania* through AQP1 is energy-independent (Brochu et al. 2003). Indeed, aquaglyceroporins are known to promote the passive transport of neutral molecules such as water, glycerol and ammonia (Hub and de Groot 2008; Phongphanphanee et al. 2010). However, the way $\text{Sb}(\text{III})$ interacts with AQP1 to get inside the parasite is still an open question, but in theory it might be as the neutral $\text{Sb}(\text{OH})_3$ specie, which presents molecular similarity to glycerol serving as substrate of AQP1 (Liu et al. 2002). The crystal structure obtained of a related AQP from the malaria parasite *Plasmodium falciparum*, revealed a tetrameric organization of functional PfAQP (Newby et al. 2008), which could also play a role in the selective mechanism of AQP1 (Hub and de Groot 2008).

The differential accumulation of $\text{Sb}(\text{V})$ and $\text{Sb}(\text{III})$ in both promastigote and amastigote stages of *Leishmania* is the major evidence that $\text{Sb}(\text{V})$ uses a distinct route of entry. Indeed, competition assays using $\text{As}(\text{III})$, shows that $\text{Sb}(\text{III})$ accumulation is inhibited but not $\text{Sb}(\text{V})$ (Brochu et al. 2003). Since $\text{Sb}(\text{V})$ -derived compounds are sugar-conjugated molecules (meglumine antimoniate and sodium stibogluconate) it has been postulated that one possible way to enter in *Leishmania* is via a receptor able to recognize a sugar-like structure. Brochu et al. (2003) reported an inhibition of 35 % in $\text{Sb}(\text{V})$ uptake by gluconate competition (Brochu et al. 2003). An alternative explanation for this result may be that a specific transport route exists for non-complexed $\text{Sb}(\text{V})$ and that added gluconate reduced the free concentration of $\text{Sb}(\text{V})$ in equilibrium with the $\text{Sb}(\text{V})$ gluconate complex.

Recently, evidence was obtained that gene deletion (Mukherjee et al. 2013) or reduced expression (Marquis et al. 2005) of *AQP1* may result in decreased $\text{Sb}(\text{III})$ influx, reduced drug accumulation, and resistance.

Complexation with intracellular thiols and possible molecular targets

The antileishmanial mechanism of $\text{Sb}(\text{III})$ is probably related to its high affinity towards sulfhydryl-containing biomolecules, including thiols, peptides, proteins, and enzymes.

$\text{Sb}(\text{III})$ was found to form stable complexes with the major intracellular thiols GSH and $\text{T}(\text{SH})_2$ as 1:3 and 1:1 Sb -thiol species, respectively (Sun et al. 2000; Yan et al. 2003).

Among the potential molecular targets of $\text{Sb}(\text{III})$, evidence was obtained that thiols, trypanothione reductase (TryR), and a zinc-finger protein may be involved. Such interaction is consistent with the modality of Cys binding of thiophilic metals such as $\text{As}(\text{III})$, $\text{Sb}(\text{III})$, and $\text{Bi}(\text{III})$.

It has been reported that $\text{Sb}(\text{III})$ interferes with the thiol metabolism by inhibiting TryR and inducing efflux of intracellular $\text{T}(\text{SH})_2$ and GSH from intact leishmania cells (Wyllie et al. 2004). This causes oxidative stress that most probably contributes to cell death. It is worth noting that similar effects in thiol metabolism were observed with $\text{Sb}(\text{V})$ on axenic amastigotes in these studies.

In other recent studies, $\text{Sb}(\text{III})$ was found to bind to zinc finger peptide models and to promote the ejection of $\text{Zn}(\text{II})$ (Demicheli et al. 2008; Frezard et al. 2012). The zinc finger domain is characterized by the coordination of a zinc atom by several amino acid residues, including cysteine and histidine. Zinc finger proteins sharing the CCHC motif have been identified in trypanosomatids and are likely to be involved in DNA replication, structure, and repair (Webb and McMaster 1993). On the other hand, CCCH zinc finger domains which are found mainly in RNA binding proteins with regulatory functions at all stages of mRNA metabolism (Lai et al. 2002), are suspected to play a crucial role in the biology of kinetoplastid protozoa, because of the unusual emphasis on post-transcriptional control of gene expression in this group of organisms (Clayton and Shapira 2007). Interestingly, $\text{Sb}(\text{III})$ promotes $\text{Zn}(\text{II})$ ejection more effectively from CCCH zinc finger peptides than CCHC peptides (Frezard et al. 2012), suggesting that the action of antimonial drugs could be related mainly to interaction of $\text{Sb}(\text{III})$ with CCCH zinc finger proteins.

Despite the well-accepted prodrug model, $\text{Sb}(\text{V})$ itself may exhibit additional antileishmania effect by host immune response modulation (Pathak and Yi 2001; Muniz-Junqueira and de Paula-Coelho 2008) and interfere with DNA dynamics (Lucumi et al. 1998). Additionally, $\text{Sb}(\text{V})$ /ribonucleoside complexes may act interfering with purine transport and salvage pathway in *Leishmania* (Frezard et al. 2009).

Efflux and sequestration pathways of antimony

Once the $\text{Sb}(\text{III})$ is within the parasite, it can form conjugates with $\text{T}(\text{SH})_2$ or GSH and the resulting complex can be extruded from the cell or sequestered into intracellular vesicles by ATP-binding cassette (ABC) transporters (Mukhopadhyay et al. 1996; Legare et al. 2001).

An ATP-dependent efflux activity of thiol conjugates of $\text{Sb}(\text{III})$ and $\text{As}(\text{III})$ has been identified in the plasma membrane of both $\text{As}(\text{III})$ -sensitive and -resistant leishmania strains (Mukhopadhyay et al. 1996; Legare et al. 2001). However, the responsible pump has not yet been identified. At least, it is not expected to belong to the ABCC subfamily,

since ABCC proteins of *Leishmania* all have intracellular localization (Leprohon et al. 2009a).

Evidence for the existence of ABC transporters in the plasma membrane of *Leishmania* was also obtained from functional studies using fluorescent substrates of mammalian MRP1 and Pgp pumps including calcein, pirarubicin, and rhodamine-123 and the effect of classical inhibitors such as verapamil and phenothiazines on the transport of these fluorescent markers (Sharom 2008; Essodaigui et al. 1999; Messaritakis et al. 2013). However, in these studies, no clear relationship between these transport pathways and antimony resistance was established. Recently, Rai et al. (2013) established for the first time the link between functional characterization of drug efflux-associated pumps and overexpression of genes previously related to Sb resistance profiling, in different Sb(V)-resistant clinically isolated *L. donovani* (Rai et al. 2013). Using fluorescent substrate for Pgp- and MRPA-like transporters, the authors highlighted the presence of these pumps in Sb(V)-resistant *L. donovani* field isolates together with overexpression of *MRPA* gene, downregulation of *AQP1* and increased intracellular thiol levels associated to upregulation of thiol metabolism-related genes (Rai et al. 2013).

A new ABC transporter, a member of the ABCI subfamily (LABCI4), capable of promoting efflux of Sb(III) and thiols, thereby conferring resistance to antimonials, has been recently identified in *L. major* parasites (Manzano et al. 2013). This transporter was located in both mitochondrial and plasma membrane. The authors postulated that LABCI4 might be a thiol-X-pump that is able to recognize metal-conjugated thiols.

The ABCC3 transporter (also known as PGPA or MRPA) was clearly identified in As(III)- and Sb(III)-resistant leishmania strains, as being located in intracellular vesicular membranes close to the flagellar pocket and evidence was obtained that this transporter presents the ability to transport thiol-conjugated metals. It has also been postulated that the MRPA-enriched vesicles may be involved in a secretion pathway that may account for reduced antimony accumulation (Legare et al. 2001). It is also noteworthy that either decreased influx of Sb(III) or increased efflux has been reported in leishmania-resistant mutants overexpressing MRPA (Callahan et al. 1994). Furthermore, no correlation between the level of MRPA expression and the intracellular accumulation of Sb was observed (Papadopoulou et al. 1994). Thus, the latter observations leave doubts about the precise transport routes involved.

It is noteworthy that overexpression of MRP1-like and Pgp-like proteins was observed not only in the parasites but also in the plasma membrane of macrophages infected with antimony-resistant *L. donovani*, after in vivo selection of Sb(V) resistance in hamsters (Mookerjee et al. 2008). This parasite-induced modulation allows the drug efflux leading to a reduced intracellular Sb(III) concentration, favoring parasite survival. Interestingly, efflux pump overexpression was not

observed in Sb-sensitive *Leishmania*-infected macrophages (Mookerjee et al. 2008). This evidence supports the vesicle-mediated cross-talking between leishmania parasites and host cells (Silverman et al. 2010; Ghosh et al. 2013) and is a fascinating open research field for drug resistance mechanisms in leishmaniasis.

These findings taken all together indicate the possible contribution of distinct ABC transporter members and transport pathways to the drug resistance phenomenon in either field isolates or laboratory-selected leishmania mutants.

Molecular changes in antimony-resistant leishmania parasites

Unlike what happens in other drug resistant-microorganisms such as in bacteria, antimony resistance in leishmania parasites is rarely associated with single-nucleotide polymorphisms in their genome. Instead, the chromosomal plasticity allows structural changes resulting in chromosomal copy number variations and extra chromosomal DNA amplification, as revealed by whole-genome sequencing (Downing et al. 2011). Indeed, aneuploidy is a well-documented phenomenon observed in antimony-resistant *Leishmania* (Ubeda et al. 2008; Leprohon et al. 2009b). Increased gene copy number due to extra- and/or intrachromosomal amplification may contribute to the overexpression of genes resulting in metabolic adaptations that would help the parasites to survive in the presence of antimony.

The gene amplification associated with antimonial resistance phenotype usually occurs by homologous recombination of direct or inverted repeated sequences in the genome (Ouellette and Borst 1991; Beverley 1991; Grondin et al. 1996; Leprohon et al. 2009b), leading to the formation of circular or linear extrachromosomal DNA elements (Leprohon et al. 2009b; Mukherjee et al. 2007; do Monte-Neto et al. 2011). Recently, Mukherjee et al. (2013) have demonstrated a subtelomeric intrachromosomal amplification in an Sb(III)-resistant *L. major* mutant. The amplified locus revealed the presence of an ascorbate-dependent peroxidase (APX) and glucose-6-phosphate dehydrogenase (G6PDH) encoding genes, which were implicated in antimonial resistance after functional analysis in a revertant mutant background (Mukherjee et al. 2013). Since the amplification occurs at regions flanked by repeated sequences, the amplified locus in Sb-resistant *Leishmania* contains co-amplified genes whose direct contribution to the phenotype is often unknown. For example, the amplification of the H locus-containing *MRPA* also presents pteridine reductase 1 (PRT1) encoding gene and a terbinafine-associated encoding gene (*HBTF*) (Mukherjee et al. 2007; Marchini et al. 2003).

Table 1 presents an updated compilation of protein-coding genes frequently associated with Sb resistance phenotype, combining fold-change data in Sb-resistant *Leishmania*.

Table 1 Genes frequently associated with antimony resistance phenotype in both laboratory-selected and field/clinical isolates *Leishmania* mutants

Gene ID	Product	Fold Change	<i>Leishmania</i> spp	Reference
Biological process: Transport (GO: 0006810)				
LmjF.23.0250	ABC-thiol transporter (MRPA)	12.7 ~3 ^a	<i>L. tarentolae</i> TarII Sb ^{III} 400.1 <i>L. tropica</i> Field isolates	(Guimond et al. 2003) (Kazemi-Rad et al. 2013)
LmxM.23.0250		~20 ^a ~3 ^a	<i>L. amazonensis</i> BA199 SbR <i>L. amazonensis</i> PH8 SbR	(do Monte-Neto et al. 2011) (Moreira et al. 2013)
LinJ.23.0290		5.39 ^a 10–15 ^a 1.74–2.48 ^a 3–5 ~10 ^a	<i>L. infantum</i> Sb ^{III} 2000.1 <i>L. donovani</i> GE1-R; NS2-R; CK2-R Field isolates <i>L. donovani</i> Field isolates	(El Fadili et al. 2005; Leprohon et al. 2009b) (Mukherjee et al. 2007) (Rai et al. 2013) (Mittal et al. 2007) (Kumar et al. 2012)
LtaP.23.0250		>10 ^a	<i>L. tarentolae</i> TarII As20.3; TarIVa As20	(Ouellette and Borst 1991)
LbrM.23.0280		Functional (upregulated) ND ~10 ^a	<i>L. tarentolae</i> TarII Sb-R <i>L. guyanensis</i> M9945/R-21 <i>L. braziliensis</i> M2904 SbR	(Haimeur et al. 2000) (Anacleto et al. 2003) (Moreira et al. 2013)
LmjF.31.0020	Aquaglyceroporin 1 (AQP1)	Functional (downregulated)	<i>L. major</i> LV39; <i>L. infantum</i> As200.1; <i>L. tarentolae</i> TarII As50.1; <i>L. tarentolae</i> TarII Sb ^{III} 400.1	(Gourbal et al. 2004)
LinJ.31.0030		~−4 ^a −6 to −7 ^a ~−3 ^a −1.29 to −1.6 ^a 1.48 ^a	<i>L. tropica</i> Field isolates <i>L. donovani</i> Field isolates <i>L. donovani</i> Field isolates <i>L. donovani</i> SbR	(Kazemi-Rad et al. 2013) (Decuypere et al. 2005) (Kumar et al. 2012) (Rai et al. 2013)
LmxM.30.0020		~−1.7 ^a	<i>L. amazonensis</i> PH8 SbR	(Moreira et al. 2013)
LbrM.31.0020		~−3 ^a	<i>L. guyanensis</i> M9945 SbR	
LmxM.36.6300	Glucose transporter (Imgt1)	−2.56 to 17.15 ^a	<i>L. amazonensis</i> Sb ^{III} 2700.2;	(do Monte-Neto et al. 2011)
LmxM.36.6290	Glucose transporter (Imgt2)	−3.10 to 19.87 ^a	Sb ^{III} 2700.3	
LmxM.32.0290	Glucose transporter/membrane transporter D2	−1.82 to 2.46 ^a		
LinJ.11.0520	Nucleobase transporter (NT4)	2.49 ^a	<i>L. infantum</i> Sb ^{III} 2000.1	(Leprohon et al. 2009b)
LmxM.13.1210	Nucleobase transporter (NT3)	13.32 ^a	<i>L. amazonensis</i> Sb ^{III} 2700.2	(do Monte-Neto et al. 2011)
LmjF.33.3260	ABC half-transporter LABC14	Functional (upregulated)	<i>L. major</i> Friedlin	(Manzano et al. 2013)
Thiol metabolism and oxidation-reduction process-related genes (Polyamine biosynthetic process GO: 0006596; Glutathione biosynthetic process GO: 0006750; Response to reactive oxygen species GO: 0000302; One-carbon metabolic process GO: 0006730; Cell redox homeostasis GO: 0045454; Oxidation-reduction process GO:00055114)				
LmjF.14.0910	Glutathione synthetase (GS) (<i>GSH2</i>)	3.6 ^a	<i>L. tarentolae</i> TarII Sb ^{III} 400.1	(Guimond et al. 2003)
LmjF.18.1660	γ-Glutamylcysteine synthetase (γ-GCS) (<i>GSH1</i>)	3.9 3.9 ^a	<i>L. guyanensis</i> Field isolates	(Torres et al. 2010)
LbrM.18.1700		ND 3–4 ^a ~13 ^a 1.47–1.73 ^a	<i>L. donovani</i> 41-R <i>L. donovani</i> Field isolates	(Mukherjee et al. 2007) (Decuypere et al. 2005) (Kumar et al. 2012) (Rai et al. 2013)
LtaP.18.1640		Functional (upregulated)	<i>L. tarentolae</i> TarII Sb-R	(Haimeur et al. 2000)
LmjF.36.3910	S-Adenosylhomocysteine hydrolase (SAHH)	2.2 1.94–2.56 ^a	<i>L. tarentolae</i> TarII Sb ^{III} 400.1 <i>L. amazonensis</i> Sb ^{III} 2700.2; Sb ^{III} 2700.3	(Guimond et al. 2003) (do Monte-Neto et al. 2011)
LmxM.36.3910		1.4 ^a	<i>L. donovani</i> GE1-R	(Biyani et al. 2011)
LinJ.36.4100		ND	<i>L. infantum</i> Sb ^{III} 2000.1	(El Fadili et al. 2005)
LbrM.35.4140		ND	<i>L. panamensis</i>	(Walker et al. 2012)
LinJ.12.0100	Omithine decarboxylase (ODC)	ND 2–3 ^a 1.46–2.7 ^a	<i>L. donovani</i> GE1-R; NS2-R; 41-R; CK2-R Field isolates <i>L. donovani</i> Field isolates	(Mukherjee et al. 2007) (Decuypere et al. 2005) (Rai et al. 2013)
LbrM.12.0300		≥2		(Adaui et al. 2011)

Table 1 (continued)

Gene ID	Product	Fold Change	<i>Leishmania</i> spp	Reference
LbrM.05.0350	Trypanothione reductase (TryR)		<i>L. braziliensis</i> Sb ^V -R field isolates	
LinJ.05.0350		2–2.5	<i>L. donovani</i> Field isolates	(Mittal et al. 2007)
LmxM.05.0350		1.8 ^a	<i>L. amazonensis</i> As-R	(Lin et al. 2008) ^b
LmxM.23.0460	Trypanothione synthetase (TryS)	2.01–2.32 ^a	<i>L. amazonensis</i> Sb ^{III} 2700.2; Sb ^{III} 2700.3	(do Monte-Neto et al. 2011)
LmxM.27.1870		2.07 ^a		
LmjF.27.1870		1.7–2.06		
LmjF.15.1120	Tryparedoxin peroxidase (TryP)	2.5 ^a	<i>L. tarentolae</i> TarII Sb1.1	(Wyllie et al. 2008)
LinJ.15.1120		8.9 ^a		
LbrM.15.1100		ND	<i>L. donovani</i> Field isolates	(Wyllie et al. 2010)
LmxM.33.0070	Ascorbate-dependent peroxidase (APX)	5 ^a	<i>L. braziliensis</i> M2904 LbSbR	(Matrangolo et al. 2013)
LmjF.34.0070		2.38–2.51 ^a	<i>L. amazonensis</i> Sb ^{III} 2700.2; Sb ^{III} 2700.3	(do Monte-Neto et al. 2011)
LmjF.34.0080	Glucose-6-phosphate 1-dehydrogenase (G6PDH)	Functional (upregulated)	<i>L. major</i> Friedlin Sb ^{III} 1000.3; .4 and .5	(Mukherjee et al. 2013)
LinJ.34.0080		1.4 ^a	<i>L. donovani</i> GE1-R	(Biyani et al. 2011)
Stress-related protein-encoding genes				
LtaP.01.0620	Heat-shock protein 70 (HSP70)	Functional (upregulated)	<i>L. tarentolae</i> TarII As50.1; <i>L. infantum</i> Sb ^{III} 4000.3	(Brochu et al. 2004)
LinJ.28.3060		4.4 ^a	<i>L. donovani</i> Field isolates	(Vergnes et al. 2007)
		2 ^a		(Kumar et al. 2012)
		5.25 ^a		(Biyani et al. 2011)
		3 ^a	<i>L. infantum</i> (syn. <i>L. chagasi</i>) PP75 LcSbR	(Matrangolo et al. 2013)
LbrM.26.1260		2.8 ^a	<i>L. panamensis</i>	(Walker et al. 2012)
LinJ.35.3900	T-complex protein 1, η subunit, putative	1.2 ^a	<i>L. donovani</i> GE1-R	(Biyani et al. 2011)
LmxM.13.1660	Chaperonin TCP20, putative	2.28 ^a	<i>L. amazonensis</i> Sb ^{III} 2700.2	(do Monte-Neto et al. 2011)
LinJ.33.0350	Heat shock protein 83–1 (HSP83)	3.56–4.4	<i>L. donovani</i> Field isolates	(Vergnes et al. 2007)
		~2 ^a		(Kumar et al. 2012)
		2.4 ^a	<i>L. infantum</i> (syn. <i>L. chagasi</i>) PP75 LcSbR	(Matrangolo et al. 2013)
LbrM.33.0340		2.6 ^a	<i>L. braziliensis</i> M2904 LbSbR	
Others (Nucleosome assembly GO: 0006334; Proteolysis GO: 0006508; Protein phosphorylation GO: 0006468)				
LinJ.34.0570	Leucine-rich repeat protein, putative	Functional (upregulated)	<i>L. infantum</i>	(Genest et al. 2008)
LmxM.10.0470	Leishmaniolysin GP63	3.52 ^a	<i>L. amazonensis</i> As-R	(Lin et al. 2008) ^b
LmxM.30.2000	GP63-like protein	3.09 ^a	<i>L. amazonensis</i> Sb ^{III} 2700.2	(do Monte-Neto et al. 2011)
LinJ.36.6760	Mitogen-activated protein kinase (MAPK1)	2.14 ^a	<i>L. amazonensis</i> Sb ^{III} 2700.3	
LmjF.36.6470		~11 ^a	<i>L. donovani</i> Field isolates	(Kumar et al. 2012)
LmxM.33.3240	Histone H1-like protein	~–4 ^a	<i>L. tropica</i> Field isolates	(Kazemi-Rad et al. 2013)
LinJ.27.1120	Histone H1	1.99–2.09 ^a	<i>L. amazonensis</i> Sb ^{III} 2700.2; Sb ^{III} 2700.3	(do Monte-Neto et al. 2011)
LinJ.21.1160	Histone H2A	~11 ^a	<i>L. donovani</i> Field isolates	(Kumar et al. 2012)
LmxM.28.0210	Histone H2B	3 ^a		(Singh et al. 2010)
		2.10 ^a	<i>L. amazonensis</i> Sb ^{III} 2700.2	(do Monte-Neto et al. 2011)

GO Gene ontology; ND not determined; Functional study based on functional analysis; Sb^V-R pentavalent antimony-resistant mutants; SbR laboratory-selected antimony-resistant mutant; M2904 *L. braziliensis* cell line MHOM/BR/75/M2904; PP75 *L. infantum* (syn. *L. chagasi*) cell line MHOM/BR/74/PP75

^a Values obtained by proteome/transcriptome profiling

^b Lin et al., 2008 performed the assays using the Sb-related metalloid Arsenite to select (As)-resistant *L. amazonensis* mutants (Lin et al. 2008)

Importantly, several proteins are involved directly in the transport of Sb(III), as MRPA, LABC14, and AQP1. On the other hand, most antimony-resistance associated genes are involved in metabolic process related to oxidative stress, cell redox homeostasis, and thiol biosynthesis (Table 1), which is not surprising considering the mode of action of antimonial drugs.

Intrinsic drug resistance mechanisms involving the transport of nutrients through the plasma membrane have been reported in Sb-resistant *Leishmania*. For example, the glucose transporter-encoding gene was found to be downregulated in Sb-resistant *L. amazonensis* (do Monte-Neto et al. 2011; Machuca et al. 2006), suggesting a decreased formation of

reactive oxygen species as a result of reduce glucose uptake, helping the parasite to survive under the oxidative environment triggered by the drug (Machuca et al. 2006). Similarly, the nucleobase transporter (NT4) encoding gene was found to be highly downregulated in *L. amazonensis* Sb-resistant mutants, although functional analysis did not confirm the role of this transporter in Sb resistance (do Monte-Neto et al. 2011).

A change frequently observed in resistant leishmania parasites is overexpression of rate-limiting enzymes of thiol biosynthesis, such as ornithine decarboxylase (ODC) and γ -glutamylcysteine synthetase (γ -GCS), that causes an overproduction of intracellular thiols, the decrease of free intracellular Sb(III) concentration, and extra protection against the oxidative stress upon drug exposure (Grondin et al. 1997; Haimeur et al. 1999; Mukherjee et al. 2007; Mandal et al. 2007). The higher rate of thiol synthesis may also result in enhanced rates of efflux or sequestration of Sb-thiol conjugates through ABC transporters, contributing to the resistance phenotype (Mukhopadhyay et al. 1996; Legare et al. 2001). In a recent metabolomic study, Berg et al. (2013) identified increased levels of metabolites of cysteine transsulfuration pathway in Sb(V)-resistant field isolates of *L. donovani*, indicating increased thiol production (Berg et al. 2013). In the same perspective, metabolites linked to the pentose phosphate pathway and NADPH production were found to be more abundant in Sb-resistant parasites, suggesting that anti-oxidant processes may act against drug-induced oxidative stress (Berg et al. 2013). Interestingly, Sb(V)-resistant *L. donovani* field isolates, when maintained in vitro under Sb(III) pressure, showed similar changes, as those observed at a basal level before drug addition when compared to sensitive parasites, but to a higher level in the presence of drug (Berg et al. 2013).

ABC transporters belonging to the subfamilies ABCB, ABCC, and ABCG have been associated with drug resistance in *Leishmania* (Perez-Victoria et al. 2001; Haimeur et al. 2000; Coelho et al. 2003; Castanys-Munoz et al. 2007; Castanys-Munoz et al. 2008). Antimony resistance is often associated with upregulation of the ABCC transporter MRPA, which mediates Sb(III) detoxification, presumably by transporting the TS_2Sb (or $\text{Sb}(\text{GS})_3$) complexes into intracellular vesicles (Legare et al. 2001; Haimeur et al. 2000).

More recently, a member of ABC half-transporters, LABCI4, was shown to confer 2-fold resistance to Sb(III), Sb(V), As(III), and Cd(II) when overexpressed in *L. major* (Manzano et al. 2013). Unlike ABCC transporters, LABCI4 is located in both mitochondrial and plasma membranes of the parasite, and it seem to form dimers in order to perform extrusion of metal-thiol conjugates (Manzano et al. 2013). This transporter may be the missing piece of the puzzle that

would explain the metal efflux through a thiol-X-pump in *Leishmania*. However, a mechanism conferring only 2-fold resistance cannot provide enough support in high resistance indexes presented by laboratory-selected Sb-resistant mutants.

As discussed above (part 2.2), Sb(III) uptake is a determinant step for the downstream drug metabolism, since this is the major known route of antimony entry into *Leishmania* (Gourbal et al. 2004). When transfected into promastigote forms, the gene *LmAQP1* was able to confer hypersensitivity to Sb(III) and As(III) in different *Leishmania* species (Gourbal et al. 2004). Similarly, the levels of intracellular Sb can be restored in Sb(III)-resistant *L. mexicana* overexpressing AQP1 (Marquis et al. 2005). A recent study has demonstrated a telomeric deletion on chromosome 31, including the *AQP1* gene, in three independent Sb(III)-resistant *L. major* mutants. The loss of *AQP1* correlates with the resistance profiling, and the mutants were still highly resistant even after 30 passages without drug pressure (Mukherjee et al. 2013). We have recently found a similar telomeric deletion accompanied by loss of AQP1 in three out of four independent Sb(III)-resistant *L. guyanensis* mutants. Surprisingly, gene sequencing analysis revealed a point mutation in *AQP1*, from the mutant in which chromosome 31 is intact, leading to less Sb accumulation which contributes to the Sb resistance profile (Monte-Neto, Frézard and Ouellette, in preparation). Recently, Mandal et al. (2012) have shown that the mitogen-activated protein kinase (LmjMPK2) modulates LmjAQP1 function and that there is a localization switch of AQP1 (from the flagellar membrane to the entire surface of parasite) upon phosphorylation. Additionally, a deletion of *MPK2* in *L. mexicana* leads to reduction in Sb(III) uptake when compared to wild-type parasites (Mandal et al. 2012). On the other hand, *MPK1* (encoded by gene *LinJ.36.6760*) was found to be upregulated in nine out of ten Indian Sb-resistant *L. donovani* field isolates (Kumar et al. 2012).

Despite the strong correlation between AQP1 downregulation and antimony resistance observed in laboratory-selected mutants, data from a study performed using Sb-resistant *L. donovani* field isolates from India revealed an overexpression of AQP1 and reduced Sb uptake, suggesting that AQP1 may not be involved in the resistance of these clinical isolates (Maharjan et al. 2008). Furthermore, the expression level of genes classically associated with Sb resistance, such as *MRPA*, *ODC*, and *AQP1*, was not altered in promastigotes of Sb-resistant *L. donovani* field isolates from Nepal, suggesting influence of their genetic background (Decuypere et al. 2012). On the other hand, Sb-resistant *L. donovani* field isolates presented overexpression of *MRPA* and *ODC* and downregulation of *AQP1*, consistent with in vitro-selected parasites profiling (Rai et al. 2013).

An attempt to identify genetic biomarkers for antimonial resistance phenotype in field isolates of Sb-resistant

L. donovani mutants led Kumar and co-workers to conclude that there is no specific marker but multiple genes responsible for clinical resistance resulting in a “mosaic of expression pattern” (Kumar et al. 2012). The authors recommend expression analysis of multiple genes to distinguish between Sb-sensitive and -resistant isolates before treatment.

Different models for transport of Sb in resistant *Leishmania* cells

As illustrated in Fig. 2, wild-type leishmania cells exhibit passive Sb(III) influx through AQP1 and actively extrude Sb-thiol conjugates via a plasma membrane pump.

The actual knowledge coming from functional and molecular studies indicates at least three possible transport models for Sb-resistant cells, which may also be combined in some mutants.

According to the “**influx**” model (Fig. 2), the efficiency of passive transport is reduced, for instance because of lower expression, deletion or mutation of AQP1. Due to the existence of active extrusion, such a change in the route of Sb(III), entry will result in a reduced intracellular accumulation of Sb. In this model, the intracellular to extracellular concentration ratio (C_i/C_e) should be equal to $k/(k+K_e')$, where k is the rate constant of passive transport and K_e' is the rate constant of

active efflux. Therefore, the condition for the effective reduction of drug accumulation is: $K_e' > 0$.

According to the “**efflux**” model (Fig. 2), the mutant either overexpresses the efflux pump present in the wild-type cell or expresses another additional efflux pump, so that the rate constant of active efflux (K_e+K_e') becomes greater than the rate constant of influx (k). In this model, the intracellular-to-extracellular concentration ratio (C_i/C_e) is equal to $k/(k+K_e+K_e')$, where K_e is the rate constant of active efflux by the additional transporter.

According to the “**sequestration**” model (Fig. 2), sequestration of Sb(III) occurs through MRPA into intracellular vesicles and a secretion route is involved to explain the reduced intracellular accumulation. In this model, one expects the transport rate constant by MRPA (K_e) to be greater than the rate constant of passive transport (k) and the rate constant of active efflux (K_e'). This implies that the rate-limiting step for Sb(III) entry into cells is the transport by AQP1, that intracellular Sb(III) is mainly located within intracellular vesicles, and that the level of Sb accumulation at steady state depends essentially on the influx-to-secretion rate constant ratio (k/K_{se}). Moreover, secretion should be the rate-limiting step of the efflux kinetics. In an influx experiment, a pseudo-steady state is expected to be reached rapidly, characterized by constant C_i , but the time-dependent increase of total intracellular Sb. In this condition, the rate of Sb(III) uptake could be influenced by MRPA.

Fig. 2 Possible models of drug transport in antimony-sensitive and -resistant leishmania parasites. AQP1, aquaglyceroporin 1; MRPA, multi-drug resistance-associated protein A; ATe, efflux pump; C_e , extracellular concentration of Sb; C_i , intracellular concentration of Sb; C_{si} , vacuolar

concentration of Sb; k , rate constant for passive transport; K_e , transport rate constant by MRPA or ATe; K_e' , rate constant of active efflux; K_{se} , rate constant for secretion

5. Biophysical and pharmacological approaches for elucidation of Sb transport mechanisms

Both biophysical and pharmacological approaches are useful to characterize functionally drug-transport mechanisms in a given cell. Typical biophysical approaches include uptake, influx and efflux studies, and the evaluation of the energy-dependence of the transport using metabolic inhibitor and temperature effects. Pharmacological approaches make use of competitive or non-competitive transporter inhibitor(s). In this regard, classical MRP inhibitors can be useful tools. The effect of endocytic pathways inhibitors could also be investigated. In the specific case of Sb(III) transport, the involvement of thiols in its active transport implies that inhibitors of thiol biosynthesis, such as buthionine sulfoxide (BSO), may also be helpful in the identification of the transport model. Table 2 summarizes expected biophysical and pharmacological profiles of each transport model.

According to these profiles, the systematic use of biophysical and pharmacological approaches should allow the elucidation of the precise Sb transport mechanisms.

Concluding remarks

In the present review, we covered the actual knowledge on the functional and molecular changes associated with the transport of Sb(III) in resistant leishmania cells. Thus, the scope of

this review is the intrinsic resistance of the parasite to Sb(III) in the context of the well-accepted prodrug model of pentavalent antimonials, although other mechanisms of action and drug resistance have also been suggested. Thus, Sb(V) may be active by itself, for instance through immune-modulation within the macrophage. The parasite may also modulate the uptake of Sb(III) by the host cell.

Most of the actual knowledge comes from studies with axenically-cultured promastigotes selected for resistance to Sb(III), which were described extensively in this review. Importantly, this experimental model has been validated, through demonstration that these mutants were cross-resistant to Sb(V) when presented as an intracellular amastigote form. Extrapolation of the results obtained from in vitro-selected mutants to Sb-resistant clinical isolates is another critical issue. Even though some reports have presented conflicting informations about Sb resistance mechanisms between clinical isolates and in vitro-selected strains, recent studies have demonstrated an overlap of mechanisms, especially when comparison is made on equivalent resistance index.

Because of its multifactorial feature, antimony resistance in *Leishmania* remains a complex and challenging issue that still requires extensive investigation. It is now well established that antimony transport pathways contribute to the resistance phenotype; however, functional and molecular studies are still needed to validate the different transport models proposed in this review.

Accordingly, several gaps in knowledge can be identified, towards which future research may be directed: (1) systematic biophysical and pharmacological characterization of the transport pathways in resistant strains; (2) molecular characterization of the efflux pump(s) in the plasma membrane of Sb-sensitive and -resistant strains; (3) functional and molecular characterization of the secretion route of Sb(III) in the sequestration transport model; and (4) functional and molecular characterization of the transport mechanism of Sb(V) into macrophages and amastigotes.

Ultimately, all these efforts should allow not only the elucidation of the mechanisms of antimony resistance in leishmania parasites but also the design of pharmacological strategies to overcome resistance and the identification of biomarkers to predict the treatment outcomes in clinical isolates.

Table 2 Biophysical and pharmacological characteristics^a of the different models of transport of Sb(III) in *Leishmania* resistant strains

	Influx Model	Efflux Model	Sequestration Model
Influx rate^b			
Reduction compared to WT ?	+	-	- or +
Sensitive to metabolic inhibitor ?	-	-	- or+(↓)
Sensitive to temperature decrease ?	-	-	- or+(↓)
Sensitive to MRP inhibitor ?	-	-	- or+(↓)
Sensitive to inhibitor of thiol biosynthesis ?	+(↓)	+(↓)	- or+(↓)
Efflux rate			
Increased compared to WT ?	-	+	+
Sensitive to metabolic inhibitor ?	+(↓)	+(↓)	+(↓)
Sensitive to MRP inhibitor ?	-	+(↓)	-
Sensitive to endocytic inhibitor ?	-	-	+(↓)
Sensitive to inhibitor of thiol biosynthesis ?	+(↓)	+(↓)	-

^a“-” means “no”; “+” means “yes” with either a decrease (↓) or an increase (↑)

^b“-” when true influx rate is accessible (initial condition of uptake); “+” when influx rate is determined in condition of pseudo-steady state

Acknowledgments We acknowledge the Brazilian agencies, “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (303046/2009 and studentship), “Fundação de Amparo à Pesquisa do Estado de Minas Gerais” (PPM-00382-11; REDE 40/11; CBB-APQ-01123-09) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (2447/2009 and studentship) for financial support. R.M.N. is supported by a Government of Canada DFAIT post-doctoral research fellowship. F.F. is recipient of a research fellowship from CNPq. The authors declare that they have no conflict of interest.

Conflict of Interest The authors declare that they have no conflict of Interest.

References

- Adaui V, Schnorbusch K, Zimic M, Gutierrez A, Decuypere S, Vanaerschot M, De Doncker S, Maes I, Llanos-Cuentas A, Chappuis F, Arevalo J, Dujardin JC (2011) Comparison of gene expression patterns among *Leishmania braziliensis* clinical isolates showing a different *in vitro* susceptibility to pentavalent antimony. *Parasitology* 138(2):183–193. doi:10.1017/S0031182010001095
- Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, Team WHOLC (2012) Leishmaniasis worldwide and global estimates of its incidence. *PLoS ONE* 7(5):e35671. doi:10.1371/journal.pone.0035671
- Anacleto C, Abdo MC, Ferreira AV, Murta SM, Romanha AJ, Fernandes AP, Moreira ES (2003) Structural and functional analysis of an amplification containing a PGPA gene in a glucantime-resistant *Leishmania (Viannia) guyanensis* cell line. *Parasitol Res* 90(2):110–118. doi:10.1007/s00436-002-0798-x
- Berg M, Vanaerschot M, Jankevics A, Cuypers B, Maes I, Mukherjee S, Khanal B, Rijal S, Roy S, Oppendoes F, Breitling R, Dujardin JC (2013) Metabolic adaptations of *Leishmania donovani* in relation to differentiation, drug resistance, and drug pressure. *Mol Microbiol*. doi:10.1111/mmi.12374
- Berman JD (1997) Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. *Clin Infect Dis* 24(4):684–703. doi:10.1093/clind/24.4.684
- Beverley SM (1991) Gene amplification in *Leishmania*. *Annu Rev Microbiol* 45:417–444. doi:10.1146/annurev.mi.45.100191.002221
- Biyani N, Singh AK, Mandal S, Chawla B, Madhubala R (2011) Differential expression of proteins in antimony-susceptible and -resistant isolates of *Leishmania donovani*. *Mol Biochem Parasitol* 179(2):91–99. doi:10.1016/j.molbiopara.2011.06.004
- Brochu C, Haimeur A, Ouellette M (2004) The heat shock protein HSP70 and heat shock cognate protein HSC70 contribute to antimony tolerance in the protozoan parasite leishmania. *Cell Stress Chaperones* 9(3):294–303
- Brochu C, Wang J, Roy G, Messier N, Wang XY, Saravia NG, Ouellette M (2003) Antimony uptake systems in the protozoan parasite leishmania and accumulation differences in antimony-resistant parasites. *Antimicrob Agents Chemother* 47(10):3073–3079
- Callahan HL, Roberts WL, Rainey PM, Beverley SM (1994) The PGPA gene of *Leishmania major* mediates antimony (SbIII) resistance by decreasing influx and not by increasing efflux. *Mol Biochem Parasitol* 68(1):145–149. doi:10.1016/0166-6851(94)00154-5
- Carrio J, de Colmenares M, Riera C, Gallego M, Arboix M, Portus M (2000) *Leishmania infantum*: stage-specific activity of pentavalent antimony related with the assay conditions. *Exp Parasitol* 95(3):209–214. doi:10.1006/expr.2000.4537
- Castanys-Munoz E, Alder-Baerens N, Pomorski T, Gamarro F, Castanys S (2007) A novel ATP-binding cassette transporter from *Leishmania* is involved in transport of phosphatidylcholine analogues and resistance to alkyl-phospholipids. *Mol Microbiol* 64(5):1141–1153. doi:10.1111/j.1365-2958.2007.05653.x
- Castanys-Munoz E, Perez-Victoria JM, Gamarro F, Castanys S (2008) Characterization of an ABCG-like transporter from the protozoan parasite leishmania with a role in drug resistance and transbilayer lipid movement. *Antimicrob Agents Chemother* 52(10):3573–3579. doi:10.1128/AAC.00587-08
- Clayton C, Shapira M (2007) Post-transcriptional regulation of gene expression in trypanosomes and leishmanias. *Mol Biochem Parasitol* 156(2):93–101. doi:10.1016/j.molbiopara.2007.07.007
- Coelho AC, Beverley SM, Cotrim PC (2003) Functional genetic identification of PRP1, an ABC transporter superfamily member conferring pentamidine resistance in *Leishmania major*. *Mol Biochem Parasitol* 130(2):83–90. doi:10.1016/S0166-6851(03)00162-2
- Croft SL, Sundar S, Fairlamb AH (2006) Drug resistance in leishmaniasis. *Clin Microbiol Rev* 19(1):111–126. doi:10.1128/CMR.19.1.111-126.2006
- Das M, Saudagar P, Sundar S, Dubey VK (2013) Miltefosine-unresponsive *Leishmania donovani* has a greater ability than miltefosine-responsive *L. donovani* to resist reactive oxygen species. *FEBS J* 280(19):4807–4815. doi:10.1111/febs.12449
- Decuypere S, Rijal S, Yardley V, De Doncker S, Laurent T, Khanal B, Chappuis F, Dujardin JC (2005) Gene expression analysis of the mechanism of natural Sb(V) resistance in *Leishmania donovani* isolates from Nepal. *Antimicrob Agents Chemother* 49(11):4616–4621. doi:10.1128/AAC.49.11.4616-4621.2005
- Decuypere S, Vanaerschot M, Bruner K, Imamura H, Muller S, Khanal B, Rijal S, Dujardin JC, Coombs GH (2012) Molecular mechanisms of drug resistance in natural *Leishmania* populations vary with genetic background. *PLoS Negl Trop Dis* 6(2):e1514. doi:10.1371/journal.pntd.0001514
- Demicheli C, Frezard F, Mangrum JB, Farrell NP (2008) Interaction of trivalent antimony with a CCHC zinc finger domain: potential relevance to the mechanism of action of antimonial drugs. *Chem Commun (Camb)* 39:4828–4830. doi:10.1039/b809186b
- Denton H, McGregor JC, Coombs GH (2004) Reduction of anti-leishmanial pentavalent antimonial drugs by a parasite-specific thiol-dependent reductase, TDR1. *Biochem J* 381(Pt 2):405–412. doi:10.1042/BJ20040283
- Dey S, Papadopolou B, Haimeur A, Roy G, Grondin K, Dou D, Rosen BP, Ouellette M (1994) High level arsenite resistance in *Leishmania tarentolae* is mediated by an active extrusion system. *Mol Biochem Parasitol* 67(1):49–57. doi:10.1016/0166-6851(94)90095-7
- Dhillon GP, Sharma SN, Nair B (2008) Kala-azar elimination programme in India. *J Indian Med Assoc* 106 (10):664, 666–668
- do Monte-Neto RL, Coelho AC, Raymond F, Legare D, Corbeil J, Melo MN, Frezard F, Ouellette M (2011) Gene expression profiling and molecular characterization of antimony resistance in *Leishmania amazonensis*. *PLoS Negl Trop Dis* 5(5):e1167. doi:10.1371/journal.pntd.0001167
- Downing T, Imamura H, Decuypere S, Clark TG, Coombs GH, Cottom JA, Hilley JD, Sd D, Maes I, Mottram JC, Quail MA, Rijal S, Sanders M, Schönian G, Stark O, Sundar S, Vanaerschot M, Hertz-Fowler C, Dujardin JC, Berriman M (2011) Whole genome sequencing of multiple *Leishmania donovani* clinical isolates provides insights into population structure and mechanisms of drug resistance. *Genome Res* 21(12):13. doi:10.1101/gr.123430.111
- El Fadili K, Messier N, Leprohon P, Roy G, Guimond C, Trudel N, Saravia NG, Papadopolou B, Legare D, Ouellette M (2005) Role of the ABC transporter MRPA (PGPA) in antimony resistance in *Leishmania infantum* axenic and intracellular amastigotes. *Antimicrob Agents Chemother* 49(5):1988–1993. doi:10.1128/AAC.49.5.1988-1993.2005
- Ephros M, Bitnun A, Shaked P, Waldman E, Zilberstein D (1999) Stage-specific activity of pentavalent antimony against *Leishmania donovani* axenic amastigotes. *Antimicrob Agents Chemother* 43(2):278–282
- Essodaigui M, Frezard F, Moreira ES, Dagger F, Garnier-Suillerot A (1999) Energy-dependent efflux from leishmania promastigotes of substrates of the mammalian multidrug resistance pumps. *Mol Biochem Parasitol* 100(1):73–84. doi:10.1016/S0166-6851(99)00036-5
- Fairlamb AH, Cerami A (1992) Metabolism and functions of trypanothione in the Kinetoplastida. *Annu Rev Microbiol* 46:695–729. doi:10.1146/annurev.mi.46.100192.003403
- Ferreira CS, Martins PS, Demicheli C, Brochu C, Ouellette M, Frezard F (2003) Thiol-induced reduction of antimony(V) into antimony(III): a comparative study with trypanothione, cysteinyl-glycine, cysteine and glutathione. *Biomaterials* 16(3):441–446

- Frezard F, Demicheli C, Ferreira CS, Costa MA (2001) Glutathione-induced conversion of pentavalent antimony to trivalent antimony in meglumine antimoniate. *Antimicrob Agents Chemother* 45(3): 913–916. doi:10.1128/AAC.45.3.913-916.2001
- Frezard F, Demicheli C, Ribeiro RR (2009) Pentavalent antimonials: new perspectives for old drugs. *Molecules* 14(7):2317–2336. doi:10.3390/molecules14072317
- Frezard F, Silva H, Pimenta AM, Farrell N, Demicheli C (2012) Greater binding affinity of trivalent antimony to a CCCH zinc finger domain compared to a CCHC domain of kinetoplastid proteins. *Metallomics* 4(5):433–440. doi:10.1039/c2mt00176d
- Garcia-Hernandez R, Manzano JI, Castanys S, Gamarro F (2012) *Leishmania donovani* develops resistance to drug combinations. *PLoS Negl Trop Dis* 6(12):e1974. doi:10.1371/journal.pntd.0001974
- Genest PA, Haimeur A, Legare D, Sereno D, Roy G, Messier N, Papadopoulou B, Ouellette M (2008) A protein of the leucine-rich repeats (LRRs) superfamily is implicated in antimony resistance in *Leishmania infantum* amastigotes. *Mol Biochem Parasitol* 158(1): 95–99. doi:10.1016/j.molbiopara.2007.11.008
- Ghosh J, Bose M, Roy S, Bhattacharyya SN (2013) *Leishmania donovani* targets Dicer1 to downregulate miR-122, lower serum cholesterol, and facilitate murine liver infection. *Cell Host Microbe* 13(3):277–288. doi:10.1016/j.chom.2013.02.005
- Glaser TA, Baatz JE, Kreishman GP, Mukkada AJ (1988) pH homeostasis in *Leishmania donovani* amastigotes and promastigotes. *Proc Natl Acad Sci U S A* 85(20):7602–7606
- Goodwin LG, Page JE (1943) A study of the excretion of organic antimonials using a polarographic procedure. *Biochem J* 37(2):198–209
- Gourbal B, Sonuc N, Bhattacharjee H, Legare D, Sundar S, Ouellette M, Rosen BP, Mukhopadhyay R (2004) Drug uptake and modulation of drug resistance in *Leishmania* by an aquaglyceroporin. *J Biol Chem* 279(30):31010–31017. doi:10.1074/jbc.M403959200
- Grondin K, Haimeur A, Mukhopadhyay R, Rosen BP, Ouellette M (1997) Co-amplification of the gamma-glutamylcysteine synthetase gene *gsh1* and of the ABC transporter gene *pgpA* in arsenite-resistant *Leishmania tarentolae*. *EMBO J* 16(11):3057–3065. doi:10.1093/emboj/16.11.3057
- Grondin K, Roy G, Ouellette M (1996) Formation of extrachromosomal circular amplicons with direct or inverted duplications in drug-resistant *Leishmania tarentolae*. *Mol Cell Biol* 16(7):3587–3595
- Guerin PJ, Oliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P, Wasunna MK, Bryceson AD (2002) Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. *Lancet Infect Dis* 2(8):494–501. doi:10.1016/S1473-3099(02)00347-X
- Guimond C, Trudel N, Brochu C, Marquis N, Fadili AE, Peytavi R, Briand G, Richard D, Messier N, Papadopoulou B, Corbeil J, Bergeron MG, Legare D, Ouellette M (2003) Modulation of gene expression in *Leishmania* drug resistant mutants as determined by targeted DNA microarrays. *Nucleic Acids Res* 31(20):5886–5896. doi:10.1093/nar/gkg806
- Haimeur A, Brochu C, Genest P, Papadopoulou B, Ouellette M (2000) Amplification of the ABC transporter gene *PGPA* and increased trypanothione levels in potassium antimonyl tartrate (SbIII) resistant *Leishmania tarentolae*. *Mol Biochem Parasitol* 108(1):131–135. doi:10.1016/S0166-6851(00)00187-0
- Haimeur A, Guimond C, Pilote S, Mukhopadhyay R, Rosen BP, Poulin R, Ouellette M (1999) Elevated levels of polyamines and trypanothione resulting from overexpression of the ornithine decarboxylase gene in arsenite-resistant *Leishmania*. *Mol Microbiol* 34(4):726–735. doi:10.1046/j.1365-2958.1999.01634.x
- Hansen C, Hansen EW, Hansen HR, Gammelgaard B, Sturup S (2011) Reduction of Sb(V) in a human macrophage cell line measured by HPLC-ICP-MS. *Biol Trace Elem Res* 144(1–3):234–243. doi:10.1007/s12011-011-9079-9
- Hub JS, de Groot BL (2008) Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proc Natl Acad Sci U S A* 105(4):1198–1203. doi:10.1073/pnas.0707662104
- Kazemi-Rad E, Mohebbi M, Khadem-Erfan MB, Saffari M, Raoofian R, Hajjarian H, Hadighi R, Khamesipour A, Rezaie S, Abedkhozasteh H, Heidari M (2013) Identification of antimony resistance markers in *Leishmania tropica* field isolates through a cDNA-AFLP approach. *Exp Parasitol* 135(2):344–349. doi:10.1016/j.exppara.2013.07.018
- Kumar D, Singh R, Bhandari V, Kulshrestha A, Negi NS, Salotra P (2012) Biomarkers of antimony resistance: need for expression analysis of multiple genes to distinguish resistance phenotype in clinical isolates of *Leishmania donovani*. *Parasitol Res* 111(1):223–230. doi:10.1007/s00436-012-2823-z
- Lai WS, Kennington EA, Blackshear PJ (2002) Interactions of CCCH zinc finger proteins with mRNA: non-binding tristetraprolin mutants exert an inhibitory effect on degradation of AU-rich element-containing mRNAs. *J Biol Chem* 277(11):9606–9613. doi:10.1074/jbc.M110395200
- Legare D, Richard D, Mukhopadhyay R, Stierhof YD, Rosen BP, Haimeur A, Papadopoulou B, Ouellette M (2001) The *Leishmania* ATP-binding cassette protein PGPA is an intracellular metal-thiol transporter ATPase. *J Biol Chem* 276(28):26301–26307. doi:10.1074/jbc.M102351200
- Leprohon P, Legare D, Ouellette M (2009a) Intracellular localization of the ABCC proteins of *Leishmania* and their role in resistance to antimonials. *Antimicrob Agents Chemother* 53(6):2646–2649. doi:10.1128/AAC.01474-08
- Leprohon P, Legare D, Raymond F, Madore E, Hardiman G, Corbeil J, Ouellette M (2009b) Gene expression modulation is associated with gene amplification, supernumerary chromosomes and chromosome loss in antimony-resistant *Leishmania infantum*. *Nucleic Acids Res* 37(5):1387–1399. doi:10.1093/nar/gkn1069
- Lin YC, Hsu JY, Shu JH, Chi Y, Chiang SC, Lee ST (2008) Two distinct arsenite-resistant variants of *Leishmania amazonensis* take different routes to achieve resistance as revealed by comparative transcriptomics. *Mol Biochem Parasitol* 162(1):16–31. doi:10.1016/j.molbiopara.2008.06.015
- Liu Z, Shen J, Carbrey JM, Mukhopadhyay R, Agre P, Rosen BP (2002) Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9. *Proc Natl Acad Sci U S A* 99(9):6053–6058. doi:10.1073/pnas.092131899
- Lucumi A, Robledo S, Gama V, Saravia NG (1998) Sensitivity of *Leishmania (Viannia) panamensis* to pentavalent antimony is correlated with the formation of cleavable DNA-protein complexes. *Antimicrob Agents Chemother* 42(8):1990–1995
- Machuca C, Rodriguez A, Herrera M, Silva S, Ponte-Sucre A (2006) *Leishmania amazonensis*: Metabolic adaptations induced by resistance to an ABC transporter blocker. *Exp Parasitol* 114(1):1–9. doi:10.1016/j.exppara.2006.02.008
- Maharjan M, Singh S, Chatterjee M, Madhubala R (2008) Role of aquaglyceroporin (AQP1) gene and drug uptake in antimony-resistant clinical isolates of *Leishmania donovani*. *Am J Trop Med Hyg* 79(1):69–75
- Mandal G, Sharma M, Kruse M, Sander-Juelch C, Munro LA, Wang Y, Vilg JV, Tamas MJ, Bhattacharjee H, Wiese M, Mukhopadhyay R (2012) Modulation of *Leishmania major* aquaglyceroporin activity by a mitogen-activated protein kinase. *Mol Microbiol* 85(6):1204–1218. doi:10.1111/j.1365-2958.2012.08169.x
- Mandal G, Wyllie S, Singh N, Sundar S, Fairlamb AH, Chatterjee M (2007) Increased levels of thiols protect antimony unresponsive *Leishmania donovani* field isolates against reactive oxygen species generated by trivalent antimony. *Parasitology* 134(Pt 12):1679–1687. doi:10.1017/S0031182007003150
- Manzano JI, Garcia-Hernandez R, Castanys S, Gamarro F (2013) A new ABC half-transporter in *Leishmania* is involved in resistance to antimony. *Antimicrob Agents Chemother* 57(8):3719–3730. doi:10.1128/AAC.00211-13

- Marchini JF, Cruz AK, Beverley SM, Tosi LR (2003) The H region HTBF gene mediates terbinafine resistance in *Leishmania major*. *Mol Biochem Parasitol* 131(1):77–81. doi:10.1016/S0166-6851(03)00174-9
- Marquis N, Gourbal B, Rosen BP, Mukhopadhyay R, Ouellette M (2005) Modulation in aquaglyceroporin AQP1 gene transcript levels in drug-resistant *Leishmania*. *Mol Microbiol* 57(6):1690–1699. doi:10.1111/j.1365-2958.2005.04782.x
- Matlashewski G, Arana B, Kroeger A, Battacharya S, Sundar S, Das P, Sinha PK, Rijal S, Mondal D, Zilberstein D, Alvar J (2011) Visceral leishmaniasis: elimination with existing interventions. *Lancet Infect Dis* 11(4):322–325. doi:10.1016/S1473-3099(10)70320-0
- Matrangola FS, Liarte DB, Andrade LC, de Melo MF, Andrade JM, Ferreira RF, Santiago AS, Pirovani CP, Silva-Pereira RA, Murta SM (2013) Comparative proteomic analysis of antimony-resistant and -susceptible *Leishmania braziliensis* and *Leishmania infantum chagasi* lines. *Mol Biochem Parasitol* 190(2):63–75. doi:10.1016/j.molbiopara.2013.06.006
- Meheus F, Balasegaram M, Olliaro P, Sundar S, Rijal S, Faiz MA, Boelaert M (2010) Cost-effectiveness analysis of combination therapies for visceral leishmaniasis in the Indian subcontinent. *PLoS Negl Trop Dis* 4(9):e818. doi:10.1371/journal.pntd.0000818
- Messaritis I, Christodoulou V, Mazeris A, Koutala E, Vlahou A, Papadogiorgaki S, Antoniou M (2013) Drug resistance in natural isolates of *Leishmania donovani* s.l. promastigotes is dependent of Pgp170 expression. *PLoS ONE* 8(6):e65467
- Mittal MK, Rai S, Ashutosh R, Gupta S, Sundar S, Goyal N (2007) Characterization of natural antimony resistance in *Leishmania donovani* isolates. *Am J Trop Med Hyg* 76(4):681–688
- Mookerjee JB, Mookerjee A, Banerjee R, Saha M, Singh S, Naskar K, Tripathy G, Sinha PK, Pandey K, Sundar S, Bimal S, Das PK, Choudhuri SK, Roy S (2008) Inhibition of ABC transporters abolishes antimony resistance in *Leishmania* infection. *Antimicrob Agents Chemother* 52(3):1080–1093
- Moreira DS, Monte-Neto RL, Andrade JM, Santi AMM, Reis PG, Frezard F, Murta SMF (2013) Molecular characterization of the MRPA transporter and antimony uptake in four New World *Leishmania* spp. susceptible and resistant to antimony. *Int J Parasitol Drugs Drug Resist* 3:143–153. doi:10.1016/j.ijpddr.2013.08.001
- Mukherjee A, Boisvert S, Monte-Neto RL, Coelho AC, Raymond F, Mukhopadhyay R, Corbeil J, Ouellette M (2013) Telomeric gene deletion and intrachromosomal amplification in antimony-resistant *Leishmania*. *Mol Microbiol* 88(1):189–202. doi:10.1111/mmi.12178
- Mukherjee A, Padmanabhan PK, Singh S, Roy G, Girard I, Chatterjee M, Ouellette M, Madhubala R (2007) Role of ABC transporter MRPA, gamma-glutamylcysteine synthetase and ornithine decarboxylase in natural antimony-resistant isolates of *Leishmania donovani*. *J Antimicrob Chemother* 59(2):204–211. doi:10.1093/jac/dkl494
- Mukhopadhyay R, Dey S, Xu N, Gage D, Lightbody J, Ouellette M, Rosen BP (1996) Trypanothione overproduction and resistance to antimonials and arsenicals in *Leishmania*. *Proc Natl Acad Sci U S A* 93(19):10383–10387. doi:10.1073/pnas.1222579110
- Muniz-Junqueira MI, de Paula-Coelho VN (2008) Meglumine antimonate directly increases phagocytosis, superoxide anion and TNF-alpha production, but only via TNF-alpha it indirectly increases nitric oxide production by phagocytes of healthy individuals, in vitro. *Int Immunopharmacol* 8(12):1633–1638. doi:10.1016/j.intimp.2008.07.011
- Newby ZE, O'Connell J 3rd, Robles-Colmenares Y, Khademi S, Miercke LJ, Stroud RM (2008) Crystal structure of the aquaglyceroporin PfAQP from the malarial parasite *Plasmodium falciparum*. *Nat Struct Mol Biol* 15(6):619–625. doi:10.1038/nsmb.1431
- Ouellette M, Borst P (1991) Drug resistance and P-glycoprotein gene amplification in the protozoan parasite leishmania. *Res Microbiol* 142(6):737–746. doi:10.1016/0923-2508(91)90089-S
- Ouellette M, Drummelsmith J, Papadopoulou B (2004) Leishmaniasis: drugs in the clinic, resistance and new developments. *Drug Resist Updat* 7(4–5):257–266. doi:10.1016/j.drug.2004.07.002
- Papadopoulou B, Roy G, Dey S, Rosen BP, Ouellette M (1994) Contribution of the *Leishmania* P-glycoprotein-related gene ltpgpA to oxyanion resistance. *J Biol Chem* 269(16):11980–11986
- Pathak MK, Yi T (2001) Sodium stibogluconate is a potent inhibitor of protein tyrosine phosphatases and augments cytokine responses in hemopoietic cell lines. *J Immunol* 167(6):3391–3397
- Perez-Victoria JM, Perez-Victoria FJ, Parodi-Talice A, Jimenez IA, Ravelo AG, Castanys S, Gamarró F (2001) Alkyl-lysophospholipid resistance in multidrug-resistant *Leishmania tropica* and chemosensitization by a novel P-glycoprotein-like transporter modulator. *Antimicrob Agents Chemother* 45(9):2468–2474. doi:10.1128/AAC.45.9.2468-2474.2001
- Perry MR, Wyllie S, Prajapati VK, Feldmann J, Sundar S, Boelaert M, Fairlamb AH (2011) Visceral leishmaniasis and arsenic: an ancient poison contributing to antimonial treatment failure in the Indian subcontinent? *PLoS Negl Trop Dis* 5(9):e1227. doi:10.1371/journal.pntd.0001227
- Phongphanphane S, Yoshida N, Hirata F (2010) Molecular selectivity in aquaporin channels studied by the 3D-RISM theory. *J Phys Chem B* 114(23):7967–7973. doi:10.1021/jp101936y
- Purkait B, Kumar A, Nandi N, Sardar AH, Das S, Kumar S, Pandey K, Ravidas V, Kumar M, De T, Singh D, Das P (2012) Mechanism of amphotericin B resistance in clinical isolates of *Leishmania donovani*. *Antimicrob Agents Chemother* 56(2):1031–1041. doi:10.1128/AAC.00030-11
- Rai S, Bhaskar GSK, Nath Dwivedi U, Sundar S, Goyal N (2013) Role of efflux pumps and intracellular thiols in natural antimony resistant isolates of *Leishmania donovani*. *PLoS ONE* 8(9):e74862. doi:10.1371/journal.pone.0074862
- Salaun P, Frezard F (2013) Unexpectedly high levels of antimony (III) in the pentavalent antimonial drug Glucantime: insights from a new voltammetric approach. *Anal Bioanal Chem* 405(15):5201–5214. doi:10.1007/s00216-013-6947-5
- Sereno D, Cavaleyra M, Zemzoumi K, Maquaire S, Ouassiss A, Lemesre JL (1998) Axenically grown amastigotes of *Leishmania infantum* used as an in vitro model to investigate the pentavalent antimony mode of action. *Antimicrob Agents Chemother* 42(12):3097–3102
- Shaked-Mishan P, Ulrich N, Ephros M, Zilberstein D (2001) Novel Intracellular SbV reducing activity correlates with antimony susceptibility in *Leishmania donovani*. *J Biol Chem* 276(6):3971–3976. doi:10.1074/jbc.M005423200
- Sharom FJ (2008) ABC multidrug transporters: structure, function and role in chemoresistance. *Pharmacogenomics* 9(1):105–127. doi:10.2217/14622416.9.1.105
- Silverman JM, Clos J, de'Oliveira CC, Shirvani O, Fang Y, Wang C, Foster LJ, Reiner NE (2010) An exosome-based secretion pathway is responsible for protein export from *Leishmania* and communication with macrophages. *J Cell Sci* 123(Pt 6):842–852. doi:10.1242/jcs.056465
- Singh R, Kumar D, Duncan RC, Nakhasi HL, Salotra P (2010) Overexpression of histone H2A modulates drug susceptibility in leishmania parasites. *Int J Antimicrob Agents* 36(1):50–57. doi:10.1016/j.ijantimicag.2010.03.012
- Sun H, Yan SC, Cheng WS (2000) Interaction of antimony tartrate with the tripeptide glutathione implication for its mode of action. *Eur J Biochem* 267(17):5450–5457. doi:10.1046/j.1432-1327.2000.01605.x
- Sundar S, Rai M, Chakravarty J, Agarwal D, Agrawal N, Vaillant M, Olliaro P, Murray HW (2008) New treatment approach in Indian visceral leishmaniasis: single-dose liposomal amphotericin B followed by short-course oral miltefosine. *Clin Infect Dis* 47(8):1000–1006. doi:10.1086/591972
- Sundar S, Singh A, Rai M, Prajapati VK, Singh AK, Ostyn B, Boelaert M, Dujardin JC, Chakravarty J (2012) Efficacy of

- miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. *Clin Infect Dis* 55(4):543–550. doi:[10.1093/cid/cis474](https://doi.org/10.1093/cid/cis474)
- Sundar S, Sinha PK, Verma DK, Kumar N, Alam S, Pandey K, Kumari P, Ravidas V, Chakravarty J, Verma N, Berman J, Ghalib H, Arana B (2011) Ambisome plus miltefosine for Indian patients with kala-azar. *Trans R Soc Trop Med Hyg* 105(2):115–117. doi:[10.1016/j.trstmh.2010.10.008](https://doi.org/10.1016/j.trstmh.2010.10.008)
- Torres DC, Aducci V, Ribeiro-Alves M, Romero GA, Arevalo J, Cupolillo E, Dujardin JC (2010) Targeted gene expression profiling in *Leishmania braziliensis* and *Leishmania guyanensis* parasites isolated from Brazilian patients with different antimonial treatment outcomes. *Infect Genet Evol* 10(6):727–733. doi:[10.1016/j.meegid.2010.05.006](https://doi.org/10.1016/j.meegid.2010.05.006)
- Ubeda JM, Legare D, Raymond F, Ouameur AA, Boisvert S, Rigault P, Corbeil J, Tremblay MJ, Olivier M, Papadopolou B, Ouellette M (2008) Modulation of gene expression in drug resistant *Leishmania* is associated with gene amplification, gene deletion and chromosome aneuploidy. *Genome Biol* 9(7):R115. doi:[10.1186/gb-2008-9-7-r115](https://doi.org/10.1186/gb-2008-9-7-r115)
- Vergnes B, Gourbal B, Girard I, Sundar S, Drummelsmith J, Ouellette M (2007) A proteomics screen implicates HSP83 and a small kinetoplastid calpain-related protein in drug resistance in *Leishmania donovani* clinical field isolates by modulating drug-induced programmed cell death. *Mol Cell Proteomics* 6(1):88–101. doi:[10.1074/mcp.M600319-MCP200](https://doi.org/10.1074/mcp.M600319-MCP200)
- Vianna G (1912) Tratamento da Leishmaniose tegumentar por injeções intravenosas de tartaro emético In: 7 Congresso Brasileiro de Medicina Tropical de São Paulo, São Paulo, Brazil, pp 426–428
- Walker J, Gongora R, Vasquez JJ, Drummelsmith J, Burchmore R, Roy G, Ouellette M, Gomez MA, Saravia NG (2012) Discovery of factors linked to antimony resistance in *Leishmania panamensis* through differential proteome analysis. *Mol Biochem Parasitol* 183(2):166–176. doi:[10.1016/j.molbiopara.2012.03.002](https://doi.org/10.1016/j.molbiopara.2012.03.002)
- Webb JR, McMaster WR (1993) Molecular cloning and expression of a *Leishmania major* gene encoding a single-stranded DNA-binding protein containing nine "CCHC" zinc finger motifs. *J Biol Chem* 268(19):13994–14002
- Wyllie S, Cunningham ML, Fairlamb AH (2004) Dual action of antimonial drugs on thiol redox metabolism in the human pathogen *Leishmania donovani*. *J Biol Chem* 279(38):39925–39932. doi:[10.1074/jbc.M405635200](https://doi.org/10.1074/jbc.M405635200)
- Wyllie S, Fairlamb AH (2006) Differential toxicity of antimonial compounds and their effects on glutathione homeostasis in a human leukaemia monocyte cell line. *Biochem Pharmacol* 71(3):257–267. doi:[10.1016/j.bcp.2005.10.043](https://doi.org/10.1016/j.bcp.2005.10.043)
- Wyllie S, Mandal G, Singh N, Sundar S, Fairlamb AH, Chatterjee M (2010) Elevated levels of trypanothione peroxidase in antimony unresponsive *Leishmania donovani* field isolates. *Mol Biochem Parasitol* 173(2):162–164. doi:[10.1016/j.molbiopara.2010.05.015](https://doi.org/10.1016/j.molbiopara.2010.05.015)
- Wyllie S, Vickers TJ, Fairlamb AH (2008) Roles of trypanothione S-transferase and trypanothione peroxidase in resistance to antimonials. *Antimicrob Agents Chemother* 52(4):1359–1365. doi:[10.1128/AAC.01563-07](https://doi.org/10.1128/AAC.01563-07)
- Yan S, Li F, Ding K, Sun H (2003) Reduction of pentavalent antimony by trypanothione and formation of a binary and ternary complex of antimony(III) and trypanothione. *J Biol Inorg Chem* 8(6):689–697. doi:[10.1007/s00775-003-0468-1](https://doi.org/10.1007/s00775-003-0468-1)
- Zhou Y, Messier N, Ouellette M, Rosen BP, Mukhopadhyay R (2004) *Leishmania major* LmACR2 is a pentavalent antimony reductase that confers sensitivity to the drug pentostam. *J Biol Chem* 279(36):37445–37451. doi:[10.1074/jbc.M404383200](https://doi.org/10.1074/jbc.M404383200)