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REVIEW ARTICLE

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ABC transporters in the protozoan parasite *Leishmania*

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Abstract ATP-binding cassette (ABC) transporters constitute one of the biggest and most conserved protein families in the evolutionary scale. Many of them are of enormous clinical relevance, due to their relationship with genetic diseases and drug resistance during the treatment of cancer and infectious diseases. Leishmaniasis is a major and globally widespread group of parasitic diseases, whose treatment has been complicated by the expansion of resistance to conventional drugs. Here, we review the current knowledge about ABC transporters in *Leishmania* spp, with special attention to their relationship with the drug-resistance phenotype.

Keywords *Leishmania* · ABC transporters · Drug resistance · P-Glycoproteins · MDR modulators

Leishmaniasis

Diseases produced by protozoan parasites are one of the main causes of morbidity and mortality around the world, affecting millions of people and domestic animals. This problem has considerably been complicated by the expansion of parasite resistance to drugs. Among these diseases, leishmaniasis has become the second most common cause of death, with a very significant 42-fold increment during the past 15 years [30]. The disease is caused by different species of the kinetoplastid protozoon, *Leishmania*. This parasite has a complex life cycle, which includes an infective flagellar promastigote form, present in the insect vector, and a non-motile intracellular form, the amastigote, which lives in the mononuclear phagocytes of the host vertebrate. According to World Health Organization (WHO) statistics (web site http://www.who.int/emc/diseases/leish/leishmaniasis, revised March 2001), 12 million people are affected by the disease world-wide and 1.5–2 million new cases are estimated to occur annually. Leishmaniasis is endemic in 88 countries, with a total of 350 million people at risk. The disease is spreading in several areas, as a consequence of massive rural–urban migration and its association with AIDS. *Leishmania*/HIV co-infection is indeed considered by the WHO as a real threat, especially in Spain and other Mediterranean countries in south-western Europe (web site http://www.who.int/inf-fs/en/fact116.html, fact sheet 116, revised May 2000).

In the absence of effective vaccines, chemotherapy is the main weapon to control infections. Conventional clinical drugs, pentavalent antimonials in the form of Glucantime and Pentostam, are not very efficient, due to their toxicity and the increased appearance of drug resistance [19]. This high rate of therapeutic failure calls for new rational approaches to develop alternative drugs. Amphotericin B liposomes and paramomycin ointment have been proved to be effective, but they present some drawbacks, such as their very high cost and their limited availability. Other pharmaceutical agents are currently being analysed, but the most promising new leishmanicidal compounds are alkyllysophospholipids (ALPs), such as miltefosine and edelfosine. Miltefosine is indeed the first oral drug that has proved to be highly effective against visceral leishmaniasis, including antimony-resistant cases [32].

ATP-binding cassette transporters

The ATP-binding cassette (ABC) transporters represent the biggest superfamily of proteins known, being present in all studied organisms, from archaeobacteria to higher eukaryotes [28]. In addition to their physiological function, translocating a high variety of substrates across cellular membranes, ABC proteins have enormous medical relevance. Some of them are responsible for the

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multidrug resistance (MDR) phenotype during the treatment of cancer and infectious diseases (AIDS, parasitosis, etc.) and others are involved in important genetic diseases, such as cystic fibrosis, adrenoleukodistrophy, etc. ABC transporters typically consist of four associated polypeptides, either fused together or independent (Fig. 1). Two of them, the transmembrane domains (TMDs), are very hydrophobic and each one normally consists of six membrane-spanning α-helices. The other two domains, called nucleotide-binding domains (NBDs), are located on the cytosolic side of the membrane and they bind ATP and couple the hydrolysis-driven energy to the activity of the protein. The NBDs are more conserved than the TMDs and contain three consensus sequences: the walker motifs A and B, involved in Mg-ATP binding, and the ABC transporter signature, of unknown role. Important interactions between all the domains are likely to occur and have been described in a number of cases. Finally, an additional cytoplasmic domain (R, linker) can link both halves of some transporters. In the ion channel CFTR, whose defect causes cystic fibrosis, this R region incorporates multiple sites for phosphorylation by cAMP-dependent protein kinase and protein kinase C and has a major regulatory role.

A number of ABC transporters are able to induce MDR in patients with cancer by accepting a wide range of cytotoxic agents as transportable substrates, expelling them from the cell. The most studied ABC protein has been the product of the *mdr1* gene, the P-glycoprotein (Pgp), discovered in 1976 [33]. Pgp overexpression makes cells resistant against many antitumour hydrophobic drugs, such as anthracyclins, actinomycin D, *Vinca* alkaloids, colchicine, taxans, etc. Its TMDs are involved in substrate-binding and translocation through the membrane and are responsible for the low selectivity toward transported drugs. This MDR phenotype can be reversed in vitro by the administration, together with the anticancer drugs, of chemosensitizing compounds (reversal agents or modulators), such as verapamil and cyclosporin A, which compete for drug-binding to the TMDs (reviewed in [1]). However, the concentration required for an effective inhibition produces undesirable side-effects that hamper their clinical use.

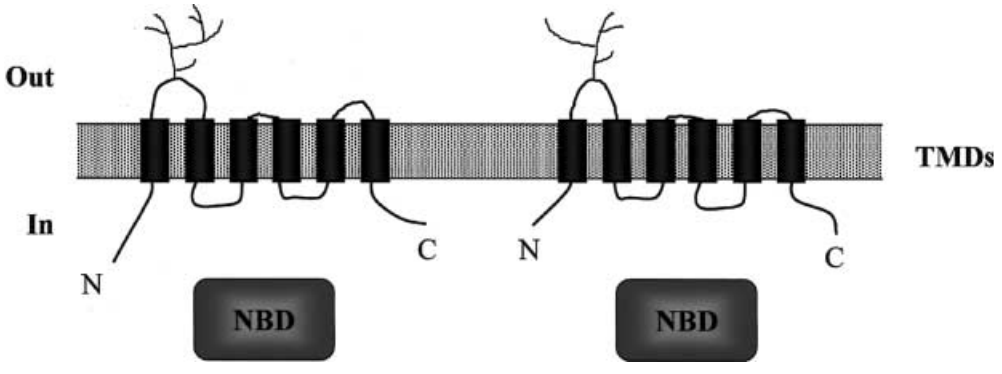
A second ABC transporter involved in MDR, the MDR-associated protein 1 (MRP1), was identified in 1992 [10]. It confers resistance to anthracyclins, *Vinca* alkaloids, oxyanions, etc. and is not inhibited by conventional Pgp modulators. Glutathione (GSH) plays an important role in the functionality of this transporter. It is considered that MRP1 cotransports hydrophobic drugs together with GSH, whereas anionic drugs are transported after their conjugation with GSH (reviewed in [29]).

In *Leishmania* spp, three different classes of ABC transporters are known. Two of them are linked with drug resistance and are homologous to the mammalian MRP1 and Pgp transporters described above. The third shows high homology with members of the mammalian ABCA family. Proteins belonging to this group, which include the mammalian ABCA1 transporter [40], seem to be involved in the dynamic distribution of lipid species across the membrane bilayer. Human ABCA1 is implicated in lipid traffic, and mutations that affect its function are responsible for Tangier disease, a disorder characterized by an almost complete absence of plasma HDL and by alterations in cholesterol and phospholipid metabolism (reviewed in [43]).

Fig. 1 Structural organisation of a typical ATP-binding cassette (ABC) protein. ABC proteins consist of four domains that can be either expressed as separate polypeptides, as shown in the scheme, or fused together in a variety of configurations. The two transmembrane domains (TMDs) are usually glycosylated in eukaryotes. Typically, each one consists of six membrane spanning α-helices. The two nucleotide-binding domains (NBD) are peripherally located at the cytoplasmic face on the membrane (adapted from [28])

MRP-like transporters in *Leishmania*

In 1990, Ouellette and co-workers identified the first ABC gene in *Leishmania*, called *pgpA* [44]. The gene is part of a family that contains four additional members, present in two loci in *Leishmania* genome. *pgpB* and *pgpC* genes are tandemly linked to *pgpA* on a 800-kb chromosome, whereas *pgpD* and *pgpE* genes are closely linked on a different chromosome, ranging from 950 kb to 1400 kb, depending on the *Leishmania* species [37]. Despite having only 22% identity with mammalian Pgp, PgpA was initially considered as a member of the same subfamily. Two years later, after the discovery of



mammalian MRP1 [10], it was noted that PgpA was its most closely related protein, with 33% identity.

pgpA was identified as part of an extrachromosomal DNA, the H-circle, amplified in *L. tarentolae* (*LtpgpA*) [44] and *L. major* (*LmpgpA*) [6] selected for resistance to the dihydrofolate reductase inhibitor methotrexate (MTX). Gene amplification is indeed a common phenomenon in *Leishmania*, often related with drug-resistance phenotypes (for review, see[3]). Arsenite-resistant parasites were often employed as a model for studying resistance to leishmanicides, because it was considered that the chemical properties and biological effects of antimonials and arsenicals were similar. Selection of several *Leishmania* spp with arsenite yielded an amplification of the H region and these lines were cross-resistant to MTX [34, 45]. Therefore, PgpA was thought to be responsible for MTX resistance. However, the molecular basis of this resistance was later elucidated with the identification of the pteridin reductase 1 gene (*ptr1*), located downstream of the *pgpA* gene in the H region, and whose product can confer MTX resistance [47].

Transfection experiments have shown the involvement of PgpA in arsenite and antimony resistance [6, 48]. However, resistance levels varied, depending on the species to which the gene was transfected [38], and never reached the high level observed in stepwise-selected mutants. For instance, transfection of *pgpA* in *L. tarentolae* only gave a two-fold increase in arsenite resistance, and this resistance level was not directly linked to the gene copy number [48]. However, disruption of the *pgpA* gene in wild-type *L. tarentolae* showed an increased sensitivity to arsenite and antimonite [49]. These experiments indicated that PgpA was involved in oxyanion resistance, but that it was not enough to confer complete resistance and required others factors to produce high levels of resistance.

L. tarentolae mutants selected for resistance to arsenite, trivalent antimony, or pentavalent antimony, showed decreased drug accumulation, caused by an increase in efflux which was not correlated with *pgpA* amplification [14]. In addition, decreased accumulation of the drug was not observed in *ltpgpA* transfectants [48]. Further studies demonstrated the existence of a pump capable of catalysing active extrusion of thiol-conjugated metals from plasma membrane vesicles prepared from *L. tarentolae*. This ATP-coupled pump was different from PgpA, because vesicles prepared from a strain with disrupted *pgpA* alleles accumulated As-GSH at wild-type levels [15].

All these results, together with the observation that arsenite-resistant *L. tarentolae* had increased levels of intracellular thiols [41] and the knowledge that MRP1 transports GSH conjugates [42], linked thiol metabolism with metal resistance. Indeed, the As-thiol pump previously described was shown to extrude As(III) or Sb(III) conjugated with trypanothione (TSH) and As(III)-resistant mutants overproduced TSH [41]. In addition, the variability of resistance in *pgpA*-transfection experiments with different *Leishmania* spp might be explained by differences in basal TSH levels among the species [4].

Low-molecular-mass thiols play an important role in the defence against damage caused by oxidants, certain heavy metals and possibly xenobiotics. In trypanosomatids, TSH (Fig. 2) is involved in the maintenance of the intracellular reducing environment, substituting for the GSH found in most other organisms [18]. The enzyme TSH reductase, absent from mammalian cells, is responsible for keeping TSH reduced; TSH is synthesized from GSH and spermidine. As shown in Fig. 2, the enzymes γ-glutamylcysteine synthetase (γ-GCS; encoded by the *gsh1* gene) and ornithine decarboxylase catalyse the rate-limiting steps in TSH biosynthesis. In *L. tarentolae* resistant to arsenite, buthionine sulfoximine (BSO), an inhibitor of γ-GCS, can partially revert the resistance phenotype [23]. Also, treatment of a Glucantime-resistant *L. tropica* line with BSO produced a thiol depletion that was accompanied by a substantial increase in the chemosensitivity to Glucantime [2].

The presence of amplicons containing both *gsh1* and *pgpA* genes in arsenite-resistant *Leishmania* lines has been described [23]. In the absence of drug, these amplicons were lost, but the resulting revertants showed a partial resistance. When *gsh1* or *pgpA* genes were independently transfected in these partial revertant lines, similarly low resistance levels were achieved in each case.

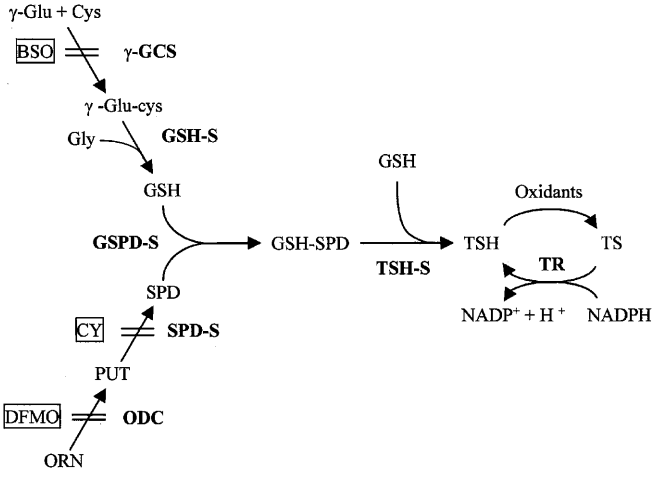


Fig. 2 Thiol synthesis in trypanosomatids. Trypanothione (TSH) is synthesized by the conjugation of glutathione (GSH), a sulfur-containing tripeptide and the polyamine spermidine (SPD). γ-Glutamylcysteine synthetase (γ-GCS), encoded by the *gsh1* gene, is the rate-limiting step in GSH biosynthesis: it catalyses the peptide linkage between the γ-carboxyl group of glutamate and the amino group of cysteine and is inhibited by buthionine sulfoximine (BSO). Ornithine decarboxylase (ODC) catalyses ornithine (ORN) decarboxylation to make putrescine (PUT) and is inhibited by difluoromethylornithine (DFMO). Linkage between two GSH molecules and one molecule of SPD results in TSH synthesis. TSH is kept in its reduced state by the activity of trypanothione reductase (TR). Other indicated enzymes are gluthathione synthetase (GSH-S), glutathionylspermidine synthetase (GSPD-S), spermidine synthase (SPD-S), inhibited by ciclohexylamine (CY), and trypanothione synthetase (TSH-S)

Interestingly, co-transfection with *gsh1* and *pgpA* in these revertants, but not in the parent *L. tarentolae* parasites, resulted in resistance levels higher than expected from the individual contribution of either gene. These results suggested a synergistic interaction between the proteins, as well as the idea that PgpA recognizes metals conjugated with TSH [23]. Thus, metal resistance in *Leishmania* required, in addition to PgpA and/or the thiol-X-pump, high levels of TSH, which can be achieved either by amplification of *gsh1* gene, as described above, or by overexpression of the *odc* gene, as observed in other arsenite-resistant *Leishmania* lines [25]. Furthermore, the partial resistance found in the revertant parasites and the fact that efficient co-transfection was only possible in this line, suggest that other factors must be involved in oxyanion resistance. It had been proposed that one of these factors could be an increased TSH transferase activity able to conjugate metal and TSH [46].

Considering the above results, Ouellette et al. [46] proposed that PgpA was localized at the membrane of an intracellular compartment, conferring resistance by sequestering metal-TSH conjugates, as shown in Fig. 3. In agreement with the role suggested for PgpA, mammalian MRP1 was shown to transport GSH-linked substrates [42] and transfection of MRP was associated with oxyanion resistance [11]. Recently, immunofluorescence, confocal and electron microscopy studies demonstrated intracellular localization of PgpA in membrane structures close to the flagellar pocket [39]. Likewise, vesicles obtained from PgpA transfectants enriched in the transporter, showed increased ATP-dependent As-GSH transport, whereas transport of non-conjugated metal was not observed [39].

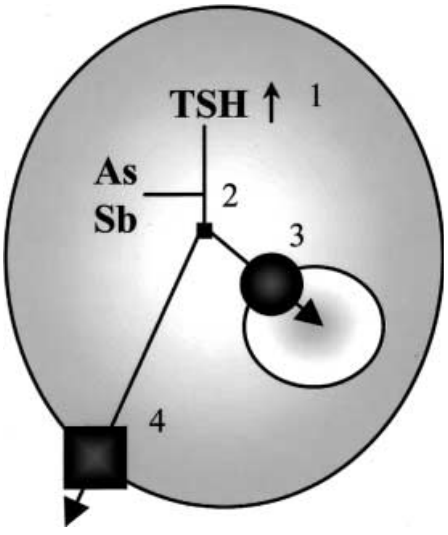


Fig. 3 Model for metal resistance in *Leishmania*. Metal resistance requires high levels of trypanothione (TSH) (1), that can be reached by amplification and/or overexpression of *gsh-1* and *odc* genes. A putative TSH-transferase conjugates the metal with TSH (*2*) and the complex is then either "sequestered" inside a vacuole, due to PgpA activity (*3*), or expelled from the cell by a X-thiol pump localized in the plasma membrane (*4*). Taken from Ouellette et al. [46]

Other members of the MRP subfamily were scarcely studied in *Leishmania* spp, probably because none of them were amplified in resistant mutants for a number of drugs, including vinblastine, puromycin, arsenite and MTX [37, 45]. In a *L. tropica* MTX-resistant line selected in vitro, which showed decreased uptake of MTX and cross-resistance to a wide range of drugs, an overexpression of the *ltpgpE* gene, as a consequence of a genomic rearrangement, has been described [21]. However, it was considered an epiphenomenon that probably does not arise from the MTX-resistance mechanism. More recently, an MRP-like transport activity has been detected in three different wild-type *Leishmania* spp [17]. The authors suggested that this activity could be the same As-thiol transporter previously described [14, 15]. However, no strong evidence has been shown and this point remains to be confirmed.

P-glycoprotein-like transporters in *Leishmania*

The second group of ABC proteins in *Leishmania* spp is constituted by transporters with a higher sequence similarity to mammalian Pgps and confers a MDR phenotype similar to that observed in cancer cells. The first *Leishmania mdr1*-like gene was described in the early 1990s by the laboratories of Ullman and Wirth, in a vinblastine-resistant *L. donovani* line (*ldmdr1*) [26]. Later, homologous genes were described in a vinblastine-resistant *L. enrietti* line (*lemdr1*) [9], in a vinblastine-resistant *L. amazonensis* line (*lamdr1*) [24] and, finally, in a daunomycin-resistant *L. tropica* line (*ltrmdr1*) [7].

All of these parasite lines were stepwise-selected with the indicated drug and presented a MDR phenotype, with a cross-resistance profile to non-related hydrophobic drugs such as puromycin [7, 9, 26], daunomycin (DNM) [26], vinblastine [7], adriamycin [24] and doxorubicine [7]. They also showed a decreased puromycin [26], DNM [7], or rhodamine 123 [24] intracellular accumulation and an increased efflux of rhodamine 123 [24], whereby it is supposed that resistance to these hydrophobic drugs is due to drug efflux, as described for mammalian Pgps. The main difference between the MDR phenotype mediated by mammalian Pgps and the phenotype conferred by the *Leishmania* Pgp-like transporters was the absence of a reversal effect of classic modulators, such as verapamil [7, 26], quinidine [26] and cyclosporine A [7]. High concentrations of verapamil and cyclosporin A, used in short-time experiments to avoid their intrinsic toxicity in these parasites, partially increased DNM accumulation in the MDR *L. tropica* line [7, 50].

The *mdr1*-like genes were overexpressed and amplified into extrachromosomal DNA circles, called V-circle or D-circle depending on the drug used in the selection step, with sizes ranging from 25 kb to 40 kb. Further demonstration of the involvement of this gene in the

MDR phenotype came from *ldmdr1*, *lemdr1* and *lamdr1* transfection assays in wild-type parasites [9, 26, 35]. Transfected parasites showed a resistance phenotype, although with lower levels to that found in the stepwise-selected resistant lines (especially in the case of *lemdr1* and *lamdr1*). This difference was not due to a lower copy number of the gene or a lower level of mRNA expressed in the transfectant line than those found in the stepwise-selected line [9, 26, 35]. In contrast, transfection of drug-sensitive *L. enrietti* parasites with the whole V-circle containing the *lemdr1* gene, converted in a shuttle vector, fully restored the drug-resistance phenotype [55]. Interestingly, the size of the *mdr1* mRNA was unusually long in all the stepwise selected lines (10.5–13 kb), compared with the predicted coding region (around 4 kb). In the case of *lemdr1*, this size was shown to be determinant in conferring resistance [55]. These observations might indicate that additional information could be present in the native transcripts, which explains the differences in the level of resistance obtained. Indeed, in the MDR *L. enrietti* line, a *cis* regulatory element present in the V-circle upstream of the *lemdr1* gene was found to regulate gene expression [56].

Parasites knocked-out for this gene showed a four-fold higher sensitivity to vinblastine than the wild-type line and, in the absence of the drug, promastigotes were able to growth at normal rate [8, 54]. Although its physiological function is unknown, these data could indicate that the *mdr1* gene is not essential for the parasite, at least under the conditions of these experiments [8, 54].

Amino acid alignment of the *Leishmania* Pgp-like transporters, with sizes ranging over 1,280–1,341 amino acids and 140–147 kDa, showed around 37% identity and 53% homology to mammalian Pgps and 78–95% identity between themselves. Hydropathy profiles revealed a typical Pgp topology, with two homologous halves, each comprising a TMD containing six transmembrane α-helices and a cytosolic NBD. The sizes of externally exposed and cytoplasmic loops between each of the predicted transmembrane helices were similar to mammalian Pgps [27]. However, in contrast to its mammalian homologue, none of the Asn-linked glycosylation consensus sites predicted from the primary structure was found in the external side of the protein [7, 9, 27, 35]. In addition, Western blot analysis of the *L. tropica* transporter showed a molecular weight around 150 kDa, consistent with a non-glycosylated protein [7]. The homology between the two halves of these transporters was 49% and that between the two NBDs was 70%. Each half showed higher homology with the equivalent half of the mammalian transporter than with its duplicated partner, indicating that the original gene duplication and subsequent fusion occurred before the separation of *Leishmania* from other members of the eukaryotic line [27].

Not all of the MDR spectra of drugs are used to treat leishmaniasis and, consequently, clinical interest in these proteins has not been well established. However, we have recently shown that the *L. tropica* Pgp-like transporter also confers resistance to the most promising new anti-*Leishmania* agents, ALPs, such as miltefosine and edelfosine (Pérez-Victoria JM et al., unpublished data). Cross-resistance to these ALPs depended on Pgp overexpression and correlated with a decreased intracellular accumulation of bodipy-C_5-PC, a fluorescent analogue of phosphatidylcholine, whose structure resembles that of edelfosine. It is possible that the mechanism of ALP resistance produced by the *Leishmania* multidrug transporter could be related to the flippase mechanism of phosphatidylcholine transport by mammalian Pgps [5]. A direct interaction between human Pgp and ALP has indeed been recently described, using purified transporter reconstituted into proteoliposomes [53]. In addition, although contradictory results have been obtained with different Pgp-overexpressing MDR cell lines, *mdr1* transfection produced cross-resistance to ALP [31, 53]. However, this resistance could not be reverted by potent Pgp inhibitors [31, 53] indicating that the mechanism by which Pgp confers resistance to ALP and classic MDR drugs could be different.

Rational reversion of the *Leishmania* MDR phenotype

The implication of the *Leishmania* multidrug transporter in resistance to ALP, together with the fact that classic mammalian Pgp inhibitors, such as verapamil and cyclosporine A are not very efficient in this parasite, support the importance for developing new inhibitors of this ABC transporter. The main problem related with reversal agents in humans is associated with the side-effects produced, which hamper their clinical use. We therefore decided to study natural products with low expected toxicity as putative MDR reversal agents, using the MDR *L. tropica* line described above as a model.

Since ATP hydrolysis is necessary for the functionality of the transporter, we first chose NBDs as a new pharmacological target for the design of inhibitors. Flavonoids, a class of natural compounds with a high presence in the Mediterranean diet and with contradictory results as MDR reversal agents, are known to interact with ATP-binding proteins (reviewed in [16]). Interestingly, they were recently shown to interact with both NBDs from mammalian Pgp in a bifunctional way, partially overlapping the ATP site and a neighbouring, hydrophobic-interacting region [12, 13, 16]. The binding affinity depended on the flavonoid class [12, 16].

Flavonoids were also able to interact with a recombinant C-terminal NBD (NBD2) from the *L. tropica* multidrug transporter in a class-dependent manner, flavones showing the highest affinity [50, 52]. Hydrophilic flavone substitutions, which increased flavonoid mimetism with the adenine group of ATP, raised their affinity-binding to the recombinant NBD2 [50], as also showed with the NBDs of mammalian Pgp [12, 16]. In addition, hydrophobic substitutions considerably improved the binding affinity [50, 52], probably by

increasing interaction with the hydrophobic-interacting region, which has also been found in the *Leishmania* NBD2 [50]. Interestingly, there was a strong correlation between the affinity of in vitro binding to the NBD2 and the efficiency of both in vivo modulation of drug accumulation [50] and reversion of the MDR phenotype [52], including ALP resistance (unpublished data). This correlation suggests that cytosolic domains are materials well suited for the rational design of inhibitors against Pgp-like transporters and that flavonoids are interesting potential MDR modulators in these parasites.

A second group of possible modulators consisted of sesquiterpene esters based on the dihydro-β-agarofuran skeleton. These compounds are chemotaxonomic indicators of the Celastraceae family, a class of plants which have a long history in traditional medicine and which have attracted great interest, due to their pharmacological activities (reviewed in [22]). They are particularly promising modulators of the MDR phenotype mediated by human Pgp [36].

We have shown that some compounds from the same family also revert the MDR phenotype in *Leishmania*. A number of agarofuran sesquiterpene derivatives from *Crossopetalum tonduzii* [51], *Maythenus chubutensis* and *Maythenus magellanica* (Cortés-Selva et al., unpublished data) efficiently revert the Pgp-like transporter-mediated DNM resistance in this MDR *L. tropica* line, by increasing intracellular drug accumulation (Pérez-Victoria et al., unpublished data; Cortés-Selva et al., unpublished data). Some of them also completely reverted ALP resistance at concentrations that did not produce any toxic effect in the parent wild-type line (Pérez-Victoria et al., unpublished data). Sesquiterpenes maintain neither a conjugated planar ring nor a substituted tertiary amino group, as described for other MDR reversal agents [20]. However, they share a significant hydrophobicity. Therefore, it could be possible that these modulators bind to the hydrophobic-interacting region characterised near to the ATP-binding site of the NBDs, as previously suggested for other hydrophobic modulators [13]. However, we found no correlation between the very low binding of these compounds to the purified NBD from the *Leishmania* Pgp-like transporter and their in vivo effect. These results suggest that the ability of dihydro-β-agarofuran sesquiterpenes to revert the *Leishmania* MDR phenotype was related to their binding to the TMDs of the *Leishmania* Pgp-like transporter and blocking DNM efflux, as classic reversal agents do. Further efforts are underway to elucidate the specific target of dihydro-β-agarofuran sesquiterpenes in the Pgp-like transporter, using photoaffinity-labelling competitive experiments.

Finally, preliminary results indicate that a combination of flavonoids and sesquiterpenes, at concentrations which did not produce a significant reversal effect when used independently, efficiently overcame the MDR phenotype in these parasites. This additive reversal effect is probably due to their binding to both the NBDs and TMDs of the Pgp-like transporter (Fig. 4). In addition, this sub-dose combination avoids any side-effect in the parent wild-type line. The toxicity of reversal agents is caused by a less specific binding to other cellular targets. These targets are probably different for each class of modulator, thus explaining the absence of additive toxicity when using the combinatorial strategy.

ABCA-like transporters

More recently we identified ABCA-like genes in the parasites *L. tropica* and *Trypanosoma cruzi* (unpublished data). Whereas in *T. cruzi*, this family appears to be represented by a single copy gene, in *Leishmania* there is a multigenic family with at least four members. We are interested in the molecular and functional characterization of these genes in parasites, using transfected and null-mutant cells. Interestingly, one *Leishmania* member, *Ltrabc1.1*, is duplicated in tandem on a 1.3 Mb chromosome and is flanked by inverted repeated sequences. *Leishmania* is very efficient in using repeated sequences

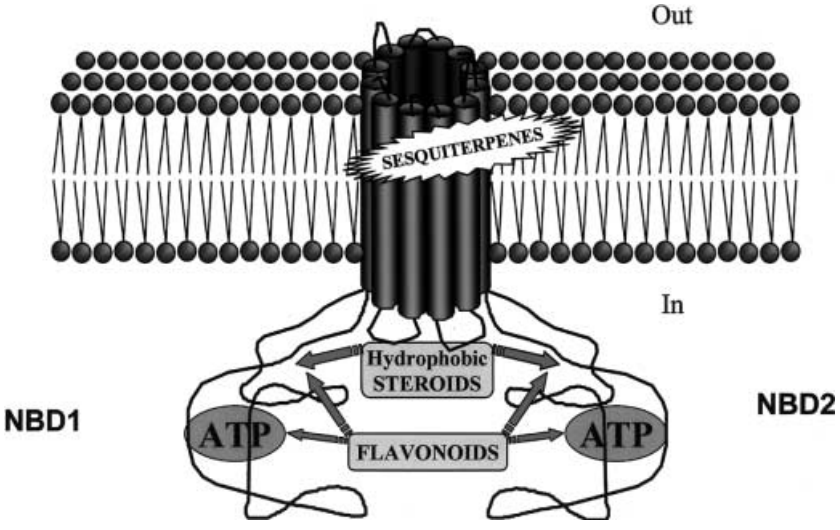


Fig. 4 Working model to explain the interaction of different modulators with the *Leishmania* Pgp-like transporter. This schematic structure of the transporter inserted in the membrane shows the transmembrane domains (TMDs) and the cytosolic nucleotide-binding domains (NBDs). Flavonoids could interact with the NBDs in a bifunctional way, with the ATP site and with a hydrophobic region close to the ATP site, as described for mammalian Pgp [12]. In contrast, sesquiterpenes probably bind to the TMDs, as most of the Pgp reversal agents do

to create amplicons, as demonstrated for the H-circle [45]. Thus, it is tempting to suggest that this locus might suffer rearrangements under drug pressure. However, we have not yet observed any amplification of this locus, and no correlation between resistance and *Ltrabc1.1* expression has been noted. Preliminary results suggest that LtrABC1.1, like the mammalian ABCA1 transporter, could be involved in lipid-trafficking across the plasma membrane of the parasite. We are currently studying the possible involvement of this activity with respect to infectivity and the survival capability of *Leishmania*.

Concluding remarks

Drug resistance during the treatment of leishmaniasis is a problem of enormous clinical relevance. Antimonials and many new potential leishmanicidal agents, such as azoles, rifampicin, doxorubicin, taxol and especially ALP, are known substrates of ABC transporters and thus could induce a drug-resistance phenotype. Research into this family of proteins in *Leishmania*, together with the rational development of inhibitors that block these transporters, is therefore a promising way to circumvent resistance to drugs. These studies also provide useful models to understand how similar defence mechanisms can be overcome in other protozoan parasites, such as *Entamoeba* and *Plasmodium*, where ABC transporters have also been associated with drug resistance.

Finally, we can assume that the ABC transporter superfamily, as one of the biggest and most conserved protein families in the evolutionary scale, must also be well represented in trypanosomatids, such as *Leishmania*. The genome project for this parasite, currently in progress, will lead to knowledge of new sequences related with ABC transporters. Taking into consideration the number of genes coding for ABC proteins in sequenced genomes (for instance 79 in *Escherichia coli*, 55 in *Drosophila melanogaster*, 30 in *Saccharomyces cerevisiae*, 51 in humans), we can advance the discovery of many new *Leishmania* ABC genes from now on. A most exciting focus of research in a near future will be the identification of the physiological role of these proteins.

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