# P-Glycoprotein-Like Transporters in *Leishmania*: A Search for Reversal Agents

14

Bruno Pradines

**Abstract**

Until now, chemotherapy has been the main line of defense against *Leishmania* infections. However, drug use and abuse have resulted in the selection and development of resistance mechanisms which strongly limit the number of antiprotozoal agents that are effective for the treatment of this disease. The emergence and spread of resistance to drugs currently in use and available for leishmaniasis emphasize that new compounds need to be identified and developed and that novel chemotherapeutic targets must be characterized. Mechanisms of drug resistance are often associated with decreased uptake of the drug into the parasite, poor drug activation, physiological alterations in the drug target, and overexpression of drug transporter proteins. One mechanism of resistance to antimony in *Leishmania* involves a decrease in its accumulation by either reduced uptake or increased efflux, mediated by P-glycoprotein (Pgp)-like transporters, which belong to the ATP-binding cassette (ABC) superfamily of proteins. The inhibition of the function of these proteins represents an attractive way to control drug resistance in clinical environments. New natural or synthetic sesquiterpenes, flavonoids, acridonecarboxamide derivative modulators of human Pgp (zosuquidar and elacridar), statins, pyridine analogs, 8-aminoquinolines, or phenothiazines revert in *Leishmania* the resistance phenotype to antimony, pentamidine, sodium stibogluconate, and miltefosine by modulating intracellular drug concentrations. In this chapter, we review some concepts concerning the reversal mechanism of multidrug resistance by the use chemosensitizers which alter the capacity of Pgp.

B. Pradines (✉)
Unité Parasitologie et entomologie, Département des maladies infectieuses, Institut de Recherche Biomédicale des Armées, Marseille, France & Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, UM, Marseille, France



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## 14.1 Introduction

Arsenic- and antimony-containing drugs are still the first line of treatment for leishmaniasis. Pentavalent antimonial compounds ($Sb^V$) remain the choice of treatment for all forms of leishmaniasis, ranging from cutaneous lesions to fatal visceral infections. The emergence and spread of resistance to currently used antileishmanial drugs emphasize the fact that new compounds need to be identified and developed. Resistance to antimonial drugs is everyday more frequently reported [1–3].

A large amount of scientific effort is spent on elucidating the mechanisms underlying this resistance with the hope of restoring/improving the efficacy of existing drugs and of developing new drugs that can bypass resistance mechanisms.

Among the various drug resistance mechanisms identified, those based on drug movement through the membranes appear to play an important role by decreasing the drug concentration at the target sites. The transport proteins of the ATP-binding cassette (ABC) superfamily provide the basis of multidrug resistance in mammalian cancer cells and in pathogenic yeasts, fungi, parasites, and bacteria [4–8]. ABC proteins were also identified in resistance to antileishmanial drugs (see Table 14.1). The ABC transporters are described in Chap. 11.

But all of the ABC families are not associated with antileishmanial drug resistance, such as the ABCA family [9].

The ABCB family includes the multidrug-resistant protein 1 (MDR1) or ABCB4 protein and the multidrug-resistant protein 2 (MDR2) or ABCB2 protein, whose overexpression confers resistance to vinblastine and structurally non-related hydrophobic compounds such as puromycin, adriamycin, doxorubicin, and daunomycin [10–16]. LeMDR1 (LeABCB4) can also affect pentamidine resistance [17]. Additionally, LgMDR1 and LaMDR1 are increased in antimony-resistant strains of *L. (V.) guyanensis* or *L. (L.) amazonensis* [18]. The subcellular location of LeABCB4 and LaABCB2 (LaMDR2) in the tubular structure, a compartment that may correspond to a multivesicular tubule lysosome, suggests that mechanisms of resistance in *Leishmania* are different from those acting in the conventional mammalian efflux pump Pgp MDR1.

The ABCC family includes the multidrug-resistant protein A (MRPA) or P-glycoprotein A (PGPA) or ABCC3; the P-glycoprotein E (PGPE) or ABCC4, associated with resistance to arsenite and antimonial drugs; and the pentamidine resistance protein 1 (PRP1) or ABCC7. ABCC3 and ABCC4 are involved in the resistance of *Leishmania* toward arsenic and antimony compounds [19–22]. Overexpression of ABCC4 and ABCC5 can also confer resistance to antimonial drugs in *L. (S.) tarentolae* [23]. Additionally, field-resistant isolates to antimony exhibit upregulation in ABCC3 (MRPA or PGPA) transcript levels in *L. (L.) donovani*, *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (L.) amazonensis*, or *L. (L.) major* ($>1.5$) [18, 24, 25]. ABCC7 is shown to confer pentamidine resistance in the promastigote and amastigote form of *L. (L.) major* and is cross-resistant to trivalent antimonial drugs when overexpressed [26–28].

The ABCG family includes the ABCG4 and ABCG6 proteins. ABCG4, localized mainly to the parasite plasma membrane, reduced the accumulation of

**Table 14.1** ATP-binding cassette (ABC) transporters in *Leishmania* spp.

| ABC subfamily | *Leishmania* spp. | Protein | Involvement in drug resistance |
|---|---|---|---|
| ABCA | *L. (L.) infantum* | LiABCA4 | No |
| | | LiABCA8 | No |
| | *L. (L.) major* | LmABCA3 | No |
| | | LmABCA4 | No |
| | | LmABCA8 | No |
| | *L. (L.) tropica* | LtrABCA4 or LtrABCA2 | No |
| | | LtrABCA8 or LtrABC1.1 | No |
| ABCB PgP cluster | *L. (L.) amanozensis* | LaABCB4 or LaMDR1 | Yes |
| | | LaABCB2 or LaMDR2 | Yes |
| | *L. (L.) donovani* | LdABCB4 or LdMDR1 | Yes |
| | *L. (M.) enriettii* | LeABCB4 or LeMDR1 | Yes |
| | *L. (V.) guyanensis* | LgABCB4 or LgMDR1 | Yes |
| | *L. (L.) tropica* | LtrABCB4 or LtrMDR1 | Yes |
| ABCC MRP cluster | *L. (L.) amazonensis* | LaABCC3 or LaMRPA | Yes |
| | | LaABCC7 or LaPRP1 | Yes |
| | *L. (V.) braziliensis* | LbABCC3 or LbMRPA | Yes |
| | *L. (L.) donovani* | LdABCC3 or LdPGPA or LdMRPA | Yes |
| | *L. (V.) guyanensis* | LgABCC3 or LgMRPA | Yes |
| | *L. (L.) infantum* | LiABCC3 or LiPGPA or LiMRPA | Yes |
| | | LiABCC4 or LiPGPE | Yes |
| | | LiABCC5 | Yes |
| | | LiABCC7 or LiPRP1 | Yes |
| | | LiABCC9 | ? |
| | *L. (L.) major* | LmABCC3 or LmPGPA or LmMRPA | Yes |
| | | LmABCC7 or LmPRP1 | Yes |
| | *L. (L.) mexicana* | LmeABCC3 or LmePGPA or LmeMRPA | Yes |
| | *L. (S.) torentolae* | LtABCC2 or LtPGPB | Yes |
| | | LtABCC3 or LtPGPA or LtMRPA | Yes |
| | | LtABCC4 or LtPGPE | Yes |
| | | LtABCC5 | Yes |
| | *L. (L.) tropica* | LtrABBC4 or LtrPGPE | Yes |

(continued)

**Table 14.1** (continued)

| ABC subfamily | *Leishmania* spp. | Protein | Involvement in drug resistance |
|---|---|---|---|
| ABCG | *L. (L.) donovani* | LdABCG6 | Yes |
| | *L. (L.) infantum* | LiABCG4 | Yes |
| | | LiABCG6 | Yes |
| | *L. (L.) major* | LmABCG2 | Yes |

? Not determined

phosphatidylcholine analogs and conferred resistance to alkyl-phospholipids (miltefosine (MIL), edelfosine, and perifosine) when overexpressed. The second ABCG reported, ABCG6, also localized mainly to the parasite plasma membrane, confers resistance to MIL and sitamaquine when overexpressed in *L. (L.) infantum* [29]. ABCG6 confers also resistance to camptothecin and arsenite [30].

The inhibition of the activity of ABC proteins represents an interesting way to control drug resistance. This concept of inhibiting ABC transporters is well studied for malaria [31–33]. *Leishmania* parasites overexpressing ABCG2 are resistant to antimony, as they demonstrate a reduced accumulation of $Sb^{III}$ due to an increase in drug efflux [34].

## 14.2 Transporter Inhibitors and Modulators of Multidrug Resistance

A number of compounds, e.g., calcium channel blockers, calmodulin antagonists, hydrophobic peptides, protein kinase inhibitors, antibiotics, hormone derivatives, and flavonoids, have been previously described to reverse in vitro multidrug resistance in mammalian cells [35]. They are called modulators or chemosensitizers; those that reverse the multidrug-resistant phenotype in *Leishmania* spp. are listed in Table 14.2.

### 14.2.1 Calcium Channel Blockers: Verapamil

Some of these compounds, like the L-type voltage-gated channel blocker verapamil, are known to efficiently overcome multidrug-resistant phenotype in vitro, not only in mammalian cells [54–56] but also in some bacteria such as *Mycobacterium* spp. [57, 58] or *Enterococcus* spp. [59] and in parasites such as nematodes like *Haemonchus contortus* [60–62] and protozoa like *Entamoeba histolytica* [63–65] or *Plasmodium falciparum* [66–68]. Verapamil is an inhibitor of the human Pgp (ABCB1) [69].

Previous studies have demonstrated that verapamil increases the in vitro antimony activity on *L. (L.) donovani* [36]. Verapamil shows efficacy in reversing several P-glycoprotein and MRP overexpression-mediated arsenite resistance

**Table 14.2** Major multidrug resistance reversal drugs investigated in *Leishmania spp.*

| Class of compound and specific modulators | Resistance to | Strains | References |
|---|---|---|---|
| Calcium channel blockers | | | |
| Verapamil | Antimonials | *L.(L.) donovani* | [36] |
| | Pentamidine | *L. (L.) mexicana* | [37] |
| | Arsenites | *L. (L.) donovani* | [30] |
| | | *L. (S.) tarentolae* | [38] |
| | Pirarubicin | *L. (V.) braziliensis* | [39] |
| | | *L. (V.) guyanensis* | [39] |
| | | *L. (L.) mexicana* | [39] |
| | | *L. (V.) peruviana* | [39] |
| | | *L. (V.) panamensis* | [39] |
| | Vinblastine | *L. (L.) amazonensis* | [13] |
| Calmodulin inhibitors: Phenothiazine derivatives | | | |
| Chlorpromazine | Antimonials | *L. (L.) donovani* | [40] |
| | | *L. (L.) major* | [40] |
| | | *L. (V.) braziliensis* | [39] |
| | | *L. (V.) guyanensis* | [39] |
| | | *L. (L.) mexicana* | [39] |
| | Pentamidine | *L. (L.) mexicana* | [37] |
| Trifluoperazine, prochlorperazine | Pirarubicin | *L. (V.) braziliensis* | [39] |
| | | *L. (V.) guyanensis* | [39] |
| | | *L. (L.) mexicana* | [39] |
| Thioridazine, trifluoropromazine | Pirarubicin | *L. (V.) braziliensis* | [39] |
| | | *L. (V ) guyanensis* | [39] |
| | | *L. (L.) mexicana* | [39] |
| Flavonoids | | | |
| Silymarin and silybin derivatives | Daunomycin | *L. (L.) tropica* | [41] |
| Quercetin | Arsenites | *L. (L.) donovani* | [30] |
| Synthetic flavonoids | Pentamidine | *L. (L.) donovani* | [42] |
| | | *L. (M.) enriettii* | [42] |
| | Sodium stiboglucanate | *L. (L.) donovan* | [42] |
| | | *L. (M.) enriettii* | [42] |
| Synthetic flavonoid derivatives | Antimonials | *L. (L.) major* | [43] |
| Trolox and derivatives | Antimonials | *L. (L.) major* | [43] |
| Sesquiterpenes | | | |
| Dihydro-β-agarofuran sesquiterpenes | Miltefosine | *L. (L.) tropica* | [41] |
| Sesquiterpene C-3 (agarofuran derivative) | Edelfosine | *L. (L.) tropica* | [41] |
| | Daunomycin | *L. (L.) tropica* | [41] |
| Nortriterpene | Daunomycin | *L. (L.) tropica* | [44] |
| Glycyrrhizic acid | Sodium stiboglucanate | *L. (L.) donovani* | [45] |

(continued)

**Table 14.2** (continued)

| | | | |
|---|---|---|---|
| Statins | | | |
| Lovastatin | Antimonials | *L. (L.) donovani* | [46] |
| Pyridine analogs | | | |
| PAK104P | Pirarubicin | *L. (V.) braziliensis* | [39] |
| | | *L. (V.) guyanensis* | [39] |
| | | *L. (L.) mexicana* | [39] |
| Oxazolo[3,2-α]pyridine | Daunomycin | *L. (L.) tropica* | [47] |
| | Miltefosine | *L. (L.) tropica* | [47] |
| Sulfonylurea | | | |
| Glibenclamide | Glucantime | *L. (L.) mexicana* | [48] |
| | | *L. (L.) major* | [49] |
| Benzoquinones | | | |
| Bis-pyranobenzoquinones | Daunomycin | *L. (L.) tropica* | [50] |
| Acridine derivatives | | | |
| Quinacrine | Pentamidine | *L. (L.) donovani* | [42] |
| | | *L. (V.) enriettii* | [51] |
| 8-aminoquinolines | | | |
| Sitamaquine | Miltefosine | *L. (L.) tropica* | [52] |
| | Antimonials | *L. (L.) tropica* | [52] |
| Acridonecarboxamide derivatives | | | |
| Elacridar, zosuquidar | Miltefosine | *L. (L.) tropica* | [53] |

phenotype in *L. (S.) tarentolae* or *L. (L.) donovani* [30, 38]. The reversion of in vitro drug resistance by verapamil is confirmed in *L. (L.) donovani* clinical isolates resistant to sodium stibogluconate [70]. This drug partially reverses the resistance in vinblastine-resistant *L. (L.) amazonensis*, which show cross-resistance to adriamycin [13]. The energy-dependent efflux of pirarubicin, an anthracycline derivative, is inhibited by verapamil in *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (L.) mexicana*, *L. (V.) peruviana*, and *L. (V.) panamensis* [39]. However, verapamil cannot revert the resistance to camptothecin, a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase-I [30]. Various studies in cancer cell lines reveal that development of resistance to topoisomerase inhibitors is a multifactorial event including altered transport, modified drug metabolism and detoxification, and change in drug-target interaction. Amino acid substitutions in topoisomerase-I confer camptothecin resistance in *L. (L.) donovani* [71]. The apparent wide substrate specificity of the *Leishmania* transport system suggests that it could be responsible for the intrinsic resistance of parasite promastigotes to drugs. Its physiological relevance is supported by the fact that it was described in at least five different *Leishmania* species. It seems that verapamil regulates drug susceptibility by downregulating Pgp expression in arsenical-resistant *Leishmania* spp. [72]. In tumor cells, the ability of verapamil to modulate multidrug resistance protein 1 (MRP1 or ABCC1)-mediated resistance seems to be link to its effect on the reduced glutathione

(GSH) status [73]. In addition to stimulate MRP1-mediated GSH transport, verapamil modulates MRP1-mediated leukotriene $C_4$ transport [74].

Verapamil also enhances pentamidine uptake into resistant *L. (L.) mexicana* and also partially reverses the drug resistance phenotype in promastigotes [37], but not in axenic amastigotes [75]. In addition, using nontoxic concentrations of verapamil, a dose-dependent reversion of pentamidine is observed in resistant parasites when compared with those not treated with verapamil in *L. (L.) amazonensis* [27]. However, verapamil has any impact either in drug uptake or drug resistance in *L. (L.) donovani* [76]. This suggests that Pgp-mediated efflux of pentamidine is not operative in *L. (L.) donovani* as it is in *L. (L.) mexicana* or *L. (L.) amazonensis*. PRP1 (ABCC7) is shown to confer pentamidine resistance in the promastigote and amastigote form of *L. (L.) major* and in *L. (L.) infantum* when overexpressed [26, 28], but not in *L. (L.) amazonensis* [27]. No difference in *PRP1* transcript levels is observed between susceptible and resistant *L. (L.) donovani* parasites to $Sb^V$ [77].

The specific Pgp inhibitor cyclosporin-A does not interfere with calcein cell retention (efflux measurement) in *L. (L.) amazonensis*, while verapamil does [78]. These results demonstrate that the drug transport systems expressed in *Leishmania* are susceptible to MRP (ABCC) inhibitors like verapamil, but not to the Pgp (ABCB) inhibitor like cyclosporin-A.

In addition, it seems that verapamil is ineffective in reverting ABCG6 overexpression-mediated resistance in *Leishmania* [30].

### 14.2.2 Calmodulin Inhibitors: Phenothiazine Derivatives

Phenothiazines and reserpine can also reverse drug resistance in mammalian cells, bacteria, and parasites [79–82]. Phenothiazine drugs, of which chlorpromazine is the leading molecule, are widely used for their antipsychotic, antianxiety, and antiemetic effects. In addition, they also possess protozoacidal activity against amastigotes and promastigotes of *L. (L.) donovani* and *L. (L.) chagasi* in vitro as well as in vivo [83–85]. Chlorpromazine is also an inhibitor of the human Pgp (ABCB1) [69].

Chlorpromazine, trifluoropromazine, thioridazine, trifluoperazine, and prochlorperazine are reported to inhibit the energy-dependent efflux of pirarubicin, an anthracycline derivative, in *L. (V.) braziliensis*, *L. (V.) guyanensis*, and *L. (L.) mexicana* [39]. A synergistic effect between chlorpromazine and N-meglumine antimoniate is observed in multidrug-resistant *L. (L.) donovani* and *L. (L.) major* cells in vitro [40]. The effect of phenothiazine derivatives on *Leishmania* drug transport may be explained by their ability to inhibit the activity of trypanothione reductase [86, 87]. Indeed, if we consider that the reduced form of trypanothione is an important co-factor for the function of the *Leishmania* drug transporter, in the same way as reduced glutathione is required for the MRP1 function [74, 88], phenothiazines may inhibit transport activity by decreasing the intracellular level of reduced trypanothione [39]. However, no significant effect is observed in vivo against amastigotes of *L. (L.) major* and *L. (L.) mexicana*, in cutaneous lesions in mice [40]. The toxic effects reported with the most frequently studied phenothiazine,

which is chlorpromazine, have impaired the investigation of other phenothiazines as potential clinical agents.

Prochlorperazine and trifluoperazine enhance pentamidine uptake into resistant *L. (L.) mexicana* and also partially reverse the drug resistance phenotype [37]. However, these drugs have any impact either in drug uptake or drug resistance in *L. (L.) donovani* [76]. This indicates that Pgp-mediated efflux of pentamidine is not operative in *L. (L.) donovani* as it is in *L. (L.) mexicana*, like for verapamil.

### 14.2.3 Flavonoids

The flavonoid class is constituted by flavones, flavonols, isoflavones, flavanones, and chacones [89]. More than 6500 different flavonoids have been identified from plant sources.

Flavonoids have shown promise to reverse multidrug-resistant phenotypes in *L. (L.) tropica* [41, 42, 90, 91]. Flavonoids constitute a well-known class of natural inhibitors of different proteins [92] with contradictory results concerning their modulation effects on different multidrug-resistant cells [93–95]. They bind to the two cytosolic NBSs of the ABC transporters. The flavanolignan silybin and its hemisynthetic derivatives exhibit good affinity to NBD2 [96]. The flavonoid interactions with the ATP-binding site and a vicinal hydrophobic region [41, 91, 97] cause the inhibition of drug efflux and reverse the resistance to daunomycin in *L. (L.) tropica*. Only flavonoids which bind with high affinity to the cytosolic domain NBD2 are able to both increase daunomycin accumulation in a *L. (L.) tropica* line overexpressing MDR1 (LtrABCB4) and inhibit the parasite growth in the presence of the drug [41]. In addition, flavonoids, such as quercetin a flavone, may modulate the multidrug transporter by decreasing Pgp synthesis and inhibiting the transcriptional activation of the *mdr* gene involved in the susceptibility to daunomycin [53, 98]. Quercetin is a human Pgp (ABCB1), MRP2 (ABCC2), and BCRP (ABCG2) transporter inhibitor [69, 99]. Quercetin reverts the resistance to camptothecin in *L. (L.) donovani* that overexpresses LdABCG6 involved in resistance to camptothecin and arsenite [30] and is associated with reduction of accumulation of alkyl-phospholipid drugs such as MIL in *Leishmania* [29]. Synthetic flavonoid dimmers exhibit a significant reversing activity on pentamidine and sodium stibogluconate resistance in *L. (S.) enriettii* and *L. (L.) donovani* [42]. This modulatory effect is dose-dependent and due to the bivalent nature of the flavonoid compounds. Compared to other MDR inhibitors such as verapamil, reserpine, quinine, quinacrine, and quinidine, these compounds are the only agents that can reverse sodium stibogluconate resistance in *L. (S.) enriettii*. These modulators exhibit reversal activity on pentamidine resistance, comparable to that of reserpine and quinacrine but whatever the level of overexpression of *Lemdr1* gene suggesting that these modulators are not specific to LeABCB4 (LeMDR1). Recently, new compounds derived from aurone, flavones, isoflavones, xanthone, chalcones, and trolox were evaluated against antimony-resistant strains of *L. (L.) major* [43]. Two trolox carboxamides induce reversion of antimony resistance in the promastigote

form of *L. (L.) major*. These two compounds are specific reversal agents targeting the *Leishmania* ABCI4 transporter. This transporter belongs to an unclassified group of proteins in the ABC family with no known homology with other eukaryotic ABC proteins but with orthologues in *Trypanosoma brucei* and *Trypanosoma cruzi* [100]. ABCI4 is a protein located in the plasma membrane and mitochondria of the parasite and efflux antimony. Overexpression of ABCI4 confers resistance to antimony.

### 14.2.4 Sesquiterpenes

Agarofuran sesquiterpenes, e.g., natural compounds isolated from *Maytenus cuzcoina* [101, 102], *M. chubutensis* [91], *M. macroparta* [103], *M. magellanica* [91], *M. apurimacensis*, [104] and *Crossopetalum tonduzii* [105], are new promising reversal agents that overcome the multidrug-resistant phenotype in *Leishmania*, including the resistance to anthracyclines (daunomycin) and alkyl-lysophospholipids (MIL and edelfosine). In *L. (L.) tropica*, dihydro-β-agarofuran sesquiterpenes enhance accumulation of calcein, a Pgp substrate, probably due to Pgp-like transporter inhibition [91]. These compounds bind to the $NBD_2$ C-terminal of *L. (L.) tropica* Pgp-like transporter, LtrMDR1 (LtrABCB4) [105]. A series of dihydro-β-agarofuran sesquiterpenes isolated from the leaves of *Maytenus cuzcoina* or semisynthetic derivatives have been tested on *L. (L.) tropica* parasites overexpressing Pgp [101]. Three-dimensional quantitative structure-activity relationship using the comparative molecular similarity indices analysis (3D-QSAR/CoMSIA) is employed to characterize the steric, electrostatic, lipophilic, and hydrogen-bond-donor and hydrogen-bond-acceptor requirements of these sesquiterpenes as modulators at Pgp-like transporter. The most salient features of requirements are the H-bond interaction between the substituents at the C-2 and C-6 positions with the receptor. The structure-activity relationship (SAR) suggests that a substituent at the C-2 position seems to be essential for reversal activity in the MDR *Leishmania* line by acting as a H-bond acceptor. The furan ring at the C-6 position seems to form a hydrogen bond with the receptor. The introduction of a carbonyl group, capable of acting as a H-bond acceptor in the H-bond with the receptor, produces a tenfold higher chemosensitization. This suggests a direct interaction with the receptor. These results would be used to design and synthesize more effective and specific new Pgp inhibitors.

Sesquiterpene C-3 remarkably sensitizes multidrug-resistant parasites to MIL and edelfosine by increasing alkyl-lysophospholipid accumulation [53]. Moreover, *mdr1* gene transfections can alter membrane fluidity in mammalian cells and change alkyl-lysophospholipid effects [106, 107].

Nortriterpene, extracted from *Maytenus chubutensis* and *M. magellanica* (Celastraceae family), shows only moderate MDR1 reversal activity in a *L. (L.) tropica* strain overexpressing LtrMDR1, involved in daunomycin resistance [64].

Glycyrrhizic acid, a triterpenoid saponin isolated from the root of the liquorice plant, limits infection with sodium antimony gluconate (SAG)-resistant *L. (L.)*

*donovani* in combination with SAG treatment [45]. Glycyrrhizic acid enhances antimony retention by inhibition of MRP1 and Pgp expression levels in splenic macrophages from infected mice. Glycyrrhizic acid acts by modulation of host ABC transporters. Glycyrrhizic acid suppresses cell surface expression of MRP1 and Pgp in host macrophages.

### 14.2.5 Statins: Lovastatin

Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, belong to a family of lipid-lowering drugs that are currently used for the control of hyperlipidemia and are considered useful for protection from cardiovascular events. Apart from the cholesterol-lowering activity of statins, the immunomodulatory and pleiotropic effects of statins may significantly impact infection-related survival [108, 109]. Statins interfered with the growth of protozoan parasites in the Trypanosomatidae family, such as *Trypanosoma cruzi* and various *Leishmania* species [110–112].

Statins are also inhibitors of Pgp in cancer cells [66, 113, 114]**.** Additionally, in Plasmodium falciparum, atorvastatin has synergistic effects in combination with antimalarial drugs such as dihydroartemisinin, quinine, or mefloquine [115–117]. atorvastatin acts probably by inhibition of MDR-like proteins, which are involved in malaria resistance.. In *Leishmania*, the combination of the antifungal drug miconazole and lovastatin is synergic in terms of inhibition of promastigote proliferation, macrophage infection, and amastigote number [118]. In promastigote cultures, the effect is more marked in *L. (L.) amazonensis* parasites than *L. (L.) donovani*. But it seems that this effect is due to inhibition of sterol biosynthesis by both lovastatin and miconazole. More recently, lovastatin, which can inhibit both Pgp and MRP1 (ABCC1), allows the accumulation of sodium antimony gluconate in resistant *L. (L.) donovani* and reversion of antimony resistance [46]. Lovastatin can induce not only the retention of antimony compounds but also that of an unrelated chemotherapeutic agent such as doxorubicin in cancer cells.

### 14.2.6 Pyridine Analog: PAK-104P

A pyridine analog, PAK-104P, was demonstrated in vitro as well as in vivo to inhibit Pgp-mediated multidrug resistance to vincristine, adriamycin, doxorubicin, paclitaxel, and antimonial and arsenical drugs [119–124]. PAK-104P partially reverses the resistance and increases the arsenite accumulation in cancer cells that overexpress MRP1 (ABCC1) [125]. PAK-104P can inhibit both Pgp and MRP [123]. PAK-104P also blocks the energy-dependent efflux of pirarubicin in *L. (V.) braziliensis*, *L. (V.) guyanensis*, and *L. (L.) mexicana* [39]. This compound probably alters the activity of trypanothione reductase and the transport activity by decreasing the intracellular level of reduced trypanothione.

Oxazolo[3,2-α]pyridine derivatives produce a significant reversion of resistance to both MIL and daunomycin in a MDR1 overexpressing *L. (L.) tropica* strain [47].

### 14.2.7 Sulfonylurea: Glibenclamide

Glibenclamide is a sulfonylurea that inhibits ABC proteins such as Pgp (ABCB1) [69, 126] and MRP1 (ABCC1) of cancer cells [127].

Glibenclamide increases calcein accumulation in *L. (L.) amazonensis*-resistant line, like verapamil [78]. Cyclosporin-A, which is a specific inhibitor of Pgp, doesn't increase calcein accumulation. These results demonstrate that the drug transport systems expressed in *L. (L.) amazonensis* are susceptible to MRP (ABCC) inhibitors like glibenclamide or verapamil, but not to the Pgp (ABCB) inhibitor like cyclosporin-A. The increased expression of MRP1 (ABCC1) at the plasma membrane of the protoplast of *Arabidopsis thaliana* is associated with an increase in the resistance of *Arabidopsis* to $Sb^{III}$ and a decrease of $Sb^{III}$ accumulation in protoplast [128]. The simultaneous administration in vitro of glibenclamide, a human MRP1 (ABCC1) inhibitor, increases the efficacy of Glucantime and decreases the infection rate of infected macrophages by *L. (L.) major* [49]. A fixed concentration of 50 μM glibenclamide in combination with various concentration of Glucantime caused an inhibition of 80–90% in cell growth. The administration of glibenclamide in experimental in vivo settings increases the potency of Glucantime when administered simultaneously and reduces the size of lesions in mice infected with drug-susceptible and drug-resistant *Leishmania* [48]. The Glucantime-glibenclamide combination could represent a novel strategy to fight against *Leishmania* infection.

### 14.2.8 Acridonecarboxamide Derivatives: Elacridar and Zosuquidar

Acridonecarboxamide derivatives, elacridar (LY335979) and zosuquidar (GF120918), modulators of human P-glycoprotein [129, 130], can overcome Pgp (LtrMDR1 or LtrABCB4)-mediated *Leishmania* MIL resistance by increasing intracellular MIL accumulation [131]. Overexpression of LtrABCB4 is involved in MIL resistance [59]. In addition, ABCG4, localized mainly to the parasite plasma membrane, reduced the accumulation of phosphatidylcholine analogs and conferred resistance to alkyl-phospholipids (MIL, edelfosine, and perifosine) when overexpressed [132]. The second ABCG reported, ABCG6, also localized mainly to the parasite plasma membrane, conferred resistance to MIL and sitamaquine when overexpressed in *L. (L.) infantum* [29]. Overexpression of ABCG6 is associated with reduction of accumulation of alkyl-phospholipid drugs into *Leishmania*.

### 14.2.9 Dithiocarbamate: Disulfiram

Disulfiram (Antabuse) is used as an adjunct in the treatment of chronic alcoholism. Disulfiram is able to potentiate the antimalarial action of subcurative doses of chloroquine and amodiaquine in *Plasmodium berghei*- and *P. vinckei petteri*-infected mice [133]. Disulfiram inhibits P-glycoproteins by covalently modifying one or more endogenous cysteine residues (Cys1074) in NBD2 [134]. Modification of only one of the Walker A cysteines is sufficient to inactive Pgp [135]. This drug could be effective in combination with Glucantime [136].

### 14.2.10 Benzoquinones

Bis-pyranobenzoquinones inhibit the activity of Pgp of mammalian cells but not MRP1 (ABCC1) [50]. In addition, these compounds increase the activity of daunorubicin in resistant *L. tropica* line. Bis-pyrano-1,4-benzoquinones are the best modulators in MDR human cancer cells, while bis-pyrano-1,2-benzoquinones exhibit the higher toxicity in combination with daunorubicin in MDR *L. (L.) tropica* line.

### 14.2.11 Quinacrine

Quinacrine is an acridine derivative with antimalarial, antileishmanial, and antitrypanosomal activities [137–139].

Quinacrine can have a synergistic effect in combination with pentamidine in *L. (M.) enriettii* and in *L. (L.) donovani* [42, 51]. Moreover, quinacrine is only effective in the pentamidine-resistant *Leishmania*, not in the sodium stibogluconate-resistant or vinblastine-resistant parasites [42]. Surprisingly, quinacrine not only restores the susceptibility of resistant parasites to pentamidine but also increases the susceptibility of susceptible parasites. This result suggests that the quinacrine target remains unaltered in susceptible and resistant parasites to pentamidine. Whatever the quinacrine target might be, it cannot be an ABC transporter in *Leishmania*.

### 14.2.12 8-Aminoquinolines: Sitamaquine

Sitamaquine (WR6026), an 8-aminoquinoline analog, overcomes the MDR1-mediated resistance to MIL by increasing intracellular MIL accumulation in a *L. (L.) tropica* strain overexpressing MDR1 and resistant to MIL [52]. Additionally, sitamaquine also modulates the activity of MRPA, involved in antimony resistance, in resistant *L. (L.) tropica* strain. Sitamaquine reverses MRPA-mediated resistance to antimony.

## 14.3 Conclusion and Future Trends

Efflux transporters play a key role in the emergence and dissemination of resistant parasites and in the acquisition of additional mechanisms of drug resistance caused by a decrease in intracellular drug concentration. Despite their noticeable divergence in structure and membrane topology, the major efflux systems share a dependence on specific key parameters including (1) the functional assembly of a membrane transporter, (2) the energy required (e.g., ATP, ion antiport, or membrane potential) for active transport, and (3) the presence of affinity sites inside the transporter that are involved in substrate recognition and transport.

The identification of functional domains and the characterization of various interactions with the transported drug may elucidate key parameters that govern efflux activity. At present, some 3D structures have been solved for bacterial drug transporters, and these have allowed the proposal of dynamic and mechanical models for drug transport [140]. The same approach must be used for *Leishmania* infection. Drug-transporter interactions have recently been shown to be an important part of multidrug resistance. In silico modeling is a powerful tool often employed to predict drug properties prior to in vitro and in vivo studies. Modeling efforts are currently being undertaken using both ligand- and transporter-based methods such as structure-activity relationship (SAR) studies, quantitative-SAR (QSAR) studies, hologram QSAR (HQSAR), comparative molecular field analysis (CoMFA) and comparative molecular similarity index analysis (CoMSIA) studies, pharmacophore modeling, homology modeling, and molecular dynamics studies. The most common approaches to discover human ABC substrates and inhibitors are development of QSAR models and SAR. This approach has been carried out in the case of human ATP-transporter multidrug resistance-associated protein 2 (MRP2 or ABCC2) [141]. The goal of QSAR modeling is to construct a mathematical relationship between descriptors and pharmacological activities of compounds. The model can then be used to predict the activity for an untested compound. The goal of SAR is usually to discern the structural features or side groups that directly lead to the desired activity under investigation. In order to use these in silico modeling techniques, compounds need to be screened to find the degree of substrate binding to inhibition. Until now, there are no or very few inhibitors or substrate datasets available for ABC transporters in *Leishmania* in literature. Some compounds with inhibitory effects toward human ABCB1 (Pgp) and ABCC1 (MRP1) transporters were studied by pharmacophore modeling, docking, and 3D QSAR to described the binding preferences of these proteins [142]. Docking of selective inhibitors into the Pgp binding cavity by the use of a structural model based on the recently resolved Pgp structure confirms the Pgp pharmacophore features identified and reveals the interactions of some functional groups and atoms in the structures with particular protein residues. However, due to the complex nature of the applied methods, useful interpretation of the models that can be directly translated into chemical structures by the medicinal chemist is rather difficult.

The aim of these efforts is to decipher the molecular basis of drug transport, to explain how differences in chemical structures modify interactions with the

transporter, or to elucidate how the transporter functions in general. In addition, original molecules have been demonstrated to restore the antileishmanial activity of drugs that are pump substrates, and these studies make it possible to identify pharmacophoric groups that are involved in efflux inhibition.

These data are crucial for the design of (1) new antileishmanial molecules that are devoid of efflux-substrate characteristics and can reach a normal intracellular accumulation level and (2) new compounds that have strong efflux pump affinity associated with a high inhibitor capability and block the pump, restoring the intracellular concentration of antileishmanial drugs.

The most prevalent mechanisms of resistance in *Leishmania* are mutations of proteins involved in the drug transport (uptake or efflux) and amplification of transporter genes. The role of ABC transporters in drug resistance in *Leishmania* is well established. Several modulators have been described to reverse multidrug resistance in vitro in *Leishmania.* Most of these drugs remain to be evaluated in vivo. Hence, clinical evaluation of therapeutic regimens is now required to validate the efficacy of these promising compounds or combinations for the treatment of leishmaniasis.

Another perspective is to modulate proteins which participate to the regulation of the expression of the level of MDR1 in *Leishmania*. Silent information regulator 2 (Sir2) is involved in *Leishmania* survival by preventing programmed cell death [143]. Sir2 plays a role in regulating the expression of MDR1 and thereby amphotericin-B (AMB) efflux from the resistant *L. (L.) donovani* [144]. Inhibition or deletion of Sir2 allele shows decreased expression levels of MDR1 and lower efflux of AMB in resistant parasites. In contrast, Sir2 overexpression in susceptible parasites leads to resistant phenotype associated with reduced activity of AMB, increased drug efflux, and increased mRNA level of MDR1. Sir2 will be used as a potent drug target for *Leishmania* treatment.

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