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MINI-REVIEW

Availability and applications of *A*TP-binding *c*assette (ABC) transporter blockers

Alicia Ponte-Sucre

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Abstract ATP-binding cassette (ABC) transporters encompass membrane transport proteins that couple the energy derived from ATP hydrolysis to the translocation of solutes across biological membranes. The functions of these proteins include ancient and conserved mechanisms related to nutrition and pathogenesis in bacteria, spore formation in fungi, and signal transduction, protein secretion and antigen presentation in eukaryotes. Furthermore, one of the major causes of drug resistance and chemotherapeutic failure in both cancer and anti-infective therapies is the active movement of compounds across membranes carried out by ABC transporters. Thus, the clinical relevance of ABC transporters is enormous, and the membrane transporters related to chemoresistance are among the best-studied members of the ABC transporter superfamily. As ABC transporter blockers can be used in combination with current drugs to increase their efficacy, the (possible) impact of efflux pump inhibitors is of great clinical interest. The present review summarizes the progress made in recent years in the identification, design, availability, and applicability of ABC transporter blockers in experimental scenarios oriented towards improving the treatment of infectious diseases caused by microorganisms including parasites.

A. Ponte-Sucre (✉)
Laboratorio de Fisiología Molecular,
Instituto de Medicina Experimental, Facultad de Medicina,
Universidad Central de Venezuela,
Caracas, Venezuela
e-mail: aiponte@gmail.com

Present address:
A. Ponte-Sucre
Institut für Molekulare Infektionsbiologie, Universität Würzburg,
Röntgenring 11,
97070 Wurzburg, Germany

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Introduction

ATP-binding cassette (ABC) transporters comprise an ancient superfamily of evolutionarily conserved proteins spanning from bacteria to humans (Dassa and Bouige 2001). They are integral membrane proteins that actively transport chemically diverse substrates across the lipid bilayer of cellular membranes and are involved in many cellular processes (Higgins 1992; Holland and Blight 1999). One of their outstanding characteristics is that the movement of molecules through ABC transporters, coupled to ATP hydrolysis, can be outward (export, secretion) or inward (import; Holland and Blight 1999; Saurin et al. 1999).

In bacteria, ABC transporters may export substrates, including drugs and antibiotics, or mediate the uptake of essential nutrients (Sheps and Ling 2006). In fact, in prokaryote systems, ABC transporters are required for the uptake of small solutes like histidine, maltose, peptides, or ribose (Ehrmann et al. 1998; Holland and Blight 1999); however, most ABC transporters found in fungi and parasites function almost exclusively as exporters, mediating the translocation of substrates from the ATP-rich cytosol out of the cell or into intracellular organelles (Saurin et al. 1999; Sheps and Ling 2006). Notably, certain ABC transporters found in bacteria, pathogenic fungi, and parasites are involved in resistance to antimicrobial drugs (Klokouzas et al. 2003; Lage 2003; McKeegan et al. 2004).

In mammals, ABC transporters are of great clinical interest, as they are associated with chemoresistance mainly through overexpression of the multidrug-resistance (MDR)

proteins (also known as ABCB1; Borst et al. 2000), MRP (also known as ABCC1; Cole and Deeley 1998; McKeegan et al. 2004), and the breast cancer resistance proteins (BCRP, ABCG2; mitoxantrone resistance proteins; Doyle et al. 1998; Maliepaard et al. 1999). Mammalian ABC transporters are also involved in diseases like cystic fibrosis where mutations in the chloride anion transporter regulator impair the movement of chloride through channels located in secretory epithelia (Holland and Blight 1999; Dean et al. 2006), secondary diabetes and children's hyperinsulinemic hypoglycemia where mutations in the sulfonylurea regulator impair the correct function of the ATP-sensitive K^+ channel present in the plasma membrane of pancreatic cells (Thomas et al. 1996; Cole and Deeley 1998; Dean et al. 2006), or the X-linked peroxysome disorder, known as adrenoleucodystrophia, where mutations in the ABCD1 gene encoding the adrenoleukodystrophy protein impair the transport of a yet unknown substrate into the peroxysome (Berger and Gartner 2006). Additionally, ABC transporters like TAP1 and TAP2 transport antigenic peptides across the endoplasmic reticulum and play a central role in antigen processing. Finally, ABC transporters may be located in epithelial cells and are, therefore, associated with drug delivery and interaction (Dean et al. 2006).

Of note, more than 100 ABC transporters have been described until now, spanning from bacteria to humans; however, the function of many of these conserved and relevant proteins still remains unknown (Pedersen 2005; Sheps and Ling 2006).

The present review summarizes the progress made in recent years in the identification, design, feasibility, and value of ABC transporter blockers in experimental and therapeutic scenarios associated with infectious diseases caused by microorganisms including parasites.

How to define an ABC transporter?

An ABC transporter is a transport ATPase. ABC specifies for "ATP binding cassette", a term used by Hyde et al. (1990) and Higgins (1992) in reference to the distinguishing feature of this transport ATPase class (Pedersen 2005). These transporters usually contain the "Walker A and B consensus motifs" twice in the same polypeptide chain, denoting the presence of two nucleotide binding sites; however, the two nucleotide binding sites can also be found in different polypeptides (McKeegan et al. 2004; Pedersen 2005). An updated description of the nomenclature on ABC transporters is provided at http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html.

Most of our knowledge about ABC transporters is derived from studies of the mammalian P-glycoprotein (P-gp), an organic cation pump that is the product of the *ABCB1* gene (Leonard et al. 2003; Higgins 2007). Comparison of the hydropathy profiles of mammalian P-gp with a multifunctional family of bacterial transporters revealed that both protein types share an extensive transmembrane region resembling that found in pore-forming plasma membrane proteins (Gerlach et al. 1986). The comparison also revealed that their C-terminals contain sequences homologous to the nucleotide-binding domains of ATP-binding proteins (Gerlach et al. 1986). These data permit the recognition of P-gp as a eukaryotic homologue of the multifunctional family of bacterial transporters, later known as the ABC superfamily (Sheps and Ling 2006). Finally, the close similarity of the P-gp with the hemolysin B transport protein supports a functional model for P-gp as an efflux pump which transports a wide variety of substrates (Gerlach et al. 1986; Hyde et al. 1990).

Due to limited writing space and as this review will cover only a specific area of the literature related to ABC transporters and their blockers, readers are referred to the following comprehensive and excellent reviews on the structure and function of ABC transporters including details on their evolution, distribution, biology, and physiology: Hyde et al. 1990; Higgins 1992; van Veen and Konings 1997; Ambudkar et al. 1999; Holland and Blight 1999; Dassa and Bouige 2001; Paulsen 2003; McKeegan et al. 2004; Jones and George 2005; Dean et al. 2006; Shilling et al. 2006; Higgins 2007.

Structure–activity relationships in ABC transporter blockers

Although the physiological functions of ABC transporters are not well known, their wide distribution through all kingdoms in nature strongly suggest that they must be crucial for cell and organism survival. Furthermore, a common characteristic of ABC transporters is their location either at the plasma membrane or at the membranes of intracellular organelles, as well as their extremely conserved role in the translocation of molecules through different cell barriers.

Despite the structural variations found among ABC transporters, most of these proteins recognize similar dyes, toxic ions, and clinically relevant antibiotics and anticancer drugs (van Veen 2001). Typically, ABC transporters possess transmembrane domains, which anchor the protein to the membrane, and form a pore through which the transport of substrates occurs, and the ATP binding domains, which provide the molecular compartment where the ATP energy is released (McKeegan et al. 2004). However, high-resolution structural data are available for only few of the ABC transporters (Holland and Blight 1999; McKeegan et al. 2004; Higgins 2007), and the three-dimensional struc-

tures that are known, although valuable, provide only a static view of the transporter (McKeegan et al. 2004). For a full understanding of drug/ABC transporter interaction mechanisms and biology, the structure of many transporters, with and without substrates, is thus required (McKeegan et al. 2004; Higgins 2007).

Furthermore, still to be elucidated are the architecture of drug and modulator binding sites, the way in which the energy released from the ABC domains is transferred to the transport module, and the details of the transport mechanism (Pedersen 2005; Sheps and Ling 2006; Higgins 2007). This information is fundamental for a rational design of drugs to block the activity of ABC transporters (van der Heide and Poolman 2002; Shilling et al. 2006).

The substrate molecules for ABC transporters exhibit a wide variety of chemical structures (Holland and Blight 1999). Inorganic ions, organic compounds, bile acids, glutathione and glucoronide conjugates, lipids, and even small peptides can be translocated through ABC transporters (Holland and Blight 1999). This wide variety of substrates impairs an understanding of the structure–activity relationship (SAR) that underlies the transport mechanisms of ABC transporters (Stouch and Gudmundsson 2002; Boumendjel et al. 2005) and hinders the design of specific inhibitors needed to block their function.

Finally, due to the wide distribution and multiple mechanisms related to ABC transporters from bacteria to humans, an effective inhibition of ABC transporters in the organisms where they have been described requires more knowledge about their physiological functions and the factors affecting their expression (van Veen and Konings 1997), as well as about fundamental principles which are common to ABC transporters (Holland and Blight 1999).

Development of ABC transporter blockers

A mechanism that reduces intracellular drug accumulation constitutes a simple and efficient way to achieve drug resistance because it reduces the amount of drug reaching the target, and thus, the level of cytotoxicity (Fojo and Bates 2003). Indeed, drug export by transporters is a common mechanism for drug resistance (Fojo and Bates 2003), and the discovery and/or design of transporter blockers is thus a clinically relevant endeavor. Unfortunately, progress in this area has been slower than expected. In fact, P-gp inhibitors should prevent drug extrusion from cells by ABC transporters, and thus, increase the cytotoxic action of drugs transported by ABC transporters, but as they act on multiple ABC transporters, they could induce an enhanced level of toxicity at different non-target sites (Sheps and Ling 2006). Nevertheless, the information obtained to date has been helpful for understanding the SAR, the architecture of the binding sites for drug and modulator, and the link between energy generation and drug translocation functions, as well as for the rational design of drugs to block the activity of ABC transporters.

P-gp modulation occurs by different means for different classes of compounds. P-gp inhibitors may interact directly with the protein through one or more of the P-gp binding sites, thereby blocking (competitively or non-competitively) the transport of substrates. Interestingly, the data suggest them to be multiple interacting drug-binding sites, but can also hold one single, large flexible pocket (Higgins 2007). Alternatively, an inhibitor may prevent ATP binding, ATP hydrolysis, or the coupling of ATP hydrolysis to the translocation of substrates (Ambudkar et al. 1999).

ABC transporter blockers are classified in three generations. First generation inhibitors are pharmaceuticals already in use for other treatments but also able to block P-gp, such as calcium channel inhibitors like verapamil, immunosupressants like cyclosporin A, anti-arrhythmics and neuroleptics like quinidine, reserpine, and yohimbine, and antiestrogens like tamoxifen and toremifene (Table 1; Varma et al. 2003). The clinical efficacy of these compounds is limited by their toxicity (Krishna and Mayer 2000; McKeegan et al. 2004). For example, verapamil produces cardiac cytotoxicity at the concentration needed to inhibit drug resistance. This fundamental drawback has stimulated the development of modulators lacking the toxic side effects of first generation compounds.

Second generation P-gp modulators like R-verapamil, GF120918, MS-209, PSC-833, VX-710, or VX-853 were derived from first generation P-gp modulators with the specific purpose of decreasing drug resistance (Table 1). For example, the compound PSC-833 (valspodar) was developed in an effort to find a non-immune-suppressant analogue of the natural chemosensitizer, cyclosporine A (Boesch et al. 1991). This compound is tenfold more active than cyclosporine in blocking the ABC transporters associated to drug resistance and has already undergone clinical trials (Fracasso 2001). Unfortunately, although second generation modulators are much less toxic than first generation modulators, they can still produce extreme side effects (Krishna and Mayer 2000; Fracasso 2001; McKeegan et al. 2004), especially due to an inhibition of multiple cell ABC transporters and to drug–drug interactions (Balayssac et al. 2005; Varma et al. 2003).

Third generation modulators such as LY335979, OC144093, R101933, and XR9576, which are highly selective inhibitors of P-gp, are still under development (Table 1; Dantzig et al. 2003).

Analysis of the large variety of compounds that can be substrates or blockers of ABC transporters revealed that the design of these chemosensitizers should, in general, include one protonable nitrogen as well as a planar aromatic region.

Table 1 Selected ABC transporter (P-gp-specific) inhibitors[a]

Pharmacological group	*Name of the individual drugs*
Analeptics	Almitrine
Antiarythmics	Amiodarone, lidocaine, quinidine[b]
Antibiotics	Clarithromycine, erythromicine
Anticancer drugs	Actinomycine D, doxorubicin, vinblastine
Antidepressants	Chlorimipramine, imipramine, paroxetine
Antiestrogens	Tamoxifen[b], toremifena[b]
Antihistamines	Terfenadine
Anti-parasitic	Chloroquine, emetine, hydroxicholoroquine, quinacrine, quinine
Calcium channel blockers	Bepridil, diltiazem, felopidine, nifedipine, nisolpidine, nitrendipine, tiapamil, verapamil[b]
Coronary vasodilators	Dipyrodamole
Diuretics	Amiloride
HIV protease inhibitors	Indinavir, retanavir, ritonavir, sequinavir
Immunosupressants	Cyclosporin A[b], FK 506, PSC833
Neuroleptics	Reserpine[b], yohimbine[b]
Local anesthetics	Bipucaine
Proton pump inhibitors	Esomeprezole, lansoprazole, omeprazole
Steroid hormones	Progesterone
Surfactants	Triton X-100, Tween 80, vremophor-EL
Toxic peptides	N-acetyl-leucyl-leucinal, gramcidine, D, valinomycin
Others	GF120918[c], LY335979[d], MS-209[c], OC144093[d], PSC-833[c], R101933[d], R-verapamil[c], VX-710[c], VX-853[c], XR9576[d]

[a] Modified from Wiese and Pajeva 2001, Lage 2003, Varma et al. 2003, Mullin et al. 2004
[b] First-generation inhibitors
[c] Second-generation inhibitors
[d] Third-generation inhibitors

In addition, the compounds should be highly lipophilic and able to form numerous and strong H-bond interactions with the ABC transporter (Seelig 1998). Following these guidelines, numerous compounds have been either synthesized or isolated from plants and then tested as ABC transporter blockers, especially against P-gp and MRP-1.

Recently designed compounds include inhibitors having the 17-azapentacyclo[6,6,5,$0^{2,7}$,$0^{9,14}$,$0^{15,19}$]-nonadeca-2,4,6,9(14),10,12-esene-16,18 dione scaffold (Bisi et al. 2006). These compounds inhibited the translocation of fluorescent rhodamine 123 through ABC transporters expressed in L5178 mouse T cell lymphoma cells infected with the pHq MDR1/A retrovirus (Bisi et al. 2006). Importantly, compounds with the dione scaffold inhibit drug translocation fivefold times more effectively than verapamil.

Natural inhibitors include flavonoids extracted from plants. They have a very low toxicity and increase the cellular accumulation of substances like the fluorescent substrate rhodamine 123 in MDR P-gp-overexpressing KB-C2 cells due to a selective inhibition of ABC transporters (Kitagawa 2006). These flavonoids are many times more active than verapamil; their inhibitory activity was found to correlate with their hydrophobicity as measured through their capacity to dissolve in octanol and are much safer than previously designed chemosensitizers (Choi et al. 2002). Of note, flavonoids do not bind to the transmembrane domains, but rather to the nucleotide-binding domain of ABC transporters, although in a site different from that occupied by ATP (Dayan et al. 1997).

Many P-gp inhibitors have also been tested against MRP-1 or BCRP, but unfortunately, most of them had no inhibitory effect (Allen et al. 2002; Boumendjel et al. 2005). This was somehow predictable, as P-gp recognizes hydrophobic compounds, while MRP-1 recognizes hydrophilic substrates (Boumendjel et al. 2005), although difficult to understand as BCRP transports structurally and functionally diverse organic substrates, including hydrophobic compounds, weak bases, organic anions, and glucuronide-, sulfate-, glutamylate- and glutathione-conjugates (van Herwaarden and Schinkel 2006).

ABC transporter blockers: difficulties associated with their design

ABC transporters have a strongly conserved primary sequence; however, few orthologous pairs of transporters are shared between phyla (Sheps et al. 2004). This characteristic, together with the extended substrate flexibility recognition of ABC transporters, suggests that redundancy in ABC transporters for the same substrate renders gene loses tolerable (Sheps et al. 2004). Furthermore, the presence of multiple binding sites complicates the understanding of a SAR for P-gp and MRP-1 and hinders the development of inhibitors (Wiese and Pajeva 2001; Varma et al. 2003; Boumendjel et al. 2005).

Additionally, the speed at which P-gp and ABC transporters in general move substrates across membranes is crucial, and the analysis of drug resistance of a cell to a given compound must thus consider the membrane and the transporter as a functionally integrated unit (Sheps and Ling 2006). For example, in the case of hydrophobic compounds and their interaction with P-gp, the deciding factor in determining whether a compound is a P-gp substrate or inhibitor is the speed at which the substrate migrates across the membrane rather than its membrane affinity (Etian et al. 1996).

Slowly permeating substances are transported out of the cell by P-gp, whereas those permeating rapidly act as P-gp inhibitors. This means that if the P-gp modulator is cycled repeatedly, it prevents the export of the accompanying drug, increasing its concentration in the cell; in this case, the

modulator acts as a competitive inhibitor (Varma et al. 2003). Furthermore, changes in membrane dynamics might allow drugs to enter the cell more rapidly than P-gp can export them, thereby reducing resistance (Kamau et al. 2005). Taken together, this means that the critical parameter determining whether a cell resists a drug is not the extracellular concentration of drug, but rather the intracellular concentration set by the equilibrium between drug inflow through the membrane and drug export by the cell's competent transporters (Sheps and Ling 2006).

The central paradigm of SAR is that compounds with similar structures act at the same site and with the same mechanism (Wiese and Pajeva 2001; Stouch and Gudmundsson 2002; Boumendjel et al. 2005). Unfortunately, as already discussed, inhibition and/or binding at P-gp, MRP-1, or ABC transporters in general can occur via different mechanisms as well as at a number of different sites. As a consequence, a general SAR of substrate or inhibitors of ABC transporters has been difficult to achieve, and a successful design of ABC transporter substrates, modulators, and blockers has thus been hindered by an incomplete understanding of the mechanisms and biology of these transporters.

Applications of ABC transporter blockers

ABC transporters expressed in wild-type cells help to maintain low levels of xenobiotics in the cells, modulate drug–drug interaction, and facilitate normal disposal of drugs. In fact, their function limits the achievements of membrane transport systems because they pump back substances into the compartments where they were originally located (Sheps and Ling 2006).

One of the purposes of the present review was to update the progress made in recent years in the identification, design, feasibility, and value of ABC transporter blockers linked to diseases caused by microorganisms including parasites. Due to the multiple mechanisms where ABC transporters are involved, their blockers are extremely valuable, and they may contribute at either the cellular or the systemic level to increase the efficiency of the primarily administered drug or to predict whether an organism is resistant to a drug or to trace the location of ABC transporters in vivo. Herein, we will briefly present some of these applications and stress the significance that ABC transporter blockers could have for the clinical scenario.

Contributions of ABC transporter blockers at the cellular level

As drug transporters are responsible for much of the increasing development of drug resistance, and thus, for one of the main impediments to successful chemotherapy, various approaches have been undertaken to overcome this problem. One approach would be to develop drugs that are minimally used as substrates by the ABC transporters. On the other hand, the design of specific blockers for ABC transporters is one of the most promising approaches to overcome the limited success of antimicrobial and anti-parasitic chemotherapy.

The search for new antibiotics and anti-parasitic drugs that are minimally used as ABC transporter substrates has been going on, as the drug efflux mediated by these proteins was identified as a mechanism of drug resistance. To this end, new ketolides, macrolides, and tetracyclines as well as fluoroquines are designed against both $Gram^+$ and $Gram^-$ bacteria (Lomovskaya and Watkins 2001). For example, the new tetracyclines (glycylcyclines) do not generate drug resistance because they are not recognized by ABC transporters, and therefore, are not transported out of the cell. Approaches to modify existing classes of drugs to enable them to avoid efflux pumps have been less successful in $Gram^-$ compared to $Gram^+$ bacteria. One of the reasons for this is that drugs have to be amphiphilic to cross both membranes of the $Gram^-$ bacteria, a property that makes them good substrates for ABC transporters (Lomovskaya et al. 2001).

On the other hand, the isolation and design of specific blockers for ABC transporters to act against microorganisms has also been attempted. For example, quinolone derivatives, as well as other alkoxyquinoline derivatives and semi-synthetic derivatives of tetracyclines, inhibit the efflux of antibiotics and restore, at least partially, the susceptibility of bacteria to different antibiotics (Nelson and Levy 1999; Mallea et al. 2003; McKeegan et al. 2004). However, the success of this approach has so far been limited. Although the activity of classical ketolides and macrolides against $Gram^+$ macrolide-resistant bacteria is enhanced by the presence of an ABC transporter blocker, this is not the case for newly designed macrolides or fluoroquinolines (Lomovskaya and Watkins 2001).

In the case of parasites, it has recently been reported that dihydroethanoanthracene derivatives (Pradines et al. 2002) and dihydro-β-agarofuran sesquiterpenes isolated from the roots of *Mayetna magallanica* and *M. chubutensis* have been tested as inhibitors of ABC transporters in both *Leishmania* and *Plasmodium* (Kennedy et al. 2001; Pradines et al. 2002). Additionally, of 21 classical anti-parasitic drugs, most of which are flavonoids, 14 inhibit ABC transporters expressed in human intestinal epithelial CaCo-2 cells. Only quinine, which is also the most active compound all 21 drugs, is simultaneously an inhibitor and a substrate of the ABC transporters expressed in these cells; the rest of the anti-parasitic drugs act only as inhibitors (Hayeshi et al. 2006). Notably *Leishmania* promastigotes resistant to ABC transporter blockers have been selected

and characterized (Ponte-Sucre et al. 1997; Silva et al. 2004; Uzcátegui et al. 2005; Machuca et al. 2006). More significantly, ABC transporter blockers have been used efficiently in vivo to increase the potency of classical drugs and reduce the size of lesions in BALB/c mice infected with drug-resistant *Leishmania* (Serrano-Martin et al. 2006), suggesting that, at least in experimental settings, the simultaneous administration of ABC transporter blockers increases the efficiency of the primarily administered drug.

Contributions of ABC transporter blockers at the systemic level

ABC transporters are extensively localized in normal tissues. Their active participation in many functions could control at the end the tissue levels of exogenous compounds, medicaments, and xenobiotics. This means that although a key issue in designing ABC transporter blockers is the identification of drugs that inhibit the efflux pump and enhance the effect of the primarily administered drug, additional (side) effects related to the physiological functions of ABC transporters should be expected. Furthermore, the combined use of ABC transporter substrates and blockers could facilitate the analysis of drug resistance in patients and help to identify where ABC transporters are located in vivo.

For example, an area currently not explored regarding the availability of antibiotics and anti-parasitic drugs is the use of ABC transporter blockers to enhance the systemic levels of drugs that have been orally administered (Sun et al. 2004). It is known that ABC transporters located in the gastrointestinal tract may limit the absorption of drugs (Leonard et al. 2003). The use of ABC transporter blockers may therefore increase the availability of drugs whose transport back to the lumen of the gastrointestinal track depends on ABC transporters (Leonard et al. 2003). The impact of this, although difficult to predict, could be an immediate change in drug toxicity and side effects, especially if the ABC transporter blocker cross the blood–brain barrier or if they are not themselves toxic (Leonard et al. 2003; Higgins 2007).

In addition, as already mentioned, ABC transporters modulate the efflux or intracellular trafficking of chemotherapeutic agents in cancer and infection. The hydrophobic derivative of calcein (Cal-AM) is a substrate of ABC transporters and can be exported from resistant cells by MDR1 or MRP1. The cellular accumulation of this fluorophore and its inhibition by blockers from any of the three generations (see Table 1) could provide a quantitative measure of cell transport activity through ABC proteins, and has been proposed to represent a proof of concept of a cellular strain being resistant. This approach has been successfully used in acute myeloid leukemia patients (Karászi et al. 2001), and it will therefore be worth standardizing it to enable an analysis of drug resistance in patients infected with bacteria or parasites.

Furthermore, the cellular localization and levels of ABC transporters are normally analyzed by measuring the corresponding mRNA and protein concentrations. However, to study the dynamic function of these proteins in vivo, more sophisticated methods are needed. By the use of ^{11}C-labeled ABC transporter substrates and inhibitors, like technetium-99 m sestamibi (Hendrickse et al. 1999), it is possible to visualize changes in the function of P-gp-mediated transport through positron emission techniques. Although this type of study has not been performed as yet in microorganisms, its standardization and implementation may offer a promising approach for obtaining quantitative measurements of ABC transporter function in vivo.

Finally, as already mentioned by Higgins (2007), probably, the time has come not to fight against drug resistance but to find alternatives to avoid and avert it. In this regard, a promising avenue is the use of ABC transporter blockers to actively prevent the emergence of drug resistance. For example, it would be interesting to explore whether the use of ABC transporter blockers prevents the selection of resistant strains with increased expression of ABC transporters like MDR or MRP. The prevention of chemoresistance through the combination of ABC transporter blockers with the selected drug has the potential drawback that resistance to the inhibitor itself could appear (Leonard et al. 2003). Hence, the risk/benefit profile of the joint use of inhibitors should be evaluated on a case-by-case basis.

Conclusions and outlook

The increasing development of drug resistance is one of the major reasons why cancer chemotherapy fails, and is predicted to constitute one of the main impediments to successful chemotherapy against microorganisms in the near future. Although the design of new drugs not transported by ABC transporters has been evaluated in detail, the isolation or design of blockers to these ABC transporters constitutes one of the most promising approaches to overcoming the lack of success in the aforementioned chemotherapeutic approaches. Several ABC transporter blockers have been successfully tried against bacteria and parasites; however, ABC transporter inhibitors are not yet in use for the treatment of bacterial or parasitic infections or for cancer diseases. Reasons for this are numerous and include the low substrate specificity and the multiple functions subserved by ABC transporters.

So far, designing an appropriate and effective clinical drug trial for P-gp blockers has been difficult, as a clear-cut outcome is not easy to achieve due to the high variability in the results. Additionally, the selection of appropriate

groups of compounds has proven to be difficult, as has been the selection of quality controls. Cross-resistance with other drugs, potential development of resistance against the ABC transporter blockers, confusing pharmacokinetics, and lack of appropriate drug-resistance markers also complicate the task of their design. Current research efforts seek to overcome these problems and to make ABC transporter blockers invaluable tools in the fight against disease as well as in prognostic and preventive applications in chemoresistance.

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