Published in final edited form as:

Clin Pharmacol Ther. 2010 January ; 87(1): 122–125. doi:10.1038/clpt.2009.116.

Transporters for Drug Delivery and as Drug Targets in Parasitic Protozoa

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Abstract

Parasitic protozoa cause devastating diseases over large regions of the globe, but the lack of economic incentives has resulted in limited development of drugs against these ‘neglected diseases’. Transporters expressed in the plasma membranes of these parasites offer potential for development of new drugs. These permeases could be employed in two distinct strategies for drug development, i) to target selective delivery of drugs to the parasite, ii) to develop drugs that inhibit essential parasite permeases.

Parasitic protozoa such as *Plasmodium falciparum*, *Trypanosoma brucei*, and *Leishmania* species are single cell eukaryotes that cause devastating diseases of global importance, including respectively malaria, African sleeping sickness, and leishmaniasis. These diseases are relatively neglected, and lack of financial incentive has resulted in a comparatively limited armamentarium of therapeutic compounds for treatment. In addition, most drugs currently in use suffer from high expense, undesirable side effects, and development of resistant strains of parasites. Hence, new strategies for drug development are urgently needed. A plethora of membrane transport proteins has been identified in these organisms that are typically employed by the parasite to scavenge nutrients from their hosts and vectors. The completed genome projects of several parasites have illustrated the large number of transporters encoded within these genomes and, along with functional studies, have provided molecular identities for some of the transport mediators. A subset of these permeases could serve as potential targets for development of novel drugs. One strategy is to employ endogenous parasite transporters to deliver cytotoxic compounds across the parasite plasma membrane. In this case, the native carrier is used as a ‘subversive transporter’ (Fig. 1, left). Alternatively, a transporter whose function is essential for parasite viability could be selectively inhibited by a compound, resulting in starvation of the parasite for an essential nutrient (Fig. 1, right). The existence of subversive transporters has been demonstrated for several anti-parasitic drugs currently in use. Selective inhibition of essential permeases is a less well-established strategy but one that has promise for future development. This article will focus on several examples of parasite permeases that have demonstrated or potential utility for both approaches to drug development. It is worth noting that parasite transporters, such as efflux pumps [1], can also promote drug resistance, but this topic is beyond the scope of this review.

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USE OF SUBVERSIVE PERMEASES

Drugs against microbial pathogens may enter the target organism by any of several routes, including diffusion across the plasma membrane of the microbe or import on a native pathogen transporter. A drug that utilizes an endogenous microbial permease for entry (Fig. 1) may be effective at a lower concentration than one that must enter by diffusion. Additionally, the parasite permease may provide a selective route for entry of the drug into the target organism but not the cells of the host. Thus a parasite transporter may employ a particular molecular recognition motif that is not utilized by a homologous mammalian transporter. One strategy for drug design is to incorporate a parasite-specific recognition motif into a compound and to conjugate this motif to a second cytotoxic component. Indeed, some current anti-parasitic drugs appear to function this way, although they were not designed with this strategy in mind. Several examples are provided below of drugs that are known to utilize native parasite permeases for entry into the pathogenic microorganism, and these drugs and their cognate transporters are listed in Table 1. A more extensive review of this approach to drug delivery is available in reference [2].

The P2 Purine Transporter of African Trypanosomes and Drug Import

Perhaps the best-studied example of a subversive permease is the P2 adenosine/adenine transporter from *T. brucei*. Classic studies by Carter and Fairlamb [3] identified this transporter as a major route for uptake of melarsen oxide, an active metabolite of the organo-arsenical drug melarsoprol that is used to treat late-stage African trypanosomiasis. Indeed, the P2 permease was identified as the melarsen oxide transporter by the ability of adenosine and adenine, but none of 30 other compounds tested, to block melarsen oxide killing of bloodstream form trypanosomes and subsequently by the demonstrated loss of P2 transport activity in a melarsen-resistant parasite line. Pentamidine is a diamidine drug used to treat early stage African trypanosomiasis. Remarkably, [³H]pentamidine uptake was also inhibited by adenine and adenosine and was greatly reduced in the P2-deficient melarsen oxide-resistant line, strongly suggesting that the P2 permease is a major mediator of pentamidine uptake [4]. Extensive kinetic studies of pentamidine uptake by bloodstream trypanosomes revealed that this drug is imported by the P2 permease and two other transport activities designated HAPT and LAPT for high affinity and low affinity pentamidine transporters [5]. The gene encoding the P2 permease, designated *TbAT1* [6], was cloned and determined to encode a member of the equilibrative nucleoside transporter family (SLC29) that is represented from protozoa to mammals. Two other nucleobase transporters, TbNT11 and TbNT12, are also low affinity transporters for pentamidine [7], but their relative importance in vivo and their potential relationship to HAPT and LAPT are uncertain.

The unexpected observation that P2/TbAT1 transported both melarsen-oxide and pentamidine was reconciled by the suggestion that these two drugs, plus adenosine and adenine, shared a common P2 recognition motif proposed to be essential for interaction of each substrate with the P2/TbAT1 permease (Fig. 2). This observation has led to efforts to link such a P2-targeting motif, contained within the melamine moiety, to various other toxic compounds with the purpose of improving anti-trypanosome activity by increasing selectivity of uptake [9].

Transport of Drugs by Other Purine Transporters

Various purine anti-metabolites, such as the hypoxanthine analogue allopurinol and the inosine analogue formycin B, have selective activity against African trypanosomes and/or *Leishmania* species. Purine nucleobase transporters play central roles in uptake of allopurinol and other purine anti-metabolites, in both African trypanosomes and *Leishmania* [10, 11]. Multiple nucleobase transporters mediate the uptake of allopurinol in *T. brucei* [12], providing a possible mechanism to explain the difficulty in selecting allopurinol-resistant mutants in this parasite. In *L. major*, two nucleobase transporters, LmaNT3 and LmaNT4, are able to mediate uptake of allopurinol [13, 14]. In contrast, the nucleoside analogue formycin B is imported by the single LdNT2 guanosine/inosine/xanthosine purine nucleoside transporter of *L. donovani* [15]. In general, the specificity of activity for these purine antimetabolites does not reside at the transporters but in the purine salvage enzymes of the parasites, which unlike the mammalian enzymes can use these purine analogues as substrates and funnel them into the purine salvage pathways [16]. Nonetheless, the parasite permeases provide the route for efficient entry of these compounds into the target cells. It is noteworthy that the purine permeases are typically high affinity proton-coupled active transporters [17, 18] with K_m values in the low micromolar range. These properties are consistent with the low concentrations of purines in mammalian tissues and the inability of these protozoa to synthesize purines [16], requiring that the parasites elaborate efficient import systems for these essential nutrients. High affinity concentrative transport by these permeases also provides a therapeutic advantage regarding delivery of cytotoxic purine analogue drugs.

Aquaporins

Aquaporins and aquaglyceroporins are channels that mediate the passage across the plasma membrane of water, glycerol, urea, and other low molecular weight compounds, including some metalloids. Homologues of mammalian aquaporins have been identified among parasitic protozoa such as *Plasmodia*, *Trypanosoma*, and *Leishmania* [19], and each species encodes multiple aquaporin paralogs within its genome. Pentostam is a pentavalent antimonial that is a first line drug for treatment of leishmaniasis. In *L. major*, the aquaglyceroporin LmAQP1 mediates the uptake of trivalent antimony, the active metabolite of Pentostam. Thus overexpression of the *LmAQP1* gene induces increased sensitivity to antimony and arsenic-containing compounds, and deletion of one copy of this gene confers decreased sensitivity to these compounds [20].

Miltefosine Transporter

Miltefosine (hexadecylphosphocholine) is a phospholipid analogue that was recently introduced as an orally available drug for treatment of leishmaniasis. The gene for the miltefosine transporter, *LdMT*, was cloned by functional rescue of a miltefosine-resistant mutant and revealed to be an P-type ATPase involved in uptake of aminophospholipids [21]. Resistant parasites transformed with the *LdMT* gene regained sensitivity to the drug and recovered the ability to take up [^{14}C]miltefosine and fluorescent-labeled glycerophospholipids, and the resistant mutant was shown to encompass single but distinct point mutations in both alleles of the *LdMT* gene.

ESSENTIAL TRANSPORTERS AS DRUG TARGETS

Any transporter that is essential for survival of the infectious stage of the parasite constitutes a potential target for development of drugs that inhibit the function of that permease (Fig. 1). Such drugs could represent high affinity substrate analogues, or they could be compounds unrelated in structure to any substrate that function either as competitive or non-competitive inhibitors. Given the limited space available for this review, only two examples of essential parasite permeases that could serve as drug targets are presented below. As we learn more about novel transporters in these microbes, especially with the resources of completed parasite genome projects to aid in the molecular identification of carriers, additional target permeases will be identified (e.g. see reference [22]).

Hexose Transporters

Transporters for hexoses such as glucose, fructose, and mannose have been shown to be essential for the infectious stages of *P. falciparum* [23], *T. brucei* [24], and *L. mexicana* [25]. The substrate analogue approach has been employed to investigate the hexose transporters of both *P. falciparum* and *T. brucei* as potential drug targets. Krishna and colleagues demonstrated that 3-*O*-((undec-10-en)-yl)-D-glucose (compound 3361) selectively inhibits PfHT1 with a K_i value of ~50 μ M compared to 3.3 mM for inhibition of the human glucose transporter Glut1. Furthermore, compound 3361 killed *P. falciparum* intraerythrocytic parasites in culture with an IC_{50} value of ~16 μ M, and the compound significantly reduced growth of the murine malaria parasite *P. berghei* in mouse infections [23]. The *T. brucei* hexose transporter TbHT1 was inhibited by acetylbromo-D-glucoseamine and acetylbromo-6-amino-D-fructose with K_i values in the low millimolar range, and these compounds killed bloodstream trypanosomes in culture with IC_{50} values of 4 and 8.3 μ M respectively [24]. Although the compounds employed are unlikely to serve as effective drugs, these studies establish the hexose transporters of *P. falciparum* and *T. brucei* as valid drug targets. The *L. mexicana* genome encodes three related glucose transporter genes, designated *LmGT1*, *LmGT2*, and *LmGT3*, that are clustered together on an ~14 kb segment of DNA. A knockout of this gene cluster, generated by homologous gene replacement, was unable to replicate as amastigotes (the mammalian life cycle stage) inside murine macrophages, to survive as culture form amastigotes [25], or to induce lesions in BALB/c mice (unpublished data of R. Burchmore and G. Coombs). These results indicate that hexose transporter function is essential for the infectious stage of the parasite life cycle and hence could serve as a potential drug target.

T. brucei Pyruvate Transporter

Bloodstream form African trypanosomes metabolize the abundant glucose in blood by glycolysis but do not possess a functional Krebs cycle or oxidative phosphorylation. Consequently, the parasites generate large amounts of pyruvate as the end product of glycolysis, and this organic acid is excreted by a plasma membrane pyruvate transporter [26]. Inhibition of this permease with [α -cyano- β -(1-phenylindol-3-yl)acrylate] (UK5099), an inhibitor of mammalian plasma membrane and mitochondrial pyruvate transporters, resulted in accumulation of very high levels of internal pyruvate, acidification of the trypanosome cytosol, inhibition of respiration, loss of motility, and ultimately lysis of the

parasites [27]. The marked dependency of trypanosomes on pyruvate export thus establishes this transporter as a potential drug target. The molecular identification of the trypanosome pyruvate transporter will be central to further evaluation of this permease as a drug target.

CONCLUSIONS

Recent studies on parasite transporters suggest that new drugs could be developed utilizing these permeases, either by conjugating parasite-specific transport recognition motifs onto cytotoxic moieties or by synthesizing or screening for inhibitors of essential parasite permeases. Such approaches could employ both synthetic chemistry and large-scale screening of compound libraries and provide both significant challenges and novel opportunities for development of new therapies against diseases of major importance to global public health.

ACKNOWLEDGEMENTS

This work was supported by grants AI25920 and AI44138 from the National Institutes of Health.

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Figure 1.

Use of 'Subversive Transporters' or 'Essential Transporters' for drug development against parasitic protozoa. Subversive transporters (blue oval at left) recognize drugs in addition to their natural substrates and provide a selective route for uptake of the drug into the target microbe. The transported drugs may encompass molecular recognition motifs shared with substrates that allow them to be imported by the parasite permease. Conjugation of a cytotoxic compound to such a recognition motif could provide a selective route for its uptake by the parasite. Essential transporters (red oval at right) are required by the parasite for import of crucial nutrients. Selective inhibition of the transporter (X within the red oval) by a drug would prevent import of the substrate and starve the parasite for an essential nutrient.

6-aminopurines

Melarsen oxide

Pentamidine

Figure 2.

The proposed P2 recognition motif (shaded ovals) present in several substrates of the P2/TbAT1 transporter. This figure has been reproduced from reference 8 with permission of the publisher.

Table 1

Several anti-parasitic drugs discussed in the text are imported by known transporters.

Drug	Parasite	Transporter
Melarsoprol/Melarsen oxide	<i>T. brucei</i>	P2/TbAT1
Pentamidine	<i>T. brucei</i>	P2/TbAT1, HAPT, LAPT, TbNT11, TbNT12
Pentostam	<i>L. major</i>	LmAQP1
Miltefosine	<i>L. donovani</i>	LdMT
Allopurinol	<i>L. major</i>	LmaNT3, LmaNT4
Formycin B	<i>L. donovani</i>	LdNT2