Review

Drug resistance in African trypanosomiasis: the melarsoprol and pentamidine story

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Melarsoprol and pentamidine represent the two main classes of drugs, the arsenicals and diamidines, historically used to treat the diseases caused by African trypanosomes: sleeping sickness in humans and Nagana in livestock. Cross-resistance to these drugs was first observed over 60 years ago and remains the only example of cross-resistance among sleeping sickness therapies. A *Trypanosoma brucei* adenosine transporter is well known for its role in the uptake of both drugs. More recently, aquaglyceroporin 2 (AQP2) loss of function was linked to melarsoprol–pentamidine cross-resistance. AQP2, a channel that appears to facilitate drug accumulation, may also be linked to clinical cases of resistance. Here, we review these findings and consider some new questions as well as future prospects for tackling the devastating diseases caused by these parasites.

> *'Cellular therapy is a consequence of cellular nutrition, for only those compounds can affect the cell that are actually eaten by it.'* – Paul Ehrlich, 1907 [1].

Chemotherapy against African trypanosomiasis

African trypanosomes are parasitic protists that circulate in the bloodstream and tissue fluids of their mammalian hosts. Transmitted by tsetse flies, they cause important human and animal diseases. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* cause human African trypanosomiasis (HAT), also known as sleeping sickness, which is typically fatal without chemotherapy, whereas the closely related, but human-serum sensitive, *T. b. brucei*, *Trypanosoma congolense*, and *Trypanosoma vivax* cause Nagana, an important veterinary disease. HAT affects 8.7 million km^2 of Sub-Saharan Africa, areas where the climate and environment are suitable for the tsetse fly [2]. *T. b. gambiense* is endemic in many areas of West and Central Africa and is currently responsible for the vast majority (>90%) of HAT cases.

For early-stage HAT cases in West Africa, pentamidine, an aromatic diamidine, is the drug of choice. Diagnosis is often late, however [3], revealing advanced infection with trypanosomes in the central nervous system (CNS). In these cases, eflornithine (in combination with nifurtimox) is the safest therapy [4], and availability of these drugs has increased in recent years [5]; nevertheless, the highly toxic, melaminophenyl arsenical melarsoprol is still used. This is explained by the lack of efficacy of eflornithine against *T. b. rhodesiense* [6] and the high cost and difficulty of administration for use against *T. b. gambiense* [5]. Thus, melarsoprol, a drug that causes an often fatal reactive encephalopathy in approximately 10% of patients [7], is currently the only drug active against both advanced *T. b. rhodesiense* and *T. b. gambiense* infections. Melarsoprol and pentamidine are also the most potent drugs used to treat HAT, both displaying low nanomolar 50%-effective growth-inhibitory concentrations (EC_{50}).

There have been three major epidemics of sleeping sickness recorded since the late 19th century. Tsetse control, the systematic screening for patients in at-risk populations followed by chemotherapy, and the introduction of nifurtimox–eflornithine combination therapy (NECT), have all contributed to the recent successful reduction in cases [8]. However, the WHO recently warned against neglect and complacency if further epidemics are to be avoided [9]. With no vaccine available and limited therapeutic alternatives, the emergence of drug resistance is a major threat in this regard [10], especially because loss of a single, non-essential transporter can result in eflornithine resistance [11]. In fact, the high cost and logistical burden of NECT might render this particular treatment unsustainable [5]. Therapies based on arsenicals and diamidines have been prominent in efforts to tackle HAT for over 100 years, and selected important developments during this time are summarised in Figure 1 and detailed below.

The arsenicals

The first organic arsenical, aminophenyl arsonic acid, euphemistically named atoxyl, was introduced as a treatment for HAT in the early 1900s [12]. This drug was partly replaced by its less toxic *N*-substituted derivative tryparsamide (arsonophenylglycineamide) [13] in the early 1920s, although it was not effective against *T. b. rhodesiense* or arsenic-resistant *T. b. gambiense* [14]. Both of

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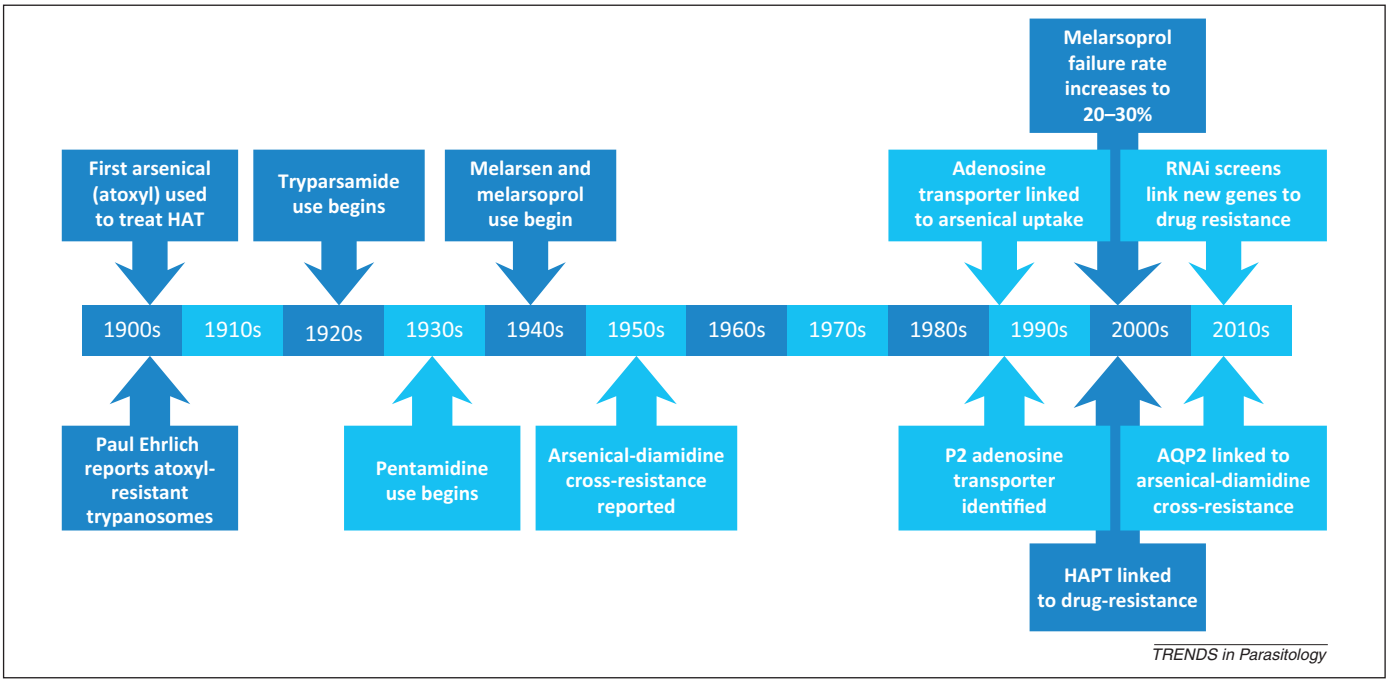

Figure 1. Timeline of arsenical and diamidine therapies against HAT. The timeline indicates selected key developments relating to these therapies and in our understanding of drug uptake and resistance.

these pentavalent arsenic compounds caused serious ocular lesions in many patients [15], and were eventually superseded by the trivalent melaminophenyl arsenicals melarsen (arsonophenylmelamine) [16,17] and, ultimately, melarsoprol [18]. Combining melarsen with British anti-Lewisite (dimercaptopropanol), melarsoprol was by far the least toxic of all the arsenicals. However, it still bears unacceptable adverse effects such as reactive encephalopathy: the presence of trypanosomes in the CNS has been correlated with the incidence of this reactive encephalopathy, suggesting that trypanosome lysis is the trigger for inflammation [19]. Melarsoprol is dissolved in 3.6% propylene glycol, itself an irritant at the site of injection, and is administered over 10 days via intravenous injection [7,20]. This drug penetrates the blood–brain barrier, but only achieves 3–4% of the maximal levels reached in plasma [21]. Melarsen oxide is thought to be the active metabolite [22] that is taken up by trypanosomes, forming a stable adduct with trypanothione known as Mel T [23]. A combination of melarsoprol with cyclodextrins has recently been proposed as an orally administered, safer alternative arsenical therapy [24].

The diamidines

Although initially the rationale for using diamidines was based on their hypoglycaemic effect, aiming to starve the trypanosomes of glucose, the diamidines were soon discovered to be directly trypanocidal [25]. Pentamidine is an aromatic diamidine which has been used in the treatment of HAT since the 1930s. The drug is administered intramuscularly once daily over a 7 day treatment period [26]. Pentamidine is unsuitable for treatment of advanced disease, in part because serum binding and tissue retention reduce blood–brain barrier traversal [27]. Pentamidine, that does cross the blood–brain barrier, is also cleared by efflux transporters such as P-glycoprotein and multidrug resistance-associated protein [28]. Even so, despite limited access to the CNS and efflux from the CNS, pentamidine has been reported to be effective against trypanosomiasis during the early phase of CNS involvement [29,30].

Diamidines are nucleic acid binding drugs [31] that typically become highly concentrated within, and destroy, the mitochondrial genome known as the kinetoplast [32], but they are also seen in the nucleus and acidocalcisomes [33,34]. Thus, pentamidine, and other diamidines, may kill trypanosomes partly through kinetoplast disruption. However, bloodstream-form cells lacking a kinetoplast are viable if they harbour a mutation in the γ subunit of the F_1 component of the mitochondrial ATP synthase, which compensates for loss of the kinetoplast-encoded A6 subunit [35]. In the case of pentamidine, toxicity by other means is certainly likely because the drug accumulates in trypanosomes to millimolar concentrations [36]. Indeed, cells apparently lacking kinetoplast DNA remain sensitive to pentamidine [36], possibly due to disruption of mitochondrial membrane potential [37,38]. Consequently, it is likely that the antitrypanosomal activity of pentamidine is the result of selective accumulation, leading to multiple deleterious effects, rather than to effects on a specific 'diamidine target' [39]. Novel diamidines [40] and pentamidine-like prodrugs [41] with improved pharmacokinetic properties are under development.

Drug resistance and the P2 adenosine transporter

Drug resistance typically emerges when a genetic change (mutation, deletion, or amplification) alters uptake, drug metabolism, drug–target interaction, or efflux. If there is a concomitant fitness cost, it will be less likely that resistant parasites will propagate and spread. The phenomenon of