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REVIEW: FRONTIERS IN PHARMACOLOGY

Human African trypanosomiasis: pharmacological re-engagement with a neglected disease

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This review discusses the challenges of chemotherapy for human African trypanosomiasis (HAT). The few drugs registered for use against the disease are unsatisfactory for a number of reasons. HAT has two stages. In stage 1 the parasites proliferate in the haemolymphatic system. In stage 2 they invade the central nervous system and brain provoking progressive neurological dysfunction leading to symptoms that include the disrupted sleep wake patterns that give HAT its more common name of sleeping sickness. Targeting drugs to the central nervous system offers many challenges. However, it is the cost of drug development for diseases like HAT, that afflict exclusively people of the world's poorest populations, that has been the principal barrier to new drug development and has led to them becoming neglected. Here we review drugs currently registered for HAT, and also discuss the few compounds progressing through clinical trials. Finally we report on new initiatives that might allow progress to be made in developing new and satisfactory drugs for this terrible disease.
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Abbreviations: AIDS, acquired immunodeficiency syndrome; apoL1, apolipoprotein L1; CSF, cerebrospinal fluid; DFMO, difluoromethylornithine; DNDi, the Drugs for Neglected Diseases initiative; FDA, US Food and Drug Administration; FIND, the Foundation for innovative new diagnostics; HAPT1, high-affinity pentamidine transporter 1; HAT, human African trypanosomiasis; LDL, low-density lipoprotein; MSF, Médecins sans Frontières; ODC, ornithine decarboxylase; PCP, *Pneumocystis carinii* pneumonia; TbAT1, *T. brucei* adenosine transporter 1 (the P2 transporter); UNC, University of North Carolina at Chapel Hill; WHO, World Health Organization

Introduction

Human African trypanosomiasis (HAT), perhaps better known as sleeping sickness once the causative trypanosome parasites have established within the central nervous system (CNS) (Barrett *et al*., 2003), is considered as a neglected disease (Remme *et al*., 2002). Neglected diseases are those ailments which affect people from among the world's poorest populations, for which satisfactory treatment does not exist, but for which the investment required to bring new compounds to market has proven a major disincentive to drug development.

At the end of the twentieth century, nearly half a million people were estimated to be afflicted by HAT (Barrett *et al*., 2003). Ironically, trypanosomes were among the first organisms to be targeted by synthetic drugs and Paul Ehrlich, 'the father of chemotherapy' (Drews, 2004), chose these organisms as a model on which to test his ideas. During the first two-thirds of the twentieth century, several compounds were introduced to treat HAT (Williamson, 1962, 1970; Apted, 1970). Sanofi-Aventis and Bayer between them currently produce all of the licensed anti-HAT drugs and donate them free of charge to the World Health Organization (WHO) who distributes them in Africa.

The African trypanosome is a popular organism for biological research. Much is known about trypanosome biochemistry and its genome sequence was published in 2005 (Berriman *et al*., 2005). The parasite is amenable to drug target validation through genetic means (Barrett *et al*., 1999) and simple screens are available to test drugs (for example, Raz *et al*., 1997). However, only a single compound, eflornithine, has been registered for use against HAT in the

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last 50 years, a reflection of the gap between our understanding about the organism and our capacity to develop new drugs. New initiatives discussed in the final part of this review promise to close the gap.

Two subspecies of the Kinetoplastid protozoans, *Trypanosoma brucei*, cause human disease. *T. b. rhodesiense* is responsible for an acute form in Eastern and Southern Africa (Fevre *et al*., 2005), while *T. b. gambiense* causes a chronic form in West and Central Africa (Figure 1). Generally the rhodesiense form of the disease is fatal within weeks to months of inoculation through the bite of an infected tsetse fly vector (Figure 2), while the gambiense form takes years to develop. There appears to be some geographical variance in disease manifestation. For example, in Malawi, patients carry *T. b. rhodesiense* and yet harbour the parasites without their causing CNS-involved disease for many years (MacLean *et al*., 2004). Sporadic reports of other trypanosome species causing disease in humans have appeared. These include *T. congolense* (Truc *et al*., 1998) and *T. evansi* (Joshi *et al*., 2005), which normally infect only animals. Generally, however, a non-immune mechanism of killing, involving a trypanosome lytic factor, believed to be apolipoprotein L1 (apoL1) (Pays *et al*., 2006) and possibly other components of high-density lipoprotein fractions (Smith *et al*., 1995), prevents most species of African trypanosome from establishing infections in humans. The single reported case of a *T. evansi* infection in man involved a patient who lacked apoL1 (Vanhollebeke *et al*., 2006). *T. b. gambiense* was responsible for the epidemics that marked the end of the twentieth century. A concerted WHO-led campaign to bring the disease under control has made an impact in recent years reducing the number of cases significantly (Barrett, 2006; Anonymous, 2006).

Figure 1 A map of countries infected with HAT. The countries shown in colour have historically reported HAT. Those countries coloured in red are currently reporting in excess of 1000 cases per year. Those in brown currently report between 50 and 1000 cases per year. Those in blue report fewer than 50 cases per year, while those in green currently report no cases of HAT. Nearly 97% of all reported cases are caused by *T. b. gambiense*. *T. b. rhodesiense* is found in East and Southern Africa. (Figure courtesy of Dr Pere Simarro at the World Health Organization.) HAT, human African trypanosomiasis.

Animal reservoirs play an important role in the epidemiology of *T. b. rhodesiense*, but are less important for *T. b. gambiense* although both wild (Njiokou *et al*., 2006) and domestic (Simo *et al*., 2006) animals have been found infected with this subspecies. Vector control can curb transmission (Allsopp, 2001) and was successfully employed in recent years to eradicate trypanosomiasis from Zanzibar using extensive trapping, followed by the sterile insect technique (Vreysen *et al*., 2000). However, it has proven difficult to implement tsetse control in a coordinated fashion on the African mainland. A process of antigenic variation, where parasites repeatedly change the surface coat that interfaces with the immune system, renders the prospects of vaccination poor (McCulloch, 2004). Drugs are central to efforts to control HAT.

Current drugs (Pepin and Milord, 1994; Barrett, 2000; Legros *et al*., 2002; Fairlamb, 2003; Burri *et al*., 2004; Brun and Balmer, 2006) all suffer drawbacks. Toxicity (sometimes severe), the need for parenteral administration, lack of a guaranteed supply and increasing incidence of treatment failure with some drugs make the situation difficult. The only compound in advanced phase III clinical trials is the orally available prodrug, pafuramidine maleate (DB289) (Boykin *et al*., 1996; Ansede *et al*., 2004), which is metabolized systemically to the diamidine, furamidine (DB75). It is the first compound whose development as a trypanocide might be considered to have approached that typical for registration of a new chemical entity for therapeutic purposes today. However, it is active only in stage 1 disease. Other drugs are urgently required, especially for stage 2 patients as it is generally only this cohort who present at HAT clinics once neurological symptoms are manifest. This review discusses those drugs already in use for HAT and also the few compounds in different stages of the development process, leading to a discussion of new initiatives in drug development for HAT.

Drugs registered for HAT chemotherapy

Four licensed compounds are used against HAT today, depending on the causative subspecies and whether parasites have initiated disease of the CNS (stage 2) or not (stage 1) (Pepin and Milord, 1994; Denise and Barrett, 2001; Keiser *et al*., 2001; Fairlamb, 2003; Burri *et al*., 2004; Brun and Balmer, 2006). Two compounds are used against stage 1 disease: suramin and pentamidine. Against stage 2 disease, melarsoprol (active against *T. b. gambiense* and *T. b. rhodesiense)* and eflornithine (only useful against *T. b. gambiense*) can be used. Nifurtimox, alone or in combination with other drugs, particularly with eflornithine, is being considered as an option for melarsoprol-refractory late-stage disease, or even more widely (Priotto *et al*., 2006).

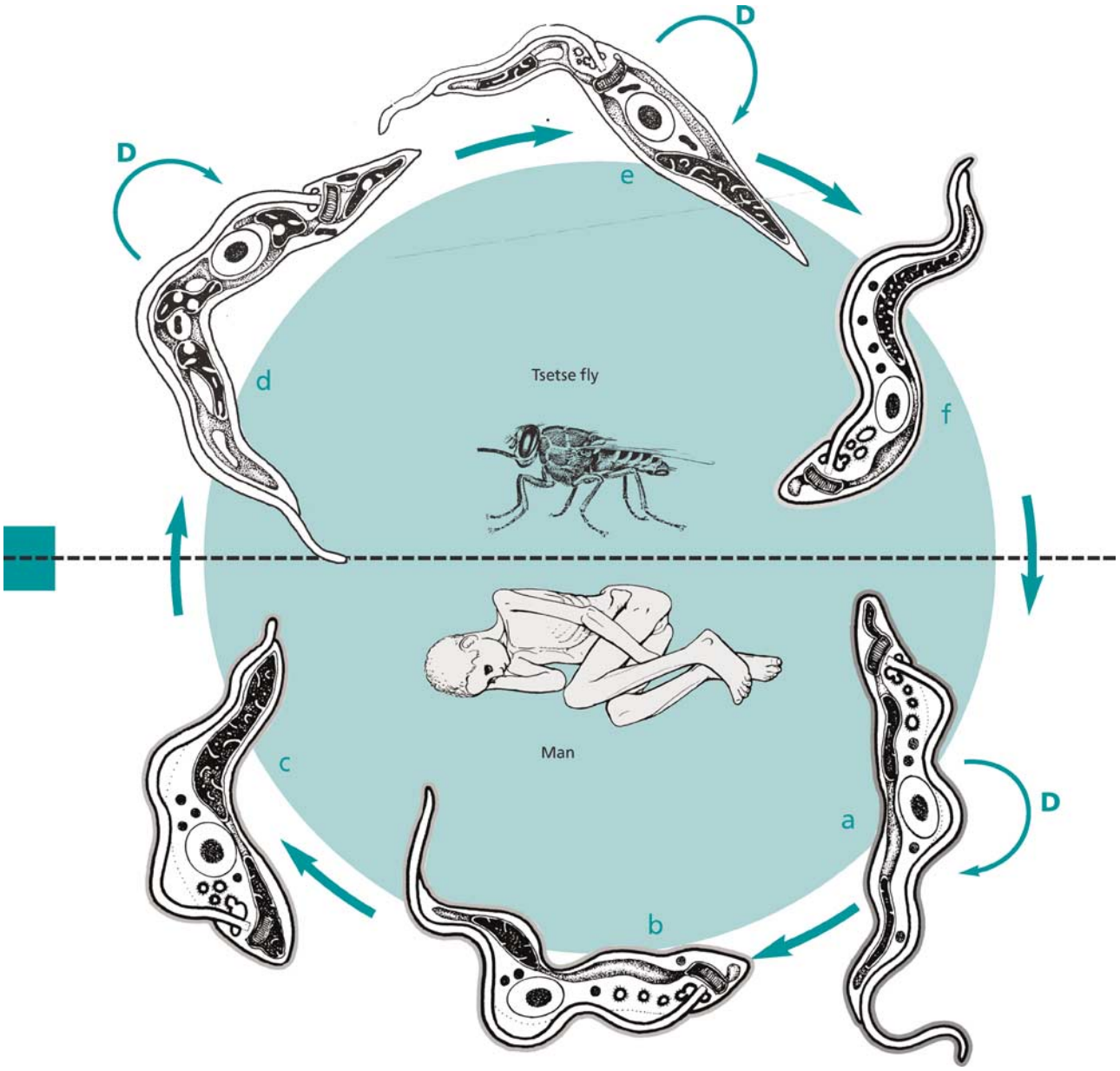

Figure 2 The life cycle of *Trypanosoma brucei.* Parasites are transmitted to man by the bite of an infected tsetse fly. Within man, the parasites proliferate first within the haemolymphatic system and later invade the CNS. Proliferative bloodstream slender form parasites (**a**), transform via an intermediate form (**b**) into non-proliferative stumpy forms (**c**). These are pre-adapted to survive within a tsetse fly where they transform into procyclic forms (**d**) that proliferate in the midgut of this environment before passing through various other stages including the epimastigote form (**e**) until transforming into metacyclic trypomastigotes (**f**) in the salivary glands. These forms are preadapted for life in the mammalian host when injected during a bloodmeal. Forms that are capable of division are labelled with a D. CNS, central nervous system.

Drugs used in early-stage disease

Provided HAT is diagnosed sufficiently early, suramin or pentamidine can be used. Their ease of administration and relative safety make them preferable to drugs used for stage 2 disease. The rapidly proliferating parasitaemia typical of the rhodesiense form of the disease can provoke early presentation by patients at clinic. However, the symptoms of early-stage gambiense disease do not normally extend beyond a general malaise common place in rural Africa. These patients are unlikely to present passively before late-stage involvement. Efforts to improve diagnosis (Chappuis *et al*., 2005a) are needed to accompany improved treatment.

Pentamidine

Background. Pentamidine isethionate (Figure 3) is currently produced by Sanofi-Aventis as pentacarinat in 200 mg ampoules for intramuscular injection. It is used against gambiense disease but not usually rhodesiense. The drug is donated, free of charge, to WHO for distribution. Four milligrams per kilogram given daily, or on alternate days,

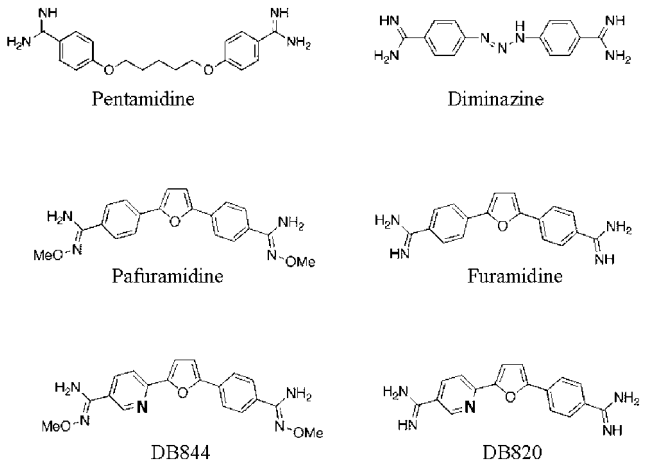

Figure 3 Structures of some important trypanocidal diamidines.

for 7–10 days are typical schedules (Sands *et al*., 1985; Burri *et al*., 2004).

Modes of action and resistance mechanisms. Pentamidine is concentrated to high levels (in the low millimolar range) by trypanosomes exposed to low micromolar concentrations of the drug (Damper and Patton, 1976a; Berger *et al*., 1995; Carter *et al*., 1995; de Koning, 2001a; Bray *et al*., 2003). Uptake is carrier mediated (Damper and Patton, 1976b). The drug enters principally via the P2 aminopurine permease which also transports melaminophenyl arsenicals (Carter *et al*., 1995). A high-affinity pentamidine transporter 1 (HAPT1) and a low-affinity pentamidine transporter 1 (LAPT1) also contribute to pentamidine uptake (de Koning, 2001a); hence parasites selected for melaminophenyl arsenical resistance that lack the P2 transporter often remain sensitive to pentamidine (Frommel and Balber, 1987; Fairlamb *et al*., 1992a; de Koning, 2001b; Matovu *et al*., 2003). One laboratory line selected for pentamidine resistance continued to accumulate drug to high levels and retained activity of the P2 transporter (Berger *et al*., 1995). This parasite line was of much reduced virulence in rodents, which might indicate that the development of resistance to pentamidine is associated with substantial fitness costs, rendering the propagation of resistant lines in the field unlikely (Berger *et al*., 1995; Bray *et al*., 2003). Another line, this time selected for pentamidine resistance in a cell line already lacking the P2 transporter (TbAT1, *T. brucei* adenosine transporter 1) (Matovu *et al*., 2003), also developed a reduced virulence phenotype, but on this occasion pentamidine uptake was greatly reduced and the HAPT1 transporter was reported to be lost (Bridges *et al*., 2007).

While pentamidine's uptake into trypanosomes has been characterized in detail, its definitive mode of action is not certain (Berger *et al*., 1993; Werbovetz, 2006). The mitochondrion appears to be a target for pentamidine in various species including yeast (Ludewig *et al*., 1994). In *Leishmania* parasites (close relatives of trypanosomes), fluorescent analogues of pentamidine accumulate preferentially in this compartment and mitochondrial damage (Hentzer and Kobayasi, 1977; Croft and Brazil, 1982) precedes cell death. Pentamidine resistance in *Leishmania* correlates to a reduction in the mitochondrial membrane potential (Basselin and Robert-Gero, 1998; Basselin *et al*., 2002; Mukherjee *et al*., 2006). As a di-cation, pentamidine interacts electrostatically with cellular polyanions. It binds DNA, including the unique intercatenated network of circular DNA molecules termed the kinetoplast, which make up the mitochondrial genome of all kinetoplastid flagellates (Simpson, 1986). However, bloodstream form *T. brucei* can retain viability, given time to adapt, when the kinetoplast has disintegrated (a state termed dyskinetoplastidy (Schnaufer *et al*., 2002)), although mitochondrial DNA can remain dispersed within the mitochondrion in some of these cases. Dyskinetoplastic parasites are slightly less sensitive than wild-type cells to diamidines. Fluorescent diamidines, for example, furamidine (Stewart *et al*., 2005; Mathis *et al*., 2006) and stilbamidine (Hawking and Smiles, 1941) also accumulate rapidly in the kinetoplast, and they also sequester in another class of organelle believed to be acidocalcisomes (Mathis *et al*., 2006). Whether the localization correlates with activity is not certain. Another suspected target, *S*-adenosylmethionine decarboxylase, was ruled out because *Leishmania* parasites overexpressing this enzyme are equally susceptible to pentamidine as wild-type cells (Roberts *et al*., 2006).

Pharmacology. Pentamidine's use against *Pneumocystis carinii* pneumonia in AIDS (acquired immunodeficiency syndrome) patients has contributed to the increased interest in understanding the pharmacokinetics of the drug (Conte, 1991). Other studies have looked specifically at the situation in HAT patients (Bronner *et al*., 1991). The drug has a large volume of distribution and long terminal half-life (elimination times running into weeks after a typical course). Extensive tissue retention and binding to serum proteins contribute to this. The long half-life of pentamidine explains why the drug had some success in prophylactic campaigns in west and central Africa in the mid-twentieth century (Waddy, 1970), a practice that is no longer recommended.

The drug is given by injection because, as expected for a highly charged molecule (pK_a of 11.4), pentamidine is not readily absorbed from the intestine. Intramuscular injection is preferred over intravenous injection since this latter route is associated with increased risk of hypotension. Although generally useful only in stage 1 disease, pentamidine might have some activity against parasites in cerebrospinal fluid (CSF), in what has been called 'early-late-stage disease', when white cell counts are still low (appearance of white blood cells in CSF accompanies stage 2 disease) and parasites have only recently entered CSF (Doua *et al*., 1996). Low levels of pentamidine (below 1% of plasma levels) have been measured in CSF (Bronner *et al*., 1991). Pentamidine is extensively metabolized (Berger *et al*., 1992) with cytochrome *P*450-dependent oxygenases playing a key role.

The drug is quite toxic. Lowering of blood glucose levels is common with hypotension evident in 10% of treated cases. Other toxic effects include pain at the site of injection, nephrotoxicity, leucopenia and liver enzyme abnormalities (Sands *et al*., 1985; Doua and Yapo, 1993).

Pentamidine is highly trypanocidal (with IC_{50} values *in vitro* in the order of 1–10 nM in a typical 3-day drug sensitivity assay). The time required to kill the parasites is dependent on dose with increasing doses causing progressively faster killing (Miezan *et al*., 1994).

Given the drug's toxicity, decreasing overall exposure is desirable. Since parasites are killed on exposure to just 10 nM for 3 days, and since pentamidine's pharmacokinetics ensures concentrations several orders of magnitude above this level are maintained for several days after the termination of drug administration, the prospects of using a 3-day course are currently being investigated.

Suramin

Background. Suramin (Figure 4) is a polysulphonated symmetrical naphthalene derivative and was first used against sleeping sickness in 1922 (Voogd *et al*., 1993). This followed Ehrlich's demonstration that other naphthalene dyes had trypanocidal activity due to selective accumulation by trypanosomes (trypan blue, named for its trypanocidal activity, is still used in mammalian cell viability assays). As there is renal clearance of these naphthalenes, suramin's colourlessness was an important factor behind its development. Suramin is currently donated to WHO by Bayer as Germanin (Bayer 205). Reports of treatment failures from gambiense foci in the 1950s and the fact that pentamidine is easier to administer led to suramin being used mainly for rhodesiense cases today. The drug has been considered for other conditions, particularly in androgen-independent prostate cancer (Kaur *et al*., 2002). The fact that the drug inhibited retroviral reverse transcriptase (Mitsuya *et al*., 1984) and also interfered with HIV binding to $CD4^+$ cells led to well-publicized, but unsuccessful, trials against HIV/AIDS.

Modes of action and resistance mechanisms. Many hypotheses as to suramin's mode of trypanocidal action have been proposed, but none proven. With six negative charges, the drug binds, by electrostatic interaction, to many enzymes. Suramin is around a hundred fold less active against procyclic form trypanosomes, that normally reside in tsetse flies, than against bloodstream forms (Scott *et al*., 1996). Since glycolysis is essential to bloodstream forms but not procyclic forms (Fairlamb and Bowman, 1977, 1980; Besteiro *et al*., 2005) and because suramin inhibits a number of glycolytic enzymes (Wierenga *et al*., 1987), glycolysis has been proposed as a likely target. However, any number of other pathways too could be targeted by the drug. For example, it is a competitive inhibitor of 6-phosphogluconate dehydrogenase, an enzyme of the pentose phosphate pathway (Hanau *et al*., 1996).

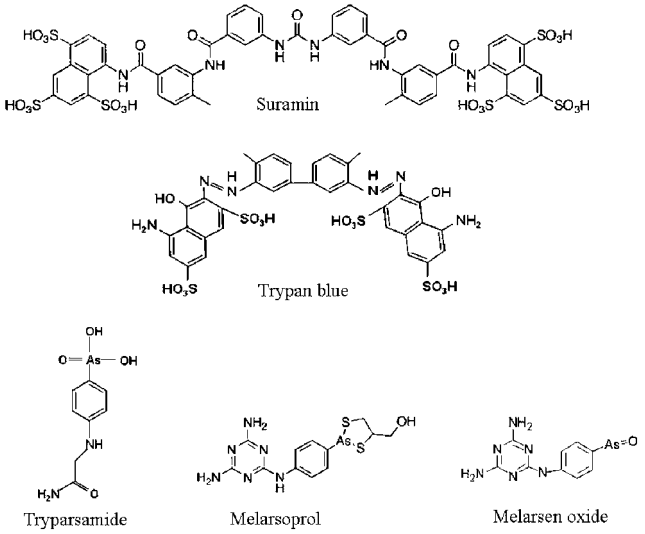

Figure 4 Structures of suramin, trypan blue and some important trypanocidal arsenicals.

Endocytosis appears to be the most likely route of entry (Fairlamb and Bowman, 1980). Because of its high avidity binding to many serum proteins, including low-density lipoprotein (LDL) (Vansterkenburg *et al*., 1993), it was suggested that it might enter while bound to LDL (Bastin *et al*., 1996; Coppens and Courtoy, 2000; Green *et al*., 2003). However, in procyclic cells, at least, manipulation of different vesicular transport systems affects uptake of LDL and suramin independently of each other (Pal *et al*., 2002). The extremely high rate of fluid-phase endocytosis displayed by bloodstream form trypanosomes (Engstler *et al*., 2004) could potentially explain uptake parameters of suramin into *T. brucei* without a requirement for a particular receptor, although this is untested.

Reports on suramin resistance in the field are rare (Barrett, 2003). However, resistance can be selected readily in the laboratory (Scott *et al*., 1996). The drug is also employed as a veterinary trypanocide and resistance has been noted in species of trypanosome, for example, *T. evansi*, that infect animals (Mutugi *et al*., 1994; El Rayah *et al*., 1999) although mechanisms of resistance are not understood.

Pharmacology. Poor intestinal absorption and a local irritation if given intramuscularly mean that suramin is best given by slow intravenous injection (Voogd *et al*., 1993). A course of five injections, every 3–7 days, over a 4-week period is typical. Most of the drug (>99%) binds to serum proteins. Suramin does not cross the blood–brain barrier to levels capable of killing trypanosomes in the CSF at doses given in treatment of stage 1 disease (although in mice, very large doses ($>80\,mg\,kg^{-1}$) are capable of curing a stage 2 model of the disease (Jennings, 1995)).

The use of the drug in AIDS and cancer clinical trials improved knowledge of its pharmacology (Collins *et al*., 1986). The elimination terminal half-life is very long (for example, 41–78 days was reported in one study (Eisenberger and Reyno, 1994)).

In vitro, exposure to $1\,\mu g\,kg^{-1}$ for 24 h is sufficient to kill trypanosomes. Thus with levels higher than $100\,\mu g\,ml^{-1}$ for several weeks after a typical course the drug's success in prophylaxis is understandable (Waddy, 1970) although no longer used.

At concentrations higher than $350\,\mu g\,ml^{-1}$, the drug induced significant neurotoxicity (Kaur *et al*., 2002) and even at lower concentrations neurotoxic effects were evident

(Bitton *et al*., 1995). Neuropathy, rash, fatigue, anaemia, hyperglycaemia, hypocalcaemia, coagulopathies, neutropaenia, renal insufficiency and transaminitis are all common. The adverse effects reported with suramin were sufficient for the US Food and Drug Administration to block approval for use in prostate cancer (Kaur *et al*., 2002), although HAT regimens are short enough to make safety tolerable.

Drugs used in late-stage disease

Stage 2 of HAT involves progressive breakdown of neurological function, including the alteration in sleep-wake patterns that defines 'sleeping sickness'. In fact, patients do not sleep more than healthy subjects, but the disrupted sleep patterns often manifest themselves in daytime somnolence (Buguet *et al*., 1993). Alterations in the 'sleep onset rapid eye movement sleep periods' may become a new tool to detect CNS involvement and 2nd stage disease (Buguet *et al*., 2005). Since the late stage is characterized by the presence of parasites within the cerebral spinal fluid and brain, any drug that is to reach the parasites must first cross the blood–brain barrier or the blood–CSF barrier (Croft, 1999; Enanga *et al*., 2002; Kennedy, 2006). Many chemicals that show good activity against models of stage 1 disease fail to cure models of stage 2 disease (Jennings *et al*., 1977; Bouteille *et al*., 1998) due to the difficulty of getting molecules to cross the blood–brain barrier.

Melarsoprol

Background. Melarsoprol (Figure 4), a melaminophenyl-based organic arsenical, was introduced in 1949 (Friedheim, 1949). It remains the most widely used drug against late-stage HAT in spite of extremely toxic side effects. In the order of 5–10% of patients taking melarsoprol suffer a reactive encephalopathy (Robertson, 1963; Pepin and Milord, 1994; Blum *et al*., 2001, 2006); half of these die!

Melarsoprol is the only available compound capable of treating stage 2 *T. b. rhodesiense* disease and the only generally affordable compound to treat stage 2 gambiense disease. It is administered as a 3.6% solution in propylene glycol and distributed in 5 ml ampoules for intravenous injection. The drug is provided by Sanofi-Aventis free to WHO for distribution and will continue to be provided for at least 5 more years.

Modes of action and resistance mechanisms. It is not known how arsenicals kill trypanosomes. When exposed to melarsoprol, the parasites lyse rapidly (Meshnick *et al*., 1978). Loss of ATP due to inhibition of glycolysis could underlie lysis although it seems that the cells lyse before ATP supplies are seriously depleted (Vanschaftingen *et al*., 1987). Arsenic forms stable interactions with thiols including trypanothione (Fairlamb *et al*., 1989), a key low molecular weight thiol (a bis-glutathionyl-spermidine adduct) found in trypanosomatids but not in mammalian cells (Fairlamb *et al*., 1985) and lipoic acid (Fairlamb *et al*., 1992b). Whether these interactions underlie trypanocidal activity is, however, not known.

In several foci, treatment failures have reached levels of 30% of those treated (Legros *et al*., 1999; Brun *et al*., 2001; Moore and Richer, 2001; Stanghellini and Josenando, 2001). Most parasites selected for resistance to melamine-based arsenicals in the laboratory have lost the P2 aminopurine transporter that carries the drug (Carter and Fairlamb, 1993; Barrett and Fairlamb, 1999; Mäser *et al*., 1999; Stewart *et al*., 2005) and several parasites isolated from relapse cases in the field are also defective in P2 transport (Mäser *et al*., 1999; Matovu *et al*., 2001; Stewart *et al*., 2005). No significant difference in intrathecal accumulation of drug between successfully treated and relapsing patients was seen (Brun *et al*., 2001), although the demonstration of parasite resistance in the field has been hampered by difficulties in retrieving *T. b. gambiense* from patients for study. Parasiteamias are generally very low and it is difficult to establish infections *in vitro* or in rodents. Cryopreservatives like Triladyl (Maina *et al*., 2006), and African rodent species that are relatively sensitive to *T. b. gambiense* parasites (Buscher *et al*., 2005; Maina *et al*., 2007), currently offer hope that collection of samples from the field can be improved.

In vitro, unmetabolized melarsoprol is believed to cross membranes by passive diffusion (Scott *et al*., 1997). Melarsen oxide, however, enters *T. brucei* principally by the P2 aminopurine transporter (Carter and Fairlamb, 1993) encoded by the *tbat1* gene (Mäser *et al*., 1999). However, trypanosomes from which the *tbat1* gene has been removed are only marginally less sensitive to melamine-based arsenicals than are wild-type cells (Matovu *et al*., 2003), indicating that secondary routes of uptake exist and loss of P2 plus these secondary routes is required for high-level resistance. Low concentrations of pentamidine inhibited a secondary, slow lysis of trypanosomes in a spectrophotometric test (Matovu *et al*., 2003), prompting suggestions that the HAPT1 (de Koning, 2001a) might be the second route of entry for melarsen oxide. Moreover, the highly pentamidine resistant line derived from the *tbat1* gene knockout line was also highly resistant to melarsen oxide and had lost the HAPT1 transporter (Bridges *et al*., 2007). However, since trypanosomes selected for high-level melarsen resistance retain sensitivity to pentamidine (Fairlamb *et al*., 1989b), it is clear that more is yet to be learned about all possible routes of uptake of these different classes of drug. Ectopic overexpression of the *tbmrpa* gene, that encodes a P-glycoprotein type pump, is capable of inducing melarsoprol resistance (Shahi *et al*., 2002).

Patients are currently treated and then subjected to follow-up for 2 years. Any recurrence of the disease over that time is considered as a treatment failure. The possibility of identifying trypanosomes that lack the P2 transporter as being melarsoprol refractory at the time of disease diagnosis has clear benefits. Tests using fluorescent diamidines (for example, furamidine) that enter via the P2 transporter can identify loss of the P2 transporter and these offer potential as a major advance in diagnosis of resistance in the field (Stewart *et al*., 2005).

Pharmacology. Treatment schedules with melarsoprol show how an understanding of pharmacokinetics can lead to radical improvements in drug regimens. A standardized

10-day course with 2.2 mg kg^{-1} given once a day is as effective as older protocols (Burri *et al*., 2000; Schmid *et al*., 2004, 2005; Pepin and Mpia, 2006). Melarsoprol was introduced to replace tryparsamide, another arsenical. Tryparsamide administration regimens took into account toxicity associated with drug accumulation in various tissues (Williamson, 1970). It was given over prolonged periods, interspersed with 'rest' periods to enable clearance of drug from tissue deposits. Similar regimens were unnecessarily retained for melarsoprol before implementation of the 10-day course.

First studies into melarsoprol's pharmacokinetics employed a bioassay (Hawking, 1962; Burri and Brun, 1992) based on *in vitro* trypanocidal activity found in various body fluids containing drug. HPLC methods (Bronner *et al*., 1998) that measure melarsoprol itself failed to identify the key active metabolite, melarsen oxide, which forms rapidly in plasma (96% clearance within 1 h). Much of the drug is bound to plasma protein. A mean elimination half-life of 3.5 h for active metabolite was determined (Burri and Brun, 1992; Burri *et al*., 1993).

Melarsoprol (or melarsen oxide) maximally accumulates across the blood–brain barrier to levels only around 1–2% of maximum plasma levels (Burri *et al*., 1993). This is sufficient to clear trypanosomes with typical 'wild-type' sensitivities to the drug. However, a drop in sensitivity of only a few fold could render parasites in the CSF non-susceptible to these levels (Brun *et al*., 2001; Burri and Keiser, 2001; de Koning, 2001b).

Side effects are severe. Convulsions and other neurological sequelae can precede coma and death in the reactive encephalopathy that afflicts 5–10% of treated patients. Other adverse events are common. These include pyrexia, headache, pruritis and thrombocytopaenia. Heart failure too has been reported. Co-administration of corticosteroids (for example, prednisolone) yields some protection against the reactive encephalopathy (Pepin *et al*., 1989a). Thiamine administration too can reportedly ameliorate melarsoprol's adverse events (Pepin and Milord, 1994). It was recently shown (Szyniarowski *et al*., 2006) that melarsoprol enters mammalian cells via a thiamine transporter, hence co-administration of this vitamin may decrease melarsoprol uptake into mammalian cells but not into trypanosomes. Conjugation of melarsoprol in complexes might improve toxicity by slowing release of the active principle (Gibaud *et al*., 2002, 2005). Topical application to mouse models of the disease was also successful and lowered toxicity (Jennings *et al*., 1993; Atouguia *et al*., 1995). However, in spite of the prospect of deriving improved melarsoprol formulations, the drug's reputation mitigates against taking new arsenical derivatives forward.

Eflornithine

Background. Eflornithine (Figure 5) or D,L-α-difluoromethylornithine (originally marketed as Ornidyl by Marion Merrell Dow) is an analogue of the amino-acid ornithine and acts as an inhibitor of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC) (Bacchi *et al*., 1980; Phillips *et al*., 1987; Bacchi and Yarlett, 1993). Eflornithine was first developed as a potential antineoplastic agent; however, it has still not been registered for use in therapy of cancer (Gerner and Meyskens, 2004). The drug has activity against *T. b. gambiense*, even in the late stage (Burri and Brun, 2003) but against *T. b. rhodesiense* activity is restricted (Iten *et al*., 1995). The usual regimen involves 100 mg kg^{-1} body weight at 6 h intervals (that is, 400 mg kg^{-1} day^{-1}) by intravenous infusion for 14 days. Shorter schedules and oral dosing have so far given lower efficacy.

Mode of action and resistance mechanisms. Eflornithine has similar affinity for both the mammalian and trypanosomal ODCs (Phillips *et al*., 1988). Its specificity against the parasite may arise because *T. b. gambiense* ODC is degraded and replenished much more slowly than its mammalian counterpart (Ghoda *et al*., 1990). A pulse of eflornithine thus deprives trypanosomes of net polyamine synthesis for a prolonged period compared with mammalian cells. Lack of activity against rhodesiense parasites may relate to the enzyme being more rapidly turned over in that subspecies (Iten *et al*., 1997).

Polyamine biosynthesis inhibition is accompanied by an increase in cellular levels of *S*-adenosyl methionine (Yarlett and Bacchi, 1988), which causes inappropriate methylation of proteins, nucleic acids, lipids and other cell components. Trypanothione levels are also diminished after eflornithine treatment (Fairlamb *et al*., 1987), which might render them more susceptible to oxidative stress and other immunological insult.

A functional immune system is required to kill the growth-arrested trypanosomes (Bitonti *et al*., 1986b), which transform into non-proliferating stumpy forms after exposure to eflornithine. *T. brucei* lacks polyamine transporters rendering them auxotrophic for polyamines (Fairlamb and Le Quesne, 1997) preventing by-pass of inhibition of polyamine biosynthesis, although the recent demonstration of variability in sensitivity of different *T. brucei* strains to eflornithine in rodents and a different potential of exogenous polyamines to antagonize eflornithine's activity (Nishimura *et al*., 2006) could indicate some variability in the parasites' capacity to accumulate polyamines. One *T. b. rhodesiense* line selected

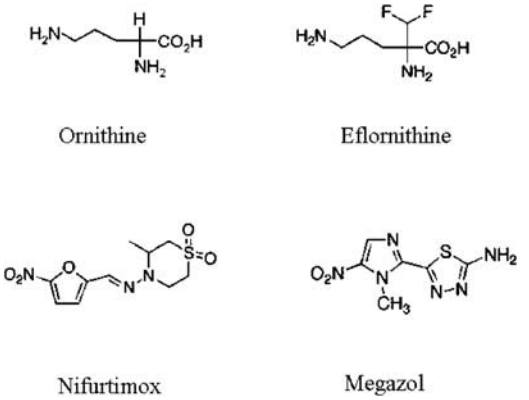

Figure 5 Structures of ornithine and eflornithine and some trypanocidal nitroheterocycles.

for eflornithine resistance showed enhanced capability to import putrescine compared with sensitive lines (Bacchi *et al*., 1993).

Eflornithine uptake in mammalian cells was reported to involve simple diffusion across the membrane (Erwin and Pegg, 1982). In *T. brucei* passive diffusion across the plasma membrane in both bloodstream (Bitonti *et al*., 1986a) and procyclic forms (Bellofatto *et al*., 1987) was proposed to account for cell entry. However, the compound is rather polar and unlikely to be lipid permeable. In the yeast, *Neurospora crassa*, uptake is mediated via a cationic amino-acid transporter (Davis *et al*., 1994). Moreover, another study in procyclic trypanosomes (Phillips and Wang, 1987) showed uptake to be temperature sensitive and to follow Michaelis–Menten-type kinetics with an apparent K_m of 244 μM. Although high concentrations (1 mM; Bitonti *et al*., 1986a, b) and 20 mM (Phillips and Wang, 1987) of unlabelled lysine, arginine and ornithine all failed to inhibit uptake, lower doses were not tested and unphysiologically high doses could lead to induction of other routes for eflornithine uptake into the cell, leading to a masking of transporter-mediated uptake. It is likely that uptake of eflornithine in trypanosomes is carrier mediated. The trypanosome's genome is replete with genes encoding amino-acid transporters (Barrett and Gilbert, 2006) of which one or more could carry eflornithine. Two reports of drug resistance (Bellofatto *et al*., 1987; Phillips and Wang, 1987) in procyclic forms show that drug uptake is diminished. Thus it seems probable that resistance can relate to loss of, or changes to, eflornithine transport into cells.

Pharmacology. The mean half-life in plasma following intravenous injection of eflornithine is only in the order of 3 h, with 80% of the drug excreted unchanged in urine after 24 h (Haegele *et al*., 1981; Griffin *et al*., 1987). Little serum protein binding occurs. Accordingly the drug must be given in large doses by prolonged intravenous infusion.

In humans, reported CSF to plasma ratios vary between 0.1 and 0.9 (Milord *et al*., 1993; Burri and Brun, 2003; Na-Bangchang *et al*., 2004) with the ratio towards the lower end of that range more commonly reported. Eflornithine monotherapy apparently fails to cure the stage 2 mouse model, perhaps because the drug fails to cross the blood–brain barrier in rodents (Levin *et al*., 1983). In combination with suramin, however, cure was possible, but only if mice were kept in a 2 h light and 4 h dark regime (Jennings, 1993), which relates to consumption of the drug in drinking water being greatly enhanced (10–15 fold) in darkness (Romijn *et al*., 1987).

In man, doses beyond 100 mg kg^{-1} given orally failed to lead to increased appearance of the drug in plasma, which suggests that eflornithine is accumulated by a saturable transporter. It is also probable that a transporter carries the drug across the blood–brain barrier, where the y^+ system (the main cationic amino-acid transporter in mammals) is the principal cationic amino-acid transporter (O'Kane *et al*., 2006) and a likely candidate.

IC_{50} growth inhibitory values for eflornithine of 81–693 μM (Zweygarth and Kaminsky, 1991) *in vitro* are very poor when compared to the other trypanocidal drugs (melarsoprol, suramin and pentamidine all have activity in the nanomolar range). Oral dosing gave average plasma levels of 234–528 μM and CSF levels at 22.3–64.7 μM in patients receiving 100 or 125 mg kg^{-1} (Na-Bangchang *et al*., 2004). *In vitro* activities do not take into account the role of the immune system in killing trypanosomes (Bitonti *et al*., 1986b); however, the relative inefficacy of eflornithine, coupled to its poor pharmacokinetic profile, renders the drug far from ideal for use in a tropical setting and it is unlikely that eflornithine would have been pursued had protocols in use today, that begin with efficacy testing against trypanosomes *in vitro*, been in place when the drug was first explored for use against trypanosomiasis.

Side effects include fever, headache, hypertension, macular rash, peripheral neuropathy and tremor, gastrointestinal problems including diarrhoea (Chappuis *et al*., 2005b). The incidence and severity of these adverse reactions are considerably lower than similar reactions in melarsoprol therapy, and Médecins sans Frontières (MSF) are currently promoting eflornithine as first-line treatment for stage 2 disease (Chappuis *et al*., 2005b; Balasegaram *et al*., 2006), although the risk of resistance emerging must be taken seriously.

Drugs in clinical trials

Pafuramidine maleate (DB289)

Background. The only new drug in advanced trials for introduction to use against HAT is pafuramidine maleate (DB289) (Figure 3). This *O*-methyl amidoxime prodrug (Boykin *et al*., 1996; Ansede *et al*., 2004) is converted to the diamidine furamidine (DB75) systemically.

Furamidine was first shown to be trypanocidal in the 1970s (Rane *et al*., 1976; Das and Boykin, 1977), but it offered little or no benefit over pentamidine and was not pursued at this time (Steck *et al*., 1982). The efficacy of pentamidine in treatment of PCP, the incidence of which rose sharply as the HIV/AIDS epidemic emerged in the 1980s, led to renewed interest in di-cationic microbicides. Studies into the metabolism of pentamidine (Berger *et al*., 1990, 1991, 1992; Clement and Jung, 1994) revealed that the positive charges of these molecules, which restrict intestinal absorbtion, could be neutralized by chemical modification. Pentamidine was converted to an amidoxime prodrug (Clement and Raether, 1985; Clement, 2002) with trypanocidal activity when given subcutaneously to mice. However, activity against pneumocystitis was weak (Hall *et al*., 1998) and furamidine synthesized as its prodrug, pafuramidine maleate (Boykin *et al*., 1996), was far better in this context. Phase III trials are currently underway for pafuramidine against PCP (Yeates, 2003). Pafuramidine (100 mg twice per day for 5 days) also performed well in clinical trials against malaria (Yeramian *et al*., 2005). However, a concern that widespread, uncontrolled use of the drug as an antimalarial might enhance the risk for selection of resistance to the drug in trypanosomes has contributed to its being abandoned for malaria. Since many diamidine compounds show superior

in vitro activity against *Plasmodium falciparum*, it is hoped that another candidate might emerge for malaria.

Mode of action and resistance mechanisms. As a diamidine it would be anticipated that the modes of action and resistance mechanisms for furamidine may overlap with those of pentamidine and diminazene. Uptake of the compound to high levels (Mathis *et al*., 2006) occurs principally via the same P2 aminopurine transporter that carries other diamidines into cells (Lanteri *et al*., 2006). In a 3-day *in vitro* assay, the activity of furamidine was greatly decreased against the *tbat1*/P2 knockout cell line and a line selected for resistance to the drug had lost the P2 transporter, through deletion of its gene, confirming this as a main route of uptake (Lanteri *et al*., 2006). However, these P2 defective cells fail to grow for prolonged periods, even in relatively low concentrations of drug, *in vitro*. Moreover, the P2 defective cells are only marginally less sensitive than wild type when treated in mice (Lanteri *et al*., 2006). Thus a secondary route of uptake, albeit minor compared with P2, plays a significant role in the pharmacology of the drug, and loss of the P2 transporter alone is unlikely to lead to parasites developing resistance.

As furamidine is fluorescent, it has been possible to trace its distribution in cells (Mathis *et al*., 2006). Within a minute, UV fluorescence is detectable within the DNA containing organelles (nucleus and kinetoplast). By 1 h, the drug becomes visible within organelles believed to be acidocalcisomes (Mathis *et al*., 2006). After 24 h exposure to 7.5 μM drug, the fluorescence of the kinetoplast has disappeared, as the drug appears to have caused disintegration of this structure (Mathis *et al*., 2006). Damage to the mitochondrion is also evident, leading to suggestions that the mitochondrion is a target for this drug. In yeast, furamidine (Lanteri *et al*., 2004) like pentamidine (Ludewig *et al*., 1994), inhibits the respiratory chain and it also acts as an uncoupler. The mitochondrial inner membrane is also affected by furamidine in trypanosomes (Lanteri *et al*., unpublished), making it tempting to speculate that mitochondrial disruption relates to activity of this compound.

Pharmacology. *In vitro*, pafuramidine is over one thousand fold less active against trypanosomes than furamidine (Ansede *et al*., 2004). *In vivo*, however, furamidine is active only when given by injection, while pafuramidine is active in an oral formulation. This is because pafuramidine, but not furamidine, crosses intestinal epithelium (Sturk *et al*., 2004) in quantities sufficient to reach trypanocidal levels in blood. The prodrug is metabolized by cytochrome *P*450 (CYP4F isoforms in particular) and other metabolic enzymes (Ansede *et al*., 2005; Saulter *et al*., 2005; Wang *et al*., 2006), through demethylation followed by reduction, to the active diamidine. Furamidine does not cross the blood–brain barrier. However a very close relative of furamidine, DB820, which differs by the addition of a single nitrogen into one of the phenyl rings (Figure 3) (Ismail *et al*., 2003), exerts trypanocidal activity in rodent models of stage 2 disease. The *O*-methyl amidoxime prodrug of DB820, currently coded as DB844, can be administered orally and still lead to the cure of stage 2 mouse model (Ansede *et al*., 2005), the first orally available compound to exert this effect.

Currently, little is published with regard to the clinical development of pafuramidine for trypanosomiasis. However, studies into metabolism, toxicology and pharmacokinetics in animal models including mouse, rat, vervet monkey and later cynomolgus monkey preceded its entry into phase I clinical trials. These satisfied criteria enabling entry into phase II trials in *T. b. gambiense* patients with stage 1 disease (Yeates, 2003).

An initial open label, non-controlled trial focussed on a site in Angola and one in the Democratic Republic of Congo. Patients were treated with 100 mg of pafuramidine given orally, twice daily for 5 days. Efficacy in that first trial was 83% cure (93% of the 30 patients partaking in the trial were cleared of parasites in blood and lymph 24 h after the final dose. However, after 24 month follow-up a further four patients had relapsed). The regimen was then lengthened to a 10-day treatment for a phase IIb trial. This has been more successful although follow-up must continue until 2 years is complete. A multicentre phase III trial, in which 250 patients will receive DB289 using non-inferiority to injected pentamidine as the comparator, is underway.

Some side effects were reported including intermittent fever and pruritis. These were not serious and administration of up to 600 mg in a single dose gave no adverse response.

Diminazene

Another diamidine, diminazene, is a registered veterinary trypanocide (Kinabo, 1993; Peregrine and Mamman, 1993). Unlicensed use in humans has been successfully attempted (Pepin and Milord, 1994). Dose of drug and time of exposure are important in determining the trypanocidal effect of diminazene. Iten *et al*. (1997) showed that exposure of trypanosomes to 10 μg ml^{-1} of diminazene for less than 1 min committed the cells to death (although it took several days before the cells died). At 1 μg ml^{-1}, the exposure time needed for death rose to 15 min, while at 0.1 μg ml^{-1} cells needed to be exposed for 24 h before proceeding to a delayed death.

The drug enters trypanosomes via the P2 transporter (Barrett *et al*., 1995; Ross and Barns, 1996; de Koning *et al*., 2004; Witola *et al*., 2004) and *tbat1* gene knockout cells (Matovu *et al*., 2003) are resistant to diminazene. Alternative transporters, such as HAPT1 and LATP1, play a less important role in entry of this compound than they do for pentamidine, although the drug does continue to enter TbAT1/P2 defective cells albeit very slowly (de Koning *et al*., 2004), and in *in vitro* assays the P2-deficient cells retain sensitivity to diminazene in the low micromolar range (Matovu *et al*., 2003).

Pharmacokinetic properties of diminazene deviate widely from pentamidine. For example, the volume of distribution is much lower in animals studied (probably due to less protein binding and tissue retention) (Peregrine and Mamman, 1993). Given that the orally available pafuramidine will complete phase III trials soon, it is this latter compound that is more likely to be approved than diminazene, which will be retained as a veterinary trypanocide. The possibility of selecting parasites that lack the P2 transporter in

development of diminazene resistance (Barrett *et al*., 1995) is of concern (de Koning, 2001a, b), especially where *T. b. rhodesiense* is present at significant levels in livestock (Barrett, 2001). The risk of selecting for P2 transporter-defective parasites that might enter humans must be considered before implementing any drug campaigns against veterinary trypanosomiasis.

Nifurtimox

Background. Nifurtimox, marketed as Lampit, is produced by Bayer, provided free to WHO and distributed by MSF for use in trials in HAT therapy or for compassionate use in cases of melarsoprol failure. Success as monotherapy has reportedly been limited (50–80% cure) against *T. brucei gambiense* (Janssens and Demuynck, 1977; Pepin *et al*., 1989b) but its use, particularly in combinations (Moens *et al*., 1984; Jennings, 1991), is of increasing interest, as reports of treatment failure with arsenical monotherapy rise. Recent trials in combination with eflornithine show great promise (Priotto *et al*., 2006).

Mode of action. Single electron reduction of the nitro group of nifurtimox generates a free radical, which may interact with cellular constituents or generate reduced oxygen metabolites believed to cause parasite death (Docampo and Moreno, 1986; Enanga *et al*., 2003). With a reduction potential of −260 mV, nifurtimox is relatively easily reduced in many cell types (Viode *et al*., 1999) but a typical prokaryote-related type 1 nitroreductase identified in the trypanosome's genome is likely to play a role in activation of nifurtimox and other nitroheterocycles (S Wilkinson, London School of Hygiene and Tropical Medicine, personal communication).

Numerous nitroheterocycles have been demonstrated to have potent activity against trypanosomes. Nitrofurazone (a nitrofuran) entered human trials in the mid-twentieth century, but toxicity issues halted its development (Apted, 1970). Several 2-substituted 5-nitroimidazoles (Jennings, 1991) were shown to be efficacious and cured the stage 2 mouse model when co-administered with suramin. The nitroimidazole–thiazole, megazol, too was active when administered with suramin in stage 2 mouse and rat models (Enanga *et al*., 1998; Darsaud *et al*., 2004) and also able to cross the blood–brain barrier in significant quantities in a primate model (Enanga *et al*., 2000). A group of 5-nitro-2-furancarbohydrazines were also recently shown to possess substantial trypanocidal activity (Millet *et al*., 2002).

As host cell toxicity, particularly genotoxicity, has mitigated against development of antimicrobial nitroheterocycles, the possibility of selectively targeting melamine-coupled nitroheterocycles to trypanosomes, via the P2 aminopurine transporter, was investigated (Stewart *et al*., 2004; Baliani *et al*., 2005). Several compounds were identified with excellent trypanocidal activity and they appear to enter via routes in addition to P2. The possibility of targeting trypanocides to trypanosomes via transporters has been covered extensively in a recent review (Barrett and Gilbert, 2006).

Pharmacology. Serum levels are reportedly low when nifurtimox is given orally, peaking 1–3 h after administration of a single 15 mg kg^{-1} dose, reaching a maximum of around 4 μM in healthy subjects (Paulos *et al*., 1989). Clearance is fast with a plasma elimination half-life of around 3 h (Paulos *et al*., 1989). The drug can accumulate across the blood–brain barrier (Burri *et al*., 2004) to levels around half of those found in plasma. African trypanosomes are not very susceptible to nifurtimox (IC_{50} values of around 5 μM compared with 10 nM for melarsen oxide in a typical *in vitro* assay (Enanga *et al*., 2003)). This probably explains the drug's limited efficacy as monotherapy.

Toxic effects to the central and peripheral nervous systems have been reported (Castro *et al*., 2006) and rats given high doses of the drug showed increased risk of cancer (Steinhoff and Grundmann, 1972). Typical regimens used for *T. cruzi* infections (15 mg kg^{-1} over 60 days) lead to nausea, vomiting and other problems. At 15 mg kg^{-1}, additional adverse affects including polyneuropathy were evident and the gastrointestinal problems more pronounced and more frequent (Pepin *et al*., 1992).

Combinations

Combination chemotherapy is becoming the preferred route of administration of antimalarial compounds in the hope of diminishing the probability of selecting for drug-resistant mutants (Kremsner and Krishna, 2004). Treatment failure is also a growing problem in trypanosomiasis therapy (Brun *et al*., 2001). There are other reasons for using drugs in combination. Synergistic effects can permit the lowering of doses for the combination partners thus reducing adverse effects. Jennings (1993) pioneered work into seeking synergistic effects with trypanocidal drugs in rodent models. Suramin, acts synergistically with many drugs. For example, administration of suramin 15 min before topical administration of melarsoprol led to good cure rates of the stage 2 mouse model. Suramin also altered the volume of distribution and pharmacokinetics of the experimental trypanocide megazol in mice (Enanga *et al*., 1998), possibly because it inhibits P-glycoprotein pumps (Buxbaum, 1999) that play key roles in maintaining the blood–brain barrier and also in removing xenobiotics through the renal system. Pre-treatment with other agents that induce changes to renal clearance or blood–brain barrier permeability could also influence efficacy of trypanocides *in vivo* (Croft, 1999).

Recent clinical trials using eflornithine (400 mg kg^{-1} day^{-1}) for 7 days in two periods of infusion (rather than 14 days with four infusions) and nifurtimox at 15 mg kg^{-1} for 10 days have yielded cure rates as high as 98% (Priotto *et al*., 2006). Other combinations (melarsoprol and eflornithine or melarsoprol and nifurtimox) were considered too toxic to pursue (Priotto *et al*., 2006). Nifurtimox exerts its activity through induction of oxidative stress (Docampo and Moreno, 1986; Enanga *et al*., 2003) while eflornithine diminishes levels of trypanothione, a key metabolite used in protecting against oxidative stress, thus it is possible that these drugs synergize at the level of parasite killing.

Since melarsoprol treatment failure rates have been increasing, and because resistance to eflornithine is relatively

easy to select in the laboratory (Bellofatto *et al*., 1987; Phillips and Wang, 1987; Bacchi *et al*., 1993), in the absence of alternative drugs every effort should be taken to delay the onset of resistance to eflornithine in the field. The use of a nifurtimox–eflornithine combination presently offers promise in this regard.

Phamacological re-engagement with HAT

By the end of the twentieth century it had been widely accepted that industrial input to neglected diseases like HAT was not viable on purely commercial grounds. For some diseases, like malaria and tuberculosis, with prevalences greatly in excess of HAT, public–private partnerships, the Medicines for Malaria Venture (http://www.mmv.org) and The Global Alliance for TB Drug Development (TB Alliance) (http://new.tballiance.org/home/home-live.php), were founded in 1999 and 2000, respectively.

For HAT, however, the situation reached a low point at the turn of the Century (Barrett, 1999). The prevalence of the disease had reached alarming levels. Treatment failures with melarsoprol were reported in several foci and Aventis, the manufacturer of that drug, found themselves confronted with the dilemma of whether to continue producing, at a loss, one of the most toxic compounds known to the pharmacopoeia, or to drop production leaving patients to die. MSF and other non-governmental organizations were establishing centres in many African countries to deal with the crisis and drugs for HAT soon made it to the forefront of MSF's campaign on 'Access for Essential Medicines' (Pécoul *et al*., 1999). WHO were engaged in an apparently fruitless quest to find a manufacturer for eflornithine after Hoechst Marion Roussel (which was later incorporated into Aventis) had abandoned production in the 1990s.

Then, in 2000, an extraordinary event occurred. Gillette launched eflornithine, made by Bristol-Myers Squibb, as a topical formulation under the trade name Vaniqa, to suppress the growth of unwanted facial hair. It was at this point that Aventis, now Sanofi-Aventis, were persuaded to produce the drug free for WHO to distribute to national programmes involved in HAT therapy. Aventis also agreed to provide pentamidine and melarsoprol gratis for an initial period of 5 years (2001–2006) and Bayer joined in by donating suramin and nifurtimox too. Aventis have recently extended their support for 5 more years (until 2011), and surveillance and research were also stepped up under the WHO–Aventis deal. In addition to the role of WHO other initiatives are playing a role.

In 1999, The Bill and Melinda Gates Foundation was established and has set about funding, to unprecedented levels, initiatives in global health and education. With an endowment fund of $31.7 billion the Foundation has transformed the environment with regard to funding in several tropical diseases. Late in 2000, the Foundation started funding a group of researchers under the leadership of the University of North Carolina at Chapel Hill (UNC) to develop pafuramidine for HAT. As discussed earlier, there is a genuine hope that the drug will successfully complete trials within 2 years and plans are already being drawn up for its manufacture and distribution. In addition to the development of pafuramidine, the UNC-led consortium also received funds to seek new drugs for visceral leishmaniasis and the consortium was refunded in 2006 to continue searching for new drugs for late-stage HAT and visceral leishmaniasis, as well as to take pafuramidine through clinical trials. Other initiatives are also underway. The Special Programme for Research and Training in Tropical Diseases of WHO (WHO/TDR) has long fought for new drugs for HAT and other tropical diseases. Following consultation initiated through MSF's 'Access Campaign' (Trouiller *et al*., 2002), the Drugs for Neglected Diseases initiative (DNDi) (http:www.dndi/org), a Public Private Partnership, was founded in 2003, to discover and develop new drugs to treat trypanosomatid diseases (HAT, Chagas disease and leishmaniases). The UK's Wellcome Trust has remained a major player in the field of trypanosomiasis research. They provided much of the funding required to sequence the trypanosome's genome (Berriman *et al*., 2005). With the genome known, every potential drug target within the trypanosome is now accessible. Gene knockout (Barrett *et al*., 1999) and RNA interference can both be used to provide evidence as to whether genes, and the proteins they encode are essential and thus validated drug targets. A natural progression has been for the Wellcome Trust to establish a screening centre at the University of Dundee, where a library of chemicals (containing an initial set of 62 000 compounds, selected for lead drug-like criteria) is being screened systematically for activity against validated targets (http://www.drugdiscovery.dundee.ac.uk). High-throughput screening is also underway at the Sandler Centre at the University of California, San Franciso (http://www.ucsf.edu/mckerrow/slide.html).

The *in vitro* model system to test trypanocidal efficacy in operation at a number of centres, the largest of which were originally supported by WHO/TDR (http://www.who.int/tdr/), allows rapid screening of tens of thousands of compounds for trypanocidal activity. These centres offer pharmaceutical companies an opportunity to re-engage with diseases like HAT without having to set up in-house facilities. Pfizer Animal Health, for example, has recently initiated a screening programme with their compound libraries in collaboration with WHO/TDR against a range of tropical diseases, including HAT. DNDi has linked up with agencies holding natural product libraries to enter similar screens. DNDi is also pursuing other lead compounds by bringing together groups of investigators in consortia to proceed through the development process. Potent trypanocides discovered in these screens can then enter rodent models of stages 1 and 2 disease to test *in vivo* efficacy.

The surge in activity in research into new drugs for HAT should see the emergence of numerous lead compounds over the next few years. A bottleneck in drug development, however, is likely to appear at this point. Committed chemists and pharmacologists must join the venture to optimize leads. The narrowest bottleneck will, however, involve taking compounds into clinical trials.

In addition to the advancement of pafuramidine through phase III trials, the UNC-led consortium is funded to seek new agents for stage 1 and, especially, stage 2 HAT. In addition to in-house chemistry efforts, novel compounds

emerging from other screening programmes will be considered by the UNC group to take through the latter stages of development, drawing on the experience gained in the development of pafuramidine. The development of pafuramidine provides a good model on the kinds of interaction needed to take a compound through the whole-development process. A tightly knit, multidisciplinary, multinational consortium, involving academic and industrial input to cover all aspects of drug development has been necessary. The project was founded on the premise that diamidines are highly trypanocidal and that chemical modification could allow changes that effect efficacy and pharmacokinetics in a manner to optimize use. The consortium involves chemists who can produce new compounds that are screened in assays for trypanocidal activity and for *in vivo* efficacy (and against drug-resistant trypanosomes). Uptake into trypanosomes and their ability to bind putative targets is also tested in a systematic fashion. Iterative syntheses based on structure–activity relationships are possible. Hit compounds can be tested for efficacy and pharmacokinetic properties in rodent and simian models, in an academic setting, with the best compounds then sent for GLP testing in an industrial setting.

For pafuramidine itself, its potential use against PCP meant that phase I clinical trials had already been completed by the time the UNC-led consortium commenced their effort on trypanosomiasis. This enabled its rapid introduction into phase II trials against HAT patients. Fortunately, investigators from the Swiss Tropical Institute had recently been engaged with a series of centres in Africa to test the new melarsoprol short course (Burri *et al*., 2000), thus trials in these centres, under conditions that came as close as is possible, in this environment, to Good Clinical Practise became a possibility. Clinical trials within HAT affected areas offer great challenges. For example, in conditions where electricity supplies are often absent, facilities enabling constant temperature storage of compounds become impossible. Biochemical and physiological tests considered routine in a hospital in the west cannot be conducted and so on. The pafuramidine project, having received appropriate funding for clinical trials and also having garnered support from national programmes and WHO, has managed to proceed remarkably smoothly.

The number of people capable of conducting such trials is limited. Moreover, the recent downturn in the incidence of HAT (Anonymous, 2006; Barrett, 2006), coupled to the requirement for strict exclusion criteria to reach objective conclusions from trials, offers a very major problem. Patients located in the vicinity of the few centres capable of conducting satisfactory clinical trials are presently in short supply. Logistical problems restrict the ability to reach other areas and to recruit patients into trials relatively far from their homes. Diagnostics, active case finding and the establishment of new facilities in remote areas where the disease is most prevalent will be required to ensure that meaningful clinical trials are possible once compounds begin to emerge over the next 2–10 years.

The problem has been recognized and the Bill and Melinda Gates Foundation has recently funded the Foundation for Innovative New Diagnostics (http://www.finddiagnostics.org) and WHO to seek new diagnostics. With a view to ensuring that any new drugs can be targeted most efficiently to those who need them, the UNC-led consortium has also initiated efforts to identify the precise extent and distribution of HAT, building on statistics collected by the WHO over many years. DNDi along with other key agencies, including WHO, has been formulating a 'platform for clinical trials' to standardize and coordinate efforts.

Unprecedented activity in the area of drug discovery for HAT is underway, plans to help products through development bottlenecks are being put in place and there is currently, for the first time in a generation, great optimism that novel drugs for HAT will emerge over the next decade.

Conclusions

HAT has become a *cause célèbre* among those concerned with the economic factors that have led to diseases of the world's poorest populations becoming neglected in terms of new drug development. The agents that are currently available as anti-trypanosomals are generally unsatisfactory due to a combination of their low efficacy, dangerous side effects and difficulty in administration. In the twenty-first century, however, the situation is changing. Increased knowledge about the trypanosomal genome has already led to the identification of novel drug targets. This development has occurred in parallel with the emergence of funding agencies committed to the elimination of sleeping sickness through coherent and integrated research projects. There is now unprecedented optimism that new drugs active against both stage 1 and stage 2 of the disease will emerge over the next decade.

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Conflict of interest

The authors are part of the Bill and Melinda Gates Foundation funded Consortium involved in the development of pafuramidine maleate and other new drugs for human African trypanosomiasis and leishmaniasis.

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