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Effective measures for controlling trypanosomiasis

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African trypanosomiasis, otherwise known as sleeping sickness in humans and '*Nagana*' in cattle, is a disease that is resurgent in Africa. Research on the disease suggests that the development of a vaccine is still far away; even existing drugs are becoming ineffective on account of the emergence of drug-resistant trypanosomes. All this contributes to heavy economic losses and a sociopolitical crisis in the continent, thus underscoring the pressure to intensify research for inexpensive, less toxic and affordable trypanocides. This review discusses the current treatment of trypanosomiasis and the progress made towards the effective control of trypanosomiasis.

Keywords: arsenicals, eflornithine, isometamidium, nifurtimox, sleeping sickness, suramin, trypanosomiasis

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1. Introduction

African trypanosomes are the causative agents of sleeping sickness in humans and '*Nagana*' in cattle. The parasites, which are transmitted by the bite of tsetse flies, live extracellularly in the blood and tissue fluids in the mammalian host. In 36 countries, > 50 million people are at risk of acquiring sleeping sickness [1]. Furthermore, cattle are threatened with *Nagana*, with an estimated loss of ~ US$140 million annually [2]. The chemotherapy of African trypanosomiasis still relies heavily on drugs developed decades ago, some of which are toxic [3]. In addition, the emergence of drug-resistant trypanosome strains in animals has been widely reported [4]. Unfortunately, the gradual breakdown of countermeasures introduced in the early part of the twentieth century has led to the re-emergence of sleeping sickness as a problem in sub-Saharan Africa, with the prevalence of infected individuals estimated to be approaching half a million [5].

A major focus on the discovery of novel antitrypanosomal and antileishmanial drugs during the past two decades has been the identification and characterisation of biochemical and molecular targets [5]. Rational approaches to chemotherapy have investigated several targets, including the sterol biosynthetic pathway in *Trypanosoma cruzi,* ornithine decarboxylase in *Trypanosoma brucei* complex, trypanothione reductase in *Trypanosoma* and *Leishmania* spp., folate metabolism and DNA topoisomerases [6,7]. The control of sleeping sickness requires tsetse fly control, prompt diagnosis and effective treatment of individuals. The eradication of tsetse breeding sites is particularly effective in the control of animal trypanosomiasis.

Apart from the drugs used in clinical practise, nomadic cattle owners and traditional herbalists have used ethnopharmacological practise for the treatment of the disease. The efficacy of some of these medicinal plants have been authenticated in several laboratories.

2. Goals for chemotherapy

The prospects for the future of trypanosomiasis treatment are deplorable, thus making the investigations on improved drugs mandatory. Moreover, on account of

the less privileged social class usually affected by the disease, the central goal of therapy is the discovery of inexpensive and less toxic drugs.

One particular feature of the trypanosome parasite is the dense glycoprotein coat covering the entire surface of the parasite. With 1000 different genes encoding antigenically distinct versions of the coat, the parasites have the capacity to engage in an immunoprotective process of antigenic variation. This phenomenon has rendered the prospect of a vaccine against the parasite difficult [8]. Therefore, it is obvious that the main options left for the effective control of trypanosomiasis are drug development and prevention of its spread by the tsetse vector.

3. Arsenicals: melarsoprol

Some of the compounds currently available for the treatment of trypanosomiasis include arsenicals, pentamidines, suramin isometamidium eflornithine, homidium bromide and nifurtimox. Their mode of action and the toxicity of some of them are described. There are two recognised stages in the clinical presentation of human African trypanosomiasis: the haemolytic and the encephalitic stage (when the CNS is involved).

The second stage of human trypanosomiasis is characterised by the presence of the parasites in the cerebrospinal fluid (CSF). At this stage, treatment can only be accomplished by drugs that have the ability to cross the blood–brain barrier (BBB). So far, only the arsenical compound melarsoprol (**Figure 1**) has demonstrated the ability to cross the BBB and kill the CSF-residing *T. brucei gambiense* and *T. brucei rhodesiense* parasites. Melarsoprol was first introduced in 1949 for the treatment of late-stage trypanosomiasis, and has remained the main drug of choice [9]. The compound has a trivalent arsenic element, with a marked reactive arsenoxide group, which confers the physicochemical ability of lipid solubility and allows its passage across the BBB [10]. Different modifications on the parent compound are known to produce varied effects; **Table 1** summarises the effect of such modifications [11]. The trivalent arsenicals, melarsoprol, melarsen oxide and phenylarsen, are highly active with a minimum inhibitory concentration (MIC) of 1.0 – 6.5 ng/ml. However, melarsen and inorganic arsenic oxide (the pentavalent forms) are considerably less active, with MIC 50 μg and 111 ng/ml, respectively. Melamie and dimercaprol are non-arsenic chemical constituents of melarsoprol and are completely inactive towards the parasites.

3.1 Mode of action, pharmacology and metabolism of melarsoprol

The targets of melarsoprol in trypanosomes are thiol-containing enzymes, such as the glycerol-3-phosphate dehydrogenase. In addition, trypanothione (N^1-N^8-*bis* glutathionylspermidine), an unusually low molecular weight thiol compound, forms a very stable complex with arsenic [12]. These reports demonstrate that the interaction with the thiol group could be a basis for chemotherapy.

Although melarsoprol is the trypanocide, the active metabolite predominant in the body is melarsen oxide (**Table 1**) and not melarsoprol, which has a half-life of < 30 min [11]. Melarsen oxide is rapidly formed in the body and reaches a maximum level after 15 min before disappearing from the plasma (apparent half-life of 3.8 h). Through *in vitro* studies, the breakdown of melarsoprol measured at room temperature has been shown to be a slow process, with a half-life of 3 days [13]. This strongly suggests the possible contribution of an *in vivo* enzymatic process in the fast conversion of melarsoprol to melarsen oxide.

3.2 Administration and adverse effects

The administration of melarsoprol to patients is complicated. In *T. brucei rhodesiense* infection, it is usually given in three series of four intravenous injections, with an interval of 10 days between each series [9]. The main side effect is severe reactive encephalopathy in ≤ 10% of the treated patients with a mortality rate of ≤ 5% [10]. The drug is also administered at a dose of 3.6 mg/kg on day 1, one-third of the dose on day 2, two-thirds of the dose on day 3 and a full dose on day 4. Recently, a 10-day short regimen of 2.2 mg/kg has been introduced, albeit for *T. brucei gambiense* infections only [14]. Melarsoprol is soluble only in propylene glycol, and is marketed as a 3.6% solution in propylene glycol; however, once the vial is opened, it begins to deteriorate.

The treatment of late-stage African human trypanosomiasis with melarsoprol is accompanied by some adverse effects, such as cutaneous reactions, polyneuropathy, diarrhoea and fever [15]. The worst of these side effects is encephalopathy, which affects ≤ 19% of patients [16]. Isolated cases of seizures, peripheral neuropathy, headache, tremor, fever, abdominal pain, chest pain, skin rash, cardiac, renal and hepatic toxicity, as well as possible agranulocytosis, have been reported [17,18]. The fatality ranges 2.0 – 9.8% for *T. brucei gambiense* [16] and 3.5 – 12 % for *T. brucei rhodesiense* [19]. Notwithstanding the toxic side effects, the precise origin of this toxicity is poorly understood.

3.3 Resistance

Treatment failures have been reported in late-stage sleeping sickness patients treated with melarsoprol [20]; in some regions, treatment failures have reached 30% [21]. Some other reports have shown that levels of drug are similar in the CSF of patients relapsing to those who are not; therefore, parasites resident at other extravascular sites may be key to treatment failure [22]. Parasites retrieved from the patients with this treatment failure were less responsive to melarsoprol than parasites isolated from other foci [23]. This possibly points to some form of mutation in the resistant parasite. Indeed, it has been shown that arsenic-refractory parasites do possess an unusual amino purine transporter, which accumulates melarsoprol; the loss of this transporter in the parasite leads to drug resistance [24,25]. *T. brucei* contains several of the purine

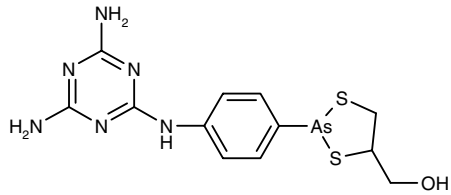


Figure 1. Chemical structure of melarsoprol.

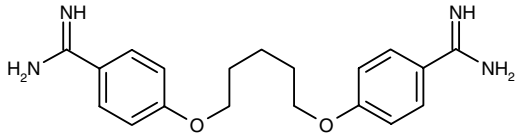


Figure 2. Chemical structure of pentamidine.

nucleoside transporter activities, including P2, which carries adenosine and a nucleobase adenine, and P1, which appears to be a general purine nucleoside transporter [24]. The P2 transporter also interacts with melaminophenyl arsenicals and diamidines, and has been confirmed to be critical in the uptake of arsenicals [24]. The resistance to melarsoprol is centred on the mutation of these transporters. Experiments on the genetic variants of the TbAT1 adenosine transporter have been confirmed in relapse infections following melarsoprol therapy [23].

3.4 Diaminidines

Pentamidine is an aromatic diamine 1,5-*bis* (4-amidi-phenoxypentane) (**Figure 2**) used for the chemotherapy of African trypanosomiasis, antimony-resistant *Leishmaniasis* and *Pneumocytis carini* pneumonia [26]. It is especially useful in the treatment of early-stage *T. brucei gambiense* sleeping sickness. The treatment of early-stage trypanosomiasis comprises 7 – 10 injections of 4 mg base/kg body weight, given daily or on alternate days. The drug is ineffective after the trypanosomes have entered the CNS.

The diffusion of pentamidine across biological membranes is very slow. It acts against the parasites independently of their physiological action on the host. The lethal intracellular concentrations of pentamidine are > 1 mM; hence, the transport of diamines is necessary for drug action [27].

The role of transporters is central in the mode of action of pentamidine in *T. brucei*. Experiments have shown that pentamidine and berenil abrogated melarsoprol-induced cell lysis in a similar manner as adenosine and adenine [28,29]. Likewise, adenine strongly inhibited [^{3}H]pentamidine uptake [28], leading to a tentative conclusion that the P2 transporter is involved in the uptake of diamines and melaminophenyl arsenicals.

3.5 Metabolism and adverse effects

Pentamidine interacts with a number of cellular anions and binds tightly to the minor groove of DNA to inhibit nucleic acid replication [30]. One possible target of pentamidine in trypanosomes is the highly intercatenated network of circular DNA molecules that comprise the mitochondrial genome or kinetoplast [31].

Pentamidine inhibits the self-splicing of GpI intron CaLSM from transcripts of the *26rRNA* gene of *Candida albicans* and prevents the formation of the catalytically active F-band conformation of the precursor RNA. This alters the ribonuclease cleavage pattern of CaLSM RNA. The drug has also been shown to specifically inhibit *S*-adenosyl methionine decarboxylase, thus suggesting that direct inhibition of polyamine synthesis could be involved. Moreover, the drug has been shown to inhibit trypanothione metabolism [32].

Treatment failures and adverse effects, such as nephrotoxicity and diabetes mellitus, have been reported [31,32]. Pentamidine binds with great avidity to imidazole receptors, which explains the hypotension and some other side effects characteristically induced by the drug [33]. The P2 transporter is capable of transporting diamidine drugs, and a selection of parasites resistant to one of these classes of drugs can often underlie cross-resistance in others [25].

4. Suramin

Suramin is a colourless polysulfonated symmetrical naphthalene derivative (**Figure 3**). It has six negative charges at physiological pH, and is, therefore, ineffective against late-stage trypanosomiasis, as it cannot cross the BBB [34]. Suramin is usually the drug of choice for early stages of African human trypanosomiasis, especially *T. brucei rhodesiense* infections.

4.1 Mode of action and resistance

The high charge property of suramin allows it to bind to many serum proteins. At 75 – 100 μM, > 75% of the drug is bound to serum proteins [35,36]. Some serum proteins, including low-density lipoproteins (LDLs), transferin, but not albumin, are taken up through a receptor-mediated endocytosis [35]; it has been shown that suramin enters the parasite specifically bound to LDL [36]. Accumulation of the drug in trypanosomes is relatively slow, thus the uptake of the drug occurs via endocytosis bound to LDL. Suramin directly interferes with the complex formation between LDL and its receptors, and inhibits the transport of LDL. The LDL receptor varies within tissues; although hardly any are present on erythrocytes, the membrane of the adrenal cortex contains a large number of receptors [37]. This probably explains why suramin does not interfere with erythrocyte function [38].

For prophylaxis, the drug is administered in doses of 1 – 2 g and repeated at 10-day intervals. Complexes of suramin with cationic drugs, especially quinapyramine, are more effective in prophylaxis than suramin alone; however, such complexes have

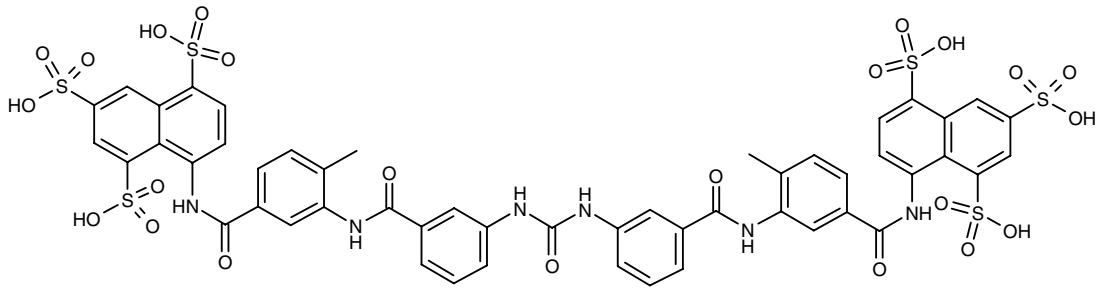


Figure 3. Chemical structure of suramin.

not been widely accepted for field purpose, because of the high cost of complex formulations and severe tissue reactions that occur at the site of injection [39].

The metabolism of suramin does not seem to be a remote cause of resistance, as it is very stable *in vivo*, remaining in the blood stream with a half-life of 44 – 54 days, after which it is finally excreted [35]. In contrast to diaminidines and melarsoprol, it is unlikely that the mechanism of suramin resistance will be a result of a dramatic change in the uptake. This is because LDL is essential for the proliferation of *T. brucei*, which is unable to synthesise fatty acid and cholesterol *de novo* [40]. Alternatively, suramin resistance has been postulated to develop as a result of changes in the drug target, by expression of a drug extrusion mechanism [41]. The induction of suramin resistance *in vivo* with a stable phenotype after transmission by the tsetse fly has been reported [42].

4.2 Action on other diseases

At physiological pH, suramin has six negative charges, thus giving it good capacity to inhibit many enzymes. A key enzyme of HIV, reverse transcriptase, is known to be strongly inhibited by suramin [43]. It also prevents HIV from penetrating $CD4^+$ cells. The observation prompted the US AIDS lobby to push for the clinical trial of suramin against AIDS in the 1980s. The drug had no impact on the progression of AIDS, although minor effects on some incidences of the AIDS-associated Kaposi's sarcoma, were observed [35]. The drug was then tested against neoplastically transformed cell lines and went into trial against a variety of cancers, where it was shown to be effective against hormone refractory prostrate cancer. The mechanism of action of the drug against cancer has been suggested to be due to antiangiogenesis effects and interaction with growth factor receptors affecting the signalling pathway.

5. Isometamidium

Isometamidium chloride, a conjugate of homidium and part of the berenil molecule, is used exclusively as a veterinary trypanocide. It is used for both prophylactic and therapeutic purposes. Isometamidium transport and resistance has largely been studied in *T. congolense*, which, together with *T. brucei* and *T. vivax,* is the main cause of trypanosomiasis in African livestock.

Resistance to isometamidium is a severe problem in many parts of sub-Saharan Africa; therefore, it is now mostly used together with periodic berenil (diminazene aceturate) to treat animal trypanosomiasis. Resistance by parasites with respect to reduced uptake has been reported [44,45]. Both reports documented that transport was energy-dependent and strongly reduced in the presence of salicylylhydrooxamic acid/glycerol.

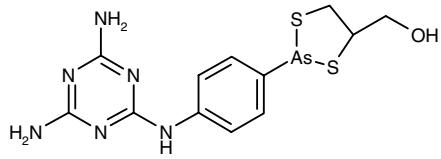
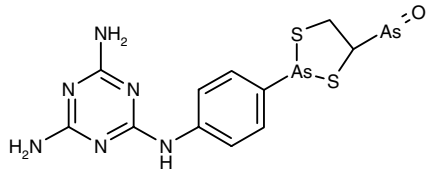
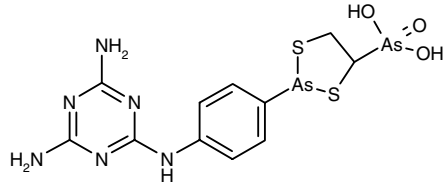
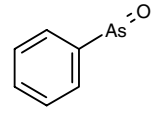
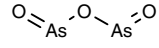
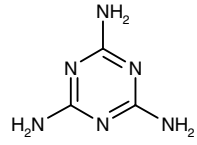
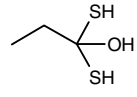
6. Eflornithine

Eflornithine (difluoromethylornithine [DFMO]) is an analogue of ornithine, which is a 'suicide inhibitor' of the enzyme ornithine decarboxylase [46]. The drug was initially developed as an anticancer agent; however, it still remains at the trial stage against neoplastic disease [47]. The drug is active against *T. brucei gambiense*, even at the late stage when the CNS is involved [48]. The uptake of DFMO in *T. brucei* occurs by passive diffusion across the plasma membrane [49]. Reduced drug accumulation has been reported in parasites resistant to eflornithine; however, whether this was due to decreased uptake or efflux was not determined [50].

DFMO inhibits ornithine decarboxylase (ODC) and has a similar affinity for the mammalian and trypanosomal enzymes. Its specificity against the parasite arises because this organism has an ODC that is degraded within the cell and replenished at a rate several orders of magnitude slower than its mammalian counterpart [51]. Thus, a pulse of DFMO can deprive trypanosomes of ODC and polyamine synthesis for a prolonged period compared with mammalian cells, leading to a cessation of growth. A functional immune system is required to kill the growth-arrested trypanosomes.

In order to be effective against sleeping sickness, DFMO is administered in large doses. An additional drawback is its

Table 1. Showing the structurally modified melarsoprol derivatives and their minimum inhibitory concentration.

Compound	Structure	Minimum inhibitory concentration
Melarsoprol		6.5 ng/ml
Melarsen oxide		6.5 ng/ml
Melarsen		50 µg/ml
Phenylarsen		1 ng/ml
Arsenic oxide		111 ng/ml
Melamine		> 111 ng/ml
Dimercaprol		11 mg/ml

Data from [11].

lack of activity against *T. brucei rhodesiense* sleeping sickness. *T. brucei rhodesiense* may be innately less susceptible to the drug than *T. brucei gambiense*, as it has a higher overall ODC activity and the enzyme has a shorter half-life than the *T. brucei gambiense* counterpart [52].

7. Nifurtimox

Nifurtimox is a 5-furan derivative that has been used for the treatment of Chagas disease since 1960 [53]. Its mechanism of action is based on its ability to generate free radicals by the

reduction of nitrofuran and their subsequent binding to proteins and DNA [54]. On account of its function as a reductant, it is postulated to be a substrate for trypanothione reductase, the main enzyme involved in oxidation reduction reactions in the trypanosome [55,56]. Nifurtimox undergoes wide distribution once administered, irrespective of its route. Studies conducted with rats, using a ^{35}S marker, revealed the presence of the drug throughout the animal, including the brain and spinal cord [57]. Thus far, nifurtimox is reported to be active only against both stages of *T. brucei gambiense* infections. As its use has yet to be approved against Human African trypanosomiasis (HAT), initial trials have involved patients refractory to melarsoprol with no treatment alternatives. A 75% cure rate among 20 patients in advanced stage who received a prior injection of suramin has been reported [58]. Furthermore, 72% of 75 patients in stage 2 were cured by nifurtimox alone, at a dose of 15 mg/kg/day for 14 – 45 days [59]. Another monotherapy trial involving 4 – 5 mg/kg t.i.d. for 60 days in 15 stage 2 patients resulted in an 87% cure rate [60]. The main observable side effects involve CNS dysfunction, such as seizures, psychotic reactions and peripheral neuropathy, and gastrointestinal disturbances, such as anorexia, nausea, vomiting and weight loss. Experiments have shown that microsomal and mitochondrial redox systems nitroreduce nifurtimox in the presence of NADPH-generating systems. Thus, the formation of reactive metabolites and nitric oxide during nifurtimox metabolism has been suggested to contribute to its toxicity [61].

8. Current best practise

It is clear that the combination of known trypanocides can produce the desired effects of reduced dosages of individual drugs [16]. Reduction of the side effects of existing drugs and extended supplies under the condition of limited availability is imperative.

Eflornithine has been used with melarsoprol, suramin and pentamidine in experimental animals with considerable success [62]. In addition, suramin has been used with megazol to clear trypanosomal infections from the CSF of experimental animals, whereas neither of these drugs alone had this effect [63,64]. Altering doses of drugs can be useful; for example, according to pharmacokinetic data, a short course of melarsoprol has similar efficacy to the original longer course [11,64].

9. Conclusion

It is obvious that the chemotherapy of African trypanosomiasis is far from satisfactory. Drugs that have been in use for a long time are being rendered ineffective due to new derivatives and resistance to drugs on account of parasite mutation. A lot has been done by a considerable number of researchers towards the identification of good drug targets in trypanosomes, and the lead compounds that inhibit these targets. However, there is currently no mechanism by which lead compounds can be taken through the costly development process required to generate clinically useful drugs. It is this gap between good science and industrial drug development that must be filled if problems associated with the chemotherapy of African trypansomiasis are to be overcome [16].

10. Expert opinion

The clinical practise of combined therapy appears to be the best current option, especially because it serves to reduce the level of dosage and produces a synergistic effect.

The poor input and insensitivity by the pharmaceutical industrial sector in the quest for trypanocides has put the pressure on traditional healers who either by luck, myth or ancestral documented evidence on ethnopharmacology practise have served to improve the fight against HAT. Their huge base of information is now exploited by scientists for the isolation of active principles from medicinal plants and improving their efficacy via biotransformation or organic synthesis using the molecules as parent compounds. This approach is especially appealing considering the current trend of functional genomics and proteomics, whereby the binding efficacy of an identified ligand can be highly improved by computer-assisted drug design.

Apart from the fact that medicinal plants are natural sources of drugs, they have the advantage of synthesising secondary metabolites specifically in response to the presence of pathogens. Prior to the advent of synthetic drugs, local herdsmen have been involved in the use of some of the common plants to control trypanosomiasis. These plants include *Adansonia digitata, Terminalia avicennoides, Khaya senegalensis, Cissus populnea, Lawsonia, Bowellia dalzielli, Pseudocedrela kotschi*, *Syzyium quinensis, Sterculia setigera* and *Afzelia africana* [65]. So far, some plants that have shown clear antitrypanosomal action from experimental chemotherapy include *Azadiracta indica* [66] and *Allium sativa* [67]. It has been shown that azanthraquinine isolated from *Mitracarpus scaber* has a strong inhibitory effect against respiration in long slender forms of *T. congolense* [68].

In addition, an experimental report has shed light on the use of some plants commonly used against HAT in Uganda. The following plants, with a median inhibitory concentration (IC_{50}) < 1 µg/ml, have strong trypanocidal effect against *T. brucei rhodesiense: Albizia gummifera, Ehretia amoena, Entada abyssinica*, *Securinega virosa* and *Vernonia subuligera* [69]. Other pertinent reports have also shown that extracts from *Enantia polycarpa (anonaceae)* have a very high antitrypanosomal action against *T. brucei rhodesiense* at 0.5 and 0.04 mg/ml, with a selectivity index of 616 and 209, respectively [70]. A major observation is that, in most cases, the active principles isolated from most of these medicinal plants are quinones. Of particular interest is that they are active in the micromolar range, suggesting that a more effective level of activity is feasible if the active principles are isolated and modified. A good example are the tryptanthrins; a group of plant alkaloids that are weakly basic. These are the active principle of a Japanese traditional remedy for fungal infections [71], and have shown to be active against

Mycobacterium tuberculosis [101]. The efficacy of tryptanthrin was highly improved by introducing a strong electron withdrawing group at position 8, yielding antitrypanosomal activity with an IC_{50} of 0.4 µM [72].

One major drawback in the use of these medicinal plants is the partial loss of activity against the parasites when administered *in vivo*. As with the cases of resistance to other trypanocides, this could also be due to a lack of uptake of the extracts by the parasites to physiologically active levels. Coupling techniques with factors that can enhance endocytosis could circumvent this major shortcoming. In addition, their use in combination with some of the established drugs could provide the desired synergistic effect against the parasites. Moreover, one other interesting property of most medicinal plants with antitrypanosomal potentials is their capacity to ameliorate anaemia, a hallmark sign of trypanosomiasis, by inhibiting the enzymes sialidase and phospholipase A_2.

10.1 Tsetse fly control

The tsetse fly is a key intermediate in effectively maintaining the cycle of transmission of trypanosomiasis. Therefore, it is vital that an effective control measure must equally include the Tsetse fly as a target. It is particularly important to identify antimicrobial peptides with antitrypanosomal properties, which can be used to block the cycle of transmission. Recent studies have revealed that a cationic base antimicrobial peptide kills both procylic and blood stream forms of trypanosomes [73]. It is interesting that this peptide is ineffective against *Sodalis glossinidins*; a bacteria symbiont of the insect vector of sleeping sickness. This can have potential application in using the bacteria as a conduit to carry genes for the expression of this peptide within the tsetse midgut to shut off the life cycle of trypanosomes ingested from an infected blood meal. Developmentally regulated structures on the parasite can also be vulnerable targets in transgenesis. The procyclins glutamic acid–proline and glycine–proline–glutamic acid–glutamic acid–threonine are the major surface glycoproteins in *T. brucei* spp. and are usually truncated by proteases in the mid-gut of the tsetse fly [74]. These trypsin-like enzymes have a pH optimum of 10. Bloodstream forms of *T. congolense* are unaffected by these proteases. The pH of the tsetse midgut is alkaline, and 48 h after blood meal the pH in the preventriculus is still alkaline and the pH of the midgut and becomes neutral–acidic. Epimastigote forms in the fly midgut are first seen in the proventriculus before they migrate to the salivary glands. All of these changes that allow the parasite to survive within this period of inset infection could be valid targets for intervention. Furthermore, with the emergence of RNA interference technology, genes identified as essential in maintaining insect infection could be suggested for silencing either by direct, or inducible, RNA interference.

With a comprehensive assessment of validated targets in both insect and mammalian stages of the disease, and the use of medicinal plants and their active components, the effective control of trypanosomiasis can be realised. However, this awaits a more serious attitude from the industrial sector, which is otherwise apathetic to a disease linked only to the poor.

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Patent

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