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Drug resistance in pathogenic African trypanosomes: what hopes for the future?

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Abstract

Trypanosomosis is a serious threat to both man and animals mostly in Africa. Although the first pathogenic trypanosome was discovered over a hundred years ago, there is still no prospect for effective control or eradication of the disease through the development and use of vaccines because of the phenomenon of antigenic variation. Control continues to rely heavily on chemotherapy and vector control strategies. This therapy and prophylaxis depends on the use of drugs which, apart from having been developed over 5 decades ago, suffer from such limitations as toxicity and with their continued use, drug resistance. Resistance to currently used drugs is a serious problem in most fields of anti-microbial chemotherapy, particularly in the case of trypanosomosis where resistance and cross-resistance in animals and man have been developing rapidly. The frequently and widely reported decreasing efficiency of available trypanocides, difficulties of maintaining tsetse control and the need for new drugs and alternative effective ways for the control of trypanosomosis. This review examines aspects of drug resistance in pathogenic trypanosomes, measures to minimise it, areas of future research in new drug targets and alternative control strategies. Based on these, it is our opinion that for now the management and control of trypanosomosis will continue to depend on proper usage of the few available trypanocides, especially strategic deployment of the sanative drugs in order to reduce the development of drug resistance, in addition to the continued use of environmentally friendly vector control programmes such as tsetse trapping. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Trypanosomes spp.; Trypanocides; Drug resistance

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1. Introduction

Trypanosomiasis is a complex debilitating and often fatal disease caused by infection with one or more of the pathogenic trypanosome transmitted protozoan parasites of the genus Trypanosoma. The most important species responsible for the disease complex, commonly known as nagana in livestock, include Trypanosoma brucei, T. congolense and T. vivax, and the said parasite T. simiae, which is responsible for acute trypanosomiasis in pigs. In Africa, Asia, the middle east and south America, T. evansi, the causative agent of surra, is important especially in draft and transport animals, and is exclusively transmitted mechanically by biting flies such as Tabanus and Stomoxys spp. On the other hand, T. gambiense and T. rhodesiense are the most important human pathogens responsible for West and East African sleeping sickness, respectively. It has long been established that Nagana renders approximately a quarter of Africa’s arable land mass unsuitable for profitable livestock farming (Molyneux, 1997). Losses in meat production, milk yield, and tractive power are estimated to cost approximately US $500 million annually and, if lost potential in livestock and crop production are also considered, then trypanosomiasis may be costing Africa an estimated US $5 billion per year (ILRAD, 1994).

There is no effective vaccine against trypanosomiasis and in the absence of coherent environmental friendly and sustainable vector control strategies, the control of trypanosomosis continues to rely principally on chemotherapy and chemoprophylaxis using the salts of three compounds: diminazene, an aromatic diamidine; homidium, a phenanthridine; and isometamidium, a phenanthridine-aromatic amidine (Leach and Roberts, 1981; ILRAD, 1990: Fig. 1). In addition, quinapyramine, suramin and recently, melarzone oxide cymene (cymelarsan) are generally used for therapy and prophylaxis of T. evansi (Leach and Roberts, 1981; Raynaud et al., 1989; Zhang et al., 1991; Ndoutumia et al., 1993). Of the six trypanocides, diminazene aceturate is the most commonly used therapeutic agent while isometamidium chloride is most commonly used as a prophylactic agent. These drugs, with the exception of cymelarsan, have been in use for at least 30 years. For instance, suramin has been in use since the 1920s, diminazene aceturate, homidium and quinapyramine were all introduced for field use in the 1950s, while isometamidium chloride came into field use in 1961 (Kinabo, 1993). Thus, cymelarsan, the trivalent water soluble analogue of the arsensal melarsoprol, introduced in 1985 for the exclusive treatment of T. evansi and other brucei-group trypanosome infections, and N,N-dimethylethylmethimizole (DFMO) dubbed the ‘resurrection drug’, are the only new trypanocides commercially available for veterinary and human use, respectively, in over 30 years (Kizoo, 1991; Raynaud et al., 1989).

The therapeutic and prophylactic use of trypanocides is beset by numerous limitations, including toxicity and the development of resistance by the parasites. The emergence of drug-resistant trypanosome strains is considered a very serious problem in trypanosomiasis control, particularly for the resource-poor, at-risk populations and farmers in Africa and in the context of sustainable parasite control. Trypanosome resistance to trypanocides increases cost, reduces the efficiency of production and depletes the stock farmers of effective control tools (Donald, 1994). This increases the risk of environmental contamination due to progressive increase in frequency of use and dose rate of drugs with declining or little beneficial effects. Moreover, there is increased risk of toxicity from the use of large doses (Donald, 1994). Thus, the urgency for development of new, effective drugs with fewer
problems associated with currently used drugs, cannot be over emphasized. Considerable work has been conducted in the last 2 decades on drug resistance in trypanosomosis and the search for alternative safe and effective therapeutic agents continues to be a future goal. In this review paper, we have highlighted some of the current status of knowledge concerning aspects of drug resistance in pathogenic trypanosomes, including strategies adopted to minimise development of resistance, possible areas of future research in new drug targets and alternative control strategies in the struggle against the human and livestock diseases caused by pathogenic trypanosomes.

2. Trypanocidal drug resistance in the field

Resistance to each of the commonly used animal trypanocides has emerged and has continued to mar effective veterinary management of trypanosomosis in Africa and elsewhere (Bacchi, 1993; Peregrine, 1994). From a historical perspective, it may be pertinent to mention that relapses were reported immediately after the introduction of suramin, the preferred drug for the treatment of camel trypanosomosis (Knowles, 1927). Nevertheless, such relapses were occasional and since a better alternative did not exist, its use was continued. How-
ever, there is now further field and experimental evidence of diminished effectiveness and in-
crative resistance of trypanosomes to suramin (Payne et al., 1992, 1994; El et al., 1999; Onah et al., 1999). This, coupled with the fact that commercial production of suramin has ceased, was recognised as a serious limitation in the treatment of surra, which can only worsen unless new anti-surra drugs are developed soon (Payne et al., 1994). Although quinotripyramine was seriously affected by drug resistance, leading to its withdrawal from the market in 1976, it was later reintroduced for the exclusive treatment of surra (Schilling and Rottcher, 1986).

Over the years, diminuzene acetate and isoetomitudinium chloride have, respectively, been regarded as the best therapeutic and prophylactic trypanocides. The former was re-
purposed as the only drug to which trypanosomes do not easily develop resistance because of its rapid elimination from the system when compared with the more persistent prophylactic drugs such as isoetomitudinium (Alim et al., 1984; Rushigijiv, et al., 1986). Unfortunately, this view is no longer accepted as field and laboratory isolates and strains of diminuzene-resistant trypanosomes have been reported, some field isolates requiring up to 45 mg/kg diminuzene acetate as the minimum required dose to achieve cote (Shitambo and Arakawa, 1992; Peregine and Masumun, 1993). Similarly, isoetomitudinium treatment failures and shortened prophylactic intervals have been attributed to infections with drug-resistant trypanosome species (Sutherl and et al., 1991; Peregine et al., 1991). Nonidazol, which was previously used extensively as a prophylactic drug was rendered almost useless by widespread develop-
ment of resistant trypanosome strains (Clausen et al., 1992; Codu et al., 1993). Even for the new trypanocide, cyclosilazane, their promises to be a break future for its field use as resistance has already been induced experimentally (Fairlamb et al., 1992; Osman et al., 1992; Ross and Barnes, 1996).

3. Mechanisms of trypanocidal drug resistance

An understanding of the mechanisms of drug resistance by trypanosomes, among others, is important as it can lead to the identification of potential novel drug targets and provide direction to how new chemotherapeutic strategies can be used to reduce development of resistance. In the latter instance rationale for combinations of existing drugs to increase ther-
apeutic activity, decrease clinical toxicity and potentially reducing the frequency of the emer-
gence of drug resistance (Barrett and Fairlamb, 1999) can be identified. Trypanocidal drug resistance could be innate, such as in resistant individuals without previous exposure to the particular drug, or acquired (induced) as a result of drug exposure/presence, cross-resistance or sometimes by mutagenesis (ILRA, 1990). Reduction in drug accumulation by the target cell or organism and diminished drug activity in immunosuppressed animals can contribute to the emergence of drug resistance (Frommel and Balber, 1987; Osman et al., 1992).

The biochemical basis of trypanosome resistance to trypanocides has not been fully characterised. However, because anti-nocerebral agents interact with a drug target, it would follow that drug resistance can arise either as a consequence of changes in drug concentrata-
in the target site or alteration in the target, or both. There is experimental evidence that drug-resistant trypanosome clones accumulate less drugs than their sensitive counterparts. Using flow cytometry, Frommel and Balber (1987) showed that resistant clones of T. brucei brucei and T. b. rhodesiense accumulated a lower intracellular quantity of the
diamidines, 1,6-diamino-2-phenyl-1H-indole (DAPI) and Hoechst 3342; the phenanthridine, etidium bromide; and the acridine, acriflavine than the sensitive clones. In addition, these authors showed that following brief treatment of the resistant and sensitive clones with the detergent Triton X-100, both accumulated identical intracellular concentrations of DAPI implicating alterations in the surface membrane of the drug-resistant trypanosome clone in the reduced accumulation of these drugs. Similarly, fluorescence microscopic and flow cytometric studies showed a reduction in uptake and accumulation of isometamidium chloride by resistant clones of *T. congoles*. Thus, while incubation for 10 min at 27 °C with 5 ng/ml isometamidium chloride resulted in 79% of susceptible IL 1180 *T. congoles* clone showing increased fluorescence over the controls, only 32% of similarly treated resistant IL 3270 clone were more fluorescent than in the corresponding control sample (Sutherland et al., 1991). Subsequently, Sutherland et al. (1992) showed that the uptake of isometamidium chloride by both sensitive and resistant clones of *T. congoles* was through an energy-dependent, specific, receptor-mediated transport system on the parasite surface. Resistance appeared multifactorial, including an alteration in a specific receptor on the cell surface of the resistant trypanosomes and a postulated enhanced efflux mechanism (Sutherland et al., 1992). This view accords with the earlier proposal of Damper and Putton (1976), that the transport of the diminazene-related drug, pentamidine, into *T. brucei* was a carrier-mediated process that is substrate specific, concentrative and energy coupled. Cutter and Fairlamb (1993) provide evidence that drug-sensitive bloodstream forms of *T. brucei* express at least two different nucleotide transporters through which they salvage adenine from their mammalian hosts. Using [3H]adenosine uptake and competitive inhibition experiments, they showed that in vitro trypanolytic effect of melarsomine oxide is specifically abrogated by adenine, adenosine and diphyridamole, all of which compete for uptake by an adenine transporter. Furthermore, they showed that melarsomine-sensitive trypanosomes have two high-affinity adenine transport systems: a P1 type, which transports adenosine and accounts for 60–70% of total adenosine uptake by *T. brucei*; and a P2 type, which also transports adenosine and accounts for 30–40% of total adenosine uptake into the cell (Fig. 2).

Fig. 2. Schematic representation of the two membrane high-affinity transport systems for adenine in drug-sensitive bloodstream forms of trypanosomes by which the parasites scaveng e low concentrations nucleotides in mammalian bloodstream (Cutter and Fairlamb, 1993). The substrate specificity for P1 and P2 transporters is shown. Note that the nucleosidophosphate adenosine and diamidines are transported via the P2 transporter and resistance and cross-resistance to these drugs have always been associated with alterations in the P2 transporters leading to a diminished trypanolytic drug accumulation and hence resistance (modified with permission from Cutter and Fairlamb, 1999).
<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>% Inhibition</th>
<th>Adapting</th>
<th>In-vivo</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>T. evansi</td>
<td>Ross and Barnes (1996)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>98</td>
<td>T. evansi</td>
<td>Ross and Barnes (1995)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>82</td>
<td>T. brucei</td>
<td>Carter and Fairlamb (1993)</td>
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</tr>
<tr>
<td>100</td>
<td>98</td>
<td>T. brucei</td>
<td>Carter and Fairlamb (1993)</td>
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<tr>
<td>20</td>
<td>82</td>
<td>T. brucei</td>
<td>Carter and Fairlamb (1993)</td>
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</tr>
<tr>
<td>60</td>
<td>98</td>
<td>T. brucei</td>
<td>Carter and Fairlamb (1993)</td>
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<tr>
<td>100</td>
<td>99</td>
<td>T. brucei</td>
<td>Carter and Fairlamb (1993)</td>
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<tr>
<td>100</td>
<td>22</td>
<td>T. brucei</td>
<td>De Koning and Jarvis (1999)</td>
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<tr>
<td>10</td>
<td>81</td>
<td>T. brucei</td>
<td>De Koning and Jarvis (1999)</td>
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<tr>
<td>100</td>
<td>90</td>
<td>T. brucei</td>
<td>De Koning and Jarvis (1999)</td>
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<tr>
<td>100</td>
<td>99</td>
<td>T. brucei</td>
<td>De Koning and Jarvis (1999)</td>
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</table>

Uptake of adenosine via the P1 and P2 transporters can be inhibited in a dose-dependent and saturable manner by theophylline and adenosine, respectively (Table 1). These findings were confirmed by De Koning and Jarvis (1999).

Several studies have shown that these purine nucleoside transporters are involved in the uptake of trypanocidal drugs and that resistance is associated with changes in them (Barrett and Fairlamb, 1999). For instance, loss or alteration of the P2-type transporter was shown to account for the resistance of T. brucei to melamathophenyl arselenate (Carter and Fairlamb, 1993) and diamidines (Carter et al., 1995). Similarly, in a diamidine-resistant T. equiperdum clone, the P2 adenine transporter was shown to have reduced transport capacity at physiological adenine concentration and decreased affinity for adenine (Barrett et al., 1995). Also resistance to cytosarum by a clone of T. evansi in which these adenine transporters were reported, was shown to be associated with an alteration in a P2-like adenine transporter (Ross and Barnes, 1996).

Studies of isometamidium uptake in the presence or absence of the metabolic inhibitor salicyldihydroxamic acid (SHAM) and glycerol, and a range of calcium flux-modulating compounds, provide indirect evidence that energy-dependent drug-efflux mechanisms may also play a role in reduced trypanocidal drug accumulation and resistance by trypanosomes to these drugs. SHAM/glycerol equilibrates steady-state isometamidium accumulation by both resistant and sensitive T. congolense clones (Sutherland et al., 1991). Uptake kinetics of isometamidium by sensitive (GRVPS 92) and resistant (GRVPS 56) T. congolense clones showed that the addition of SHAM/glycerol resulted in the uptake of similar amounts of the drug by both clones and that this was attributed to a reduction and increase in drug uptake by the sensitive and resistant clones, respectively. After 5 min incubation with labelled isometamidium alone, the sensitive and resistant clones accumulated approximately 34 and 10ng/10⁶, respectively, but on addition of SHAM/glycerol and incubation for the same period of time, the respective accumulated amounts were approximately 13.6 and 14.5 ng/10⁶ for the sensitive and resistant clones (Sutherland et al., 1992).
Furthermore, in comparison to the uptake of labelled isometizolum alone, the addition of calcium flux-modulating compounds such as verapamil and prazosin, which block drug efflux, caused significant decreases (from 33.2 to 22.6 ng/g/h/5 min for verapamil, 33.2 to 14.6 ng/g/h/5 min for prazosin) and increases (from 12.7 to 22.2 ng/g/h/5 min for verapamil, 12.7 to 24.7 ng/g/h/5 min for prazosin) in drug accumulated by the sensitive and resistant clones, respectively (Sutherland et al., 1992). Although, these results do not directly prove the involvement of drug efflux, the uptake kinetics in the presence or absence of SHAM/glycerol imply that while there is active uptake of the drug by both clones, an active process which resulted in a decreased accumulation of the drug was inhibited in the resis-
tant clone. Similarly, results of the disruption of drug transport by calcium flux-modulating agents suggest that in this case drug efflux has been inhibited and may therefore, be the possible mechanism for reduced accumulation of, and the development of resistance to, isometizolum by T. congolense (Sutherland et al., 1992).

4. Cross- and multiple drug-resistance

As earlier stated, the treatment and prophylaxis of livestock trypanosomiasis in Africa have largely depended on the use of diminazene, homidium, and isometizolum while quinuprine is recommended for use against camelid and equine T. evansi trypanoso-
mis (Leach and Roberts, 1981; Ndoutamia et al., 1993). Ever since they were introduced more than 30 years ago resistance by strains of trypanosomes to each of these compounds has been reported in the field across Africa (Leach and Roberts, 1981; Perregegne, 1984). More worrying however, are the repeated incidences of field strains that have developed multiple resistance to these trypanocidal drugs (Table 2; Leach and Roberts, 1981; Muloo and Kuta-
za, 1990; Aimoussie et al., 1992; Claassen et al., 1992; Mohamed-Ahmed et al., 1992; Codjia et al., 1993; Mulugeta et al., 1997), a situation that constitutes a particularly grave threat to livestock production and health in Africa. Eleven and 10 strains of T. congolense isolated from cattle in Ghibe valley, south-west Ethiopia in 1989 and 1993, respectively, were shown to be resistant to diminazene, isometizolum and homidium (Codjia et al., 1993; Mulugeta et al., 1997). Also, a multiple drug-resistant T. congolense was isolated from Zebu cattle in the field in Borkina Faso during an epidemiological survey (Claussen et al., 1992). In a

Table 2

Cross- and multiple drug-resistance by T. congolense (T.c) and T. evansi (T.e) in cattle and goats\(^a\)

<table>
<thead>
<tr>
<th>Parastate</th>
<th>Animal</th>
<th>D</th>
<th>I</th>
<th>H</th>
<th>Q</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.c.</td>
<td><strong>Cattle</strong></td>
<td>3.5</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>Schonfeld et al. (1987)</td>
</tr>
<tr>
<td>T.c.</td>
<td><strong>Cattle</strong></td>
<td>10.5</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>Moho and Kiruth (1990)</td>
</tr>
<tr>
<td>T.c.</td>
<td><strong>Cattle</strong></td>
<td>14</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>Prospring et al. (1991)</td>
</tr>
<tr>
<td>T.c.</td>
<td><strong>Cattle</strong></td>
<td>7.5</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>Aimoussie et al. (1992)</td>
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<tr>
<td>T.e.</td>
<td><strong>Cattle</strong></td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>Claassen et al. (1992)</td>
</tr>
<tr>
<td>T.e.</td>
<td><strong>Goats</strong></td>
<td>17.5</td>
<td>3</td>
<td>-</td>
<td>5</td>
<td>Claassen et al. (1992)</td>
</tr>
<tr>
<td>T.c.</td>
<td><strong>Cattle</strong></td>
<td>7</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>Codjia et al. (1993)</td>
</tr>
</tbody>
</table>

\(^a\) Figures represent the number of ng/kg/h of drug of the respective drugs that failed to cure the infection, D = diminazene, I = isometizolum, H = homidium, Q = quinuprine.
corresponding chemotherapy trial by these authors in previously unexposed Zebu bulls and Sabinian goats infected with one primary \textit{T. congolense} isolate from Samorø, Congo, the parasite demonstrated the high level of resistance to all three drugs in addition to quinapyramine sulphate at 3 mg/kg body weight in goats (Claussen et al., 1992).

In fact, prior to 1976 when quinapyramine ceased to be manufactured (Holmes and Scott, 1982), it was widely used in livestock as a therapeutic and prophylactic agent (Fiennes, 1953; Ndoitumia et al., 1993). It was withdrawn from the market because resistance to it in trypanosomes appears to develop quickly and easily and rapidly (Wilson, 1949; Fiennes, 1953; Unsworth, 1954; Newton, 1964; Leach and Roberts, 1967) but because the resistance is always associated with the high level of multiple resistance to diminazene, homidium and isometamidium (Mwamba and Mwapone, 1971; Whiteside, 1960). This has been confirmed experimentally by repeated treatment of infected mice with subcurative doses of quinapyramine sulphate over period of 208 days. During this period, the level of resistance of \textit{T. congolense}, IL 1180 clone increased approximately 42-fold, from a 5% cure rate dose (CD$_{50}$) of 0.023 mg/kg of body weight (b.w.) for the parental clone to 0.06 mg/kg/b.w., for the resistant IL 1180 stab 2 (Ndoitumia et al., 1993). This resistance was associated with a 6-fold increase in resistance to isometamidium (from a CD$_{50}$ of 0.011 to 0.1 mg/kg/b.w.). A 26-fold increase in resistance to homidium (from a CD$_{50}$ of 0.37 to 10.35 mg/kg/b.w.) and a 5.5-fold increase in resistance to diminazene (from a CD$_{50}$ of 2.3 to 13.74 mg/kg/b.w.) in infected mice. In goats infected with the resistant clone, one of five goats treated with 3 mg/kg/b.w. quinapyramine sulphate, none of five goats treated with 0.25 mg/kg/b.w. isometamidium chloride, two of five goats treated with 1 mg/kg/b.w. homidium chloride, and three of five goats treated with 3.5 mg/kg/b.w. diminazene succinate subsequently relapsed, showing that except for isometamidium, the clone expressed multiple drug resistance even in the definitive host (Ndoitumia et al., 1993). Similarly, resistance to isometamidium was induced and increased to 84-fold by repeated subcurative treatment of infected mice with isometamidium chloride over 14 months from a CD$_{50}$ of 0.018 mg/kg/b.w. for the parental \textit{T. congolense} IL 1180 to a CD$_{50}$ of 1.7 mg/kg/b.w. for the resistant IL 1180 stab 2 (Peregine et al., 1997). This clone also exhibited multiple drug resistance with a high level of resistance to homidium chloride (33-fold increase in CD$_{50}$ from 0.37 to 12.1 mg/kg/b.w.) but lower levels of resistance to diminazene acetate (3.4-fold increase in CD$_{50}$ from 2.3 to 7.8 mg/kg/b.w.) and quinapyramine sulphate (4.2-fold increase in CD$_{50}$ from 0.23 to 0.97 mg/kg/b.w.; Peregine et al., 1997). Nevertheless, it is known that cross-resistance between diminazene and isometamidium rarely occurs in trypanosomes in the field, thus they are used as a "saturation" combination to curtail the development of resistance to either drug (Whiteside, 1960; Molao et al., 1987). However, strains with cross-resistance to diminazene and isometamidium have been demonstrated in the field and experimentally (Molao and Katurra, 1990; Chitimba and Arakawa, 1991, 1992; Attanasi et al., 1992), although it is possible that cross-resistance in these studies occurred because the trypanosome may have consisted of two phenotypically distinct populations since heterogeneity of drug resistance to diminazene and isometamidium has been demonstrated (Peregine et al., 1991). The origin of multiple resistance to these trypanocides by trypanosomes in the field is unclear, but it has been suggested that it might be associated with cross-resistance between the different compounds as a result of their closely related molecular structures (Fig. 1; Whiteside, 1960; Williamson, 1970).
In contrast, a number of experimental drug sensitivity studies with suramin, diminazene and cycloheximide in vitro and in rodents have demonstrated that acquisition of resistance to suramin does not confer resistance to diminazene and cyclosaine, suggesting that cross-resistance may not exist between these drugs (Rossew et al., 1985; Zwang and Kumin斯基, 1989; Zhang et al., 1993; Pospichal et al., 1994; Ameen et al., 1996). Although similar observations have not been made in the field it would appear that these drugs may be potentially effective in treating field cases of trypanosomiasis resistant to each other. In fact, a single injection of either quinapyramine sulphate (5 mg/kg bw) or suramin (2 mg/kg bw) was equally effective in the treatment of experimental infections of sheep with a T. evansi isolate resistant to treatment with 10 mg/kg bw of suramin (Onuh et al., 1998, 1999). Cyclosaine was also effective against stocks of T. evansi resistant to quinapyramine and suramin in cattle (Payne et al., 1994).

5. Strategies for combating trypanocidal drug resistance

5.1. Sanative pairs

These are pairs of curative drugs which are not susceptible to cross-resistance between each other and thus could be used alternately in the field when resistance to either of them has occurred. This concept of sanative pairs of trypanocides was originally proposed by Whiteside (1958), with diminazene and homidium, or diminazene and isometamidium usually used in the field as sanative combinations (Whiteside, 1958, 1960, 1962). These pairs when strategically employed can be used to maintain herd productivity in the field without the development of resistance to either of the compounds. For instance, in northern Nigeria, the treatment regimen recommended and which has been in effective use since 1967 is the strategic administration of homidium during the rainy season months of May–October and diminazene during the dry season months of November–April (Akin, 1981). Similarly, a protocol of intramuscular diminazene acetate (3.5 mg/kg) and isometamidium chloride (0.5 mg/kg) was used, respectively, during late rainy season (June) and late dry season (October) to protect cattle from trypanosomosis in Zaire, now Republic of Congo (DeBoox, 1990). Also, since cyclosaine and suramin do not induce cross-resistance to each other experimentally, it has been suggested that both drugs could constitute a sanative pair for the treatment of T. evansi infections (Zwang and Kumin斯基, 1990; Zhang et al., 1993; Payne et al., 1994; Ameen et al., 1996; Ross and Barnes, 1996). However, as mentioned above, the effectiveness of this protocol may be questioned by reports of field isolates from many parts of Central, East, and West Africa of trypanosomes with multiple resistance to trypanocidal drugs including the sanative pairs (Rotcher and Schilling, 1985; Ngouo and Heith, 1986; Schneefeld et al., 1987; Chiabombo and Arakawa, 1991, 1992; Clausen et al., 1992; Coque et al., 1993; Kula, 1995; Ameen, 1997; Mboleka et al., 1997).

5.2. High dose and repeat treatment regimen

High dose treatment offers the best opportunity for eliminating infections with trypanosomes which express high degree of resistance to drugs (Sutherland et al., 1991;
Biowan and Hunter, 1993). However, it must be appreciated that the scope for increased drug dosage is highly dependent on the relationship between the maximal tolerated dose and the minimal dose required to effect a cure, i.e., the therapeutic index. This is a major limitation to high dose treatment with trypanocides as the margin of safety of most of them is usually quite narrow, trypanocidal drug toxicity being quite common (Wang et al., 1995; Troeberg et al., 1999). However, there are different field isolates of T. vivax from cattle in three districts of Kenya which were resistant to treatment with 3.5 mg/kg diminazene aceturate were all cured when infected cattle were treated with 7 mg/kg b.w. of the same drug (Schowfeld et al., 1987). On the other hand, studies on the efficacy of repeat treatments of T. congolense infections with diminazene aceturate indicate that such regimens may be useful especially if administered at 48 or 96 h intervals (Silayo, 1993). This tends to support the suggestion that the efficacy of trypanocides depends not only on the concentration of the drug to which the parasites are exposed but, also on the length of exposure.

For instance, repeated treatment of cattle infected with a highly resistant strain of T. congolense at 28 day intervals using isometamidium chloride had no additive therapeutic effect (Sutherland et al., 1991). When given intravenously however, it was found to be a superior regimen over routine prophylaxis in the control of drug-resistant parasites (Dowler et al., 1989).

5.3. Combination therapy

Most critical in the evaluation of the efficacy of trypanocides is the chemotherapy of late-stage t. trypanosomosis with central nervous system involvement where there is always the tendency for the reappearance of trypanosomes in the blood after treatment (Anene et al., 1989; Chibwuku et al., 1990; Onah and Uzoikwu, 1990). This phenomenon has been ascribed to the presence of extracerebral parasites sequestered in sites inaccessible to the drug. Trypanosomoses in such sites as the brain and the cerebrospinal fluids are protected from certain drugs by the blood-brain-barrier. For instance, only melarsoprol (Mel B) was available for the treatment of late stage sleeping sickness with cerebral involvement in humans before the introduction of DFPMO in 1985, which, although highly effective in curing such infections due to T. gambiense, is limited in its effectiveness against many clinical isolates of T. rhodesiense (Van Nieuwenhove et al., 1985; Barren and Fairlamb, 1999). Unfortunately, Mel B is not only toxic and also not always curative, but remains the most widely used human trypanocide. In an attempt to reduce dosage and the incidence of its toxicity, combination regimens with Mel B has been recommended in the past (Apted, 1970). Of more relevance here is that combination therapy of either 10 mg/kg b.w. of diminazene and 50 mg/kg b.w. of the 5-substituted,2-nitroimidazole (RO 15-0216) or 20 mg/kg diminazene and 25 mg/kg RO 15-0216 were equally effective in treating T. simiae infections in pigs (Zwycznik and Roterich, 1987), whereas isometamidium chloride combined with dextro sulphate was not only highly effective against T. vivax infections in cattle but also less toxic in treating camels (Alia and Samas, 1979).

As will be outlined below, experimental studies in rodents show that rational use of combination therapy enhances the potency of individual trypanocides and stems the appearance of trypanosome stains resistant to trypanocides. The efficiency of combination
therapy lies in the additive or synergistic effect of drugs. When given together, suramin and quinapyramine are more effective than when given alone, just as suramin in combination with trypanamide, proguanil or diminazene is more effective in acute T. rhodesiense infection in mice (Williams et al., 1982). Combinations of suramin with 2-substituted 5-nitroimidazoles were also highly successful in the treatment of chonic T. brucei infection in mice (Jennings et al., 1983; Jennings, 1990). Similarly, DFM0 is highly additive in combination with diminazene, Mel B, pentamidine or sarans in the treatment of acute T. brucei infections (McCann et al., 1983). In addition, combinations of DFM0 and suramin, or DFM0 and diminazene were effective in the treatment of cerebral T. brucei infections in mice (Clarkson et al., 1984; Jennings, 1992). It has been postulated that synergy in combinations of DFM0 and the diamidines (diminazene or pentamidine) may be as a result of joint interference with polymerase synthesis and function by trypanosomes (Bitonti et al., 1986). The synergy between suramin and oxygen-stress-inducing compounds such as DFMO, diminazene, Mel B and nitroimidazoles, is thought to be based on their ability to augment the sublethal effect of suramin on the uptake of low density lipoproteins by trypanosomes (Van Nieuwenhove et al., 1985). Combining DFMO with bleomycin, a cytotoxic anti-tumour antibiotic, resulted in the cure of both early- and late-stage T. brucei infections in mice (Clarkson et al., 1983). The curative activity was blocked by co-administration with polyoxymethylene, suggesting that the synergy is based on polyoxymethylene mechanism (Bucio et al., 1982).

Adjuvant therapy with anti-inflammatory drugs also enhances the efficacy of trypanocides although the mechanism by which this is brought about is not clear. The therapeutic efficacy of diminazene against T. vivax in sheep is enhanced when combined with mepronyl maleate (Joshua and Buhadola, 1983) and against T. brucei in mice and rabbits when combined with proguanil (Kiddey; Abbot, 1991). The potential of diminazene and lithium chloride combination in the treatment of relapsing T. brucei infection has also been indicated in experimental infections in rats (Odika et al., 1995a). Lithium chloride, a hyperosmolar compound, is believed to achieve osmotic opening of the blood-brain-barrier to diminazene resulting in increased concentration of the drug in the same cerebral environment as the sequestered parasites and increased therapeutic efficacy (Odika et al., 1995b). With trypanocidal drugs such as DFMO and quinapyramine, efficiency of the host's immune system is vital in the eventual destruction of the parasites. It is thus conceivable that their co-administration with immunomodulators may achieve enhanced therapeutic activity, although no synergy was obtained by combining DFMO with the immunostimulant levamisole (Arone et al., 1995). However, since trypanosomiasis induces generalised immunosuppression in infected hosts, there is need to imitate the effects of a wider range of immunostimulants in combination chemotherapy and chemophylaxis against trypanosomes.

6. Which way forward?

Due to antigenic variation, there is little or no hope for the production of anti-trypanosome vaccine in the foreseeable future. This, coupled with the limitations of current treatment methods such as toxicity and multiple drug resistance, has initiated the urgent search for
more effective and less toxic chemotherapeutic agents in the fight against the disease. In this respect, proteinases of various pathogens have received attention as potential targets for chemotherapeutic intervention (Coombs and Mottram, 1997, Treeberg et al., 1998). Bloodstream forms of *T. brucei* for instance, contain two major proteinolytic activities: first, a cysteol serine proteinase termed oligopeptidase-Tb with trypsin-like specificity and second a group of cathespin L-like cysteine proteinases localised in the lysoosomal compartments whose major enzymatic form is termed trypanopeptiase-Tb (Hin et al., 1992, Treeberg et al., 1996). The homologue of trypanopeptiase-Tb in *T. congolense* has also been isolated (Mwape et al., 1992). The potential of cysteine proteinase inhibitors as new chemotherapeutic agents for the treatment of trypanosomiasis has been investigated and they have been shown to kill *T. cruzi* and *T. congolense* (Ashall et al., 1980; Mwape et al., 1992). Two recent studies support the proposition that cysteine proteinase inhibitors have potential as trypanocidal agents. In the first, the irreversible cysteine proteinase inhibitors vinyl sulfones, and the peptidyl ketones: chloromethylketones, diazomethylketones (DMK) and a fluoroacetone were trypanocidal against cultured bloodstream forms of *T. brucei* at low micromolar concentrations, with trypanopeptiase-Tb as their major target (Treeberg et al., 1999). Perhaps more importantly, it was shown in the second study that DMK kills bloodstream forms of *T. brucei* both in vitro and in vivo, and that infected mice treated with the inhibitor survived longer than similarly infected group treated with placebo (Story et al., 1999). Again these authors showed that serine proteinase activity, probably due to oligopeptidase-Tb, was not the target of inhibition by DMK and suggested that in the absence of studies indicating the presence of other significant cysteine proteinases in the parasite, it would seem reasonable to concede that trypanopeptiase-Tb is the critical target of DMK. It seems therefore, that there may be value in targeting cysteine proteinases of trypanosomes probably with more biologically active inhibitors which may eventually provide a novel chemotheraphy for trypanosomiasis in livestock and in human beings.

As stated earlier, it was shown that drug-sensitive trypanosomes contain the two high-affinity adenine transport systems (P1 and P2) by which they scavenge low concentration of nucleosides in the mammalian bloodstream. While P1 is inhibited by inosine and other nucleosides, P2 transporter is inhibited only by adenine and melarsoprol and, a melanophenyl arsencial resistant *T. brucei* line had a greatly reduced initial rate of adenine uptake (Carter and Fairlamb, 1993), Transport proteins normally recognise common chemical features of their substrates and since a P2 recognition motif was identified in the structures of f-aminoaripines and trypanocidal drugs that interact with P2 transporter (Fig. 3), it is possible that addition of the motif to any potentially toxic moiety might generate novel trypanocides (Barrett and Fairlamb, 1999). In addition, there must be promise in exploring immunological alternatives of in silico conventional vaccine development is for now far-fetched. Game animals and livestock that usually undergo self cure exhibit class switch from IgM to IgG phenotype. Manipulating the immune system in favour of responses that will enable the host to express tolerising IgG responses following trypanosome infection is one area of future research interest. Agents or other organisms that may induce strong Th2 response and down play Th1 responses to achieve this may arm the host better to ablate infectios. Several agents including cytokines have the potential to do this and should be explored.
7. Concluding remarks

Because few trypanocidal drugs (both old and new) exist, there is no doubt that the development of individual resistance by pathogenic trypanosomes in the field portends a great danger to the control and treatment of trypanosomosis. Therefore, the emergence of multi-drug-resistant field strains of the parasite is a serious problem in the management of the disease and a grave threat to livestock development and productivity in areas where they occur. In this review, we have highlighted the mode of trypanocidal drug transport and its role in the development of drug resistance. It is evident, from the mode of the experimental induction of drug resistance in originally susceptible parent trypanosome strains, that despite the lack of the P2 transporter or the structural similarities between the trypanocides in drug resistance, the emergence of drug resistance in the field may be consistent with under-dosage and indiscriminate usage by unsupervised personnel and farmers. Such practices for sure are widespread in Africa and it is therefore surprising that drug resistance...
by trypanosomes in the field is not as widely discriminated as would be expected in such situations. These not witnessing, it would appear that the management and control of trypanosomosis will continue for now to depend on proper usage of the few available trypanocides, especially strategic deployment of the quinoline drugs, in addition to the continued use of environmentally friendly vector control programmes such as sussu trapping. However, the search for new trypanocides must continue and intensified and concerted efforts must be made to reawaken the interest of funding agencies and pharmaceutical companies in this direction which presently seems to be at its lowest ebb.

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References


