Chemotherapeutic agents against pathogenic animal trypanosomes

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Trypanosomoses are protozoan diseases, affecting both human and animals, and mainly found in tropical Africa, Latin America and Asia. In Africa, trypanosomes produce serious diseases in human beings such as West and East Sleeping Sickness caused by *T. brucei gambiense* and *T. brucei congolense* respectively, while in the Americas *T. cruzi* causes the Chagas disease. Other species of Trypanosoma affect animals and produce enormous economical impact in the endemic areas. Those species could be classified as those transmitted by tsetse flies- *(Trypanosoma vivax*, *T. congolense* and *T. brucei brucei*) producing a disease known as nagana and those non-transmitted by tsetse- (*T. evansi* –surra-, *T. equiperdum* –dourine-).

Due to antigenic variation shown by the trypanosome, prophylaxis of these diseases using vaccines is challenging; for that, most of the control and eradication programs against animal trypanosomes carried out in the infected areas in the world are based on therapeutic and prophylactic measures, using trypanocidal drugs or combining both measures. However, only three compounds are available in the market (isometamidium chloride, homidium –bromide and chloride- and diminazene aceturate) and all of them have been on the market for over 40 years. One of the most important risks for the future use of these existing trypanocides is the development and dissemination of resistances and, for that, new drugs have been developed in the recent past and are available in the market to treat *T. evansi* (melarsomine) and, on the other hand, new anti-trypanosomes candidates are being developed and tested at clinical trial level presently.

The aim of the present chapter is to review the current status of the therapeutic and prophylactic agents used for the control of pathogenic animal trypanosomes as well as the potential candidates that are tested nowadays as possible new trypanocides to be used in the next future.

Keywords Animal trypanosomosis, *Trypanosoma evansi*, brucei, vivax, congolense, equiperdum, trypanocides

1. Introduction

Trypanosomosis are protozoan diseases, affecting both human and animals, mainly found in tropical Africa, Latin America and Asia, and most of animal trypanosomosis can be considered as Neglected Tropical Diseases [1]. Species of trypanosomes infecting mammals fall into two distinct groups and, accordingly, have been divided into two sections: *Stercoraria* (subgenera *Schizotrypanum, Megatrypanum* and *Herpetosoma*), in which trypanosomes are typically processed in the hindgut of the vector and are then passed on by contaminative transmission from the posterior end of the digestive tract and *Salivaria* (subgenera *Duttonella, Nannomonas* and *Trypanozoon*), in which transmission occurs by the anterior station and is inoculative [2]. Characteristically, salivarian species, by virtue of variant surface glycoprotein (VSG) genes, are the only trypanosomes to exhibit antigenic variation [3].

From a geographical viewpoint, Africa is a particularly affected continent because the tsetse-transmitted trypanosomosis is circumscribed to sub-Saharan Africa, in an area of approximately 9 million km^2. *Trypanosoma vivax, T. congolense, T. simiae, T. brucei* are transmitted by this vector. In Africa, a daily mortality of about 100 people and 10,000 cattle is estimated to be occurring as a consequence of trypanosomosis [4]. Other trypanosomes present in the continent are *T. evansi*, which is transmitted by hematophagous flies, and *T. equiperdum*, a sexually transmitted protozoon.

In Latin America, Chagas' disease (*Trypanosoma cruzi*) is also a major human disease that particularly affects poor populations living in slum conditions; of the 90 million people exposed to the risk, 20 million are estimated to be infected, with a mortality rate of about 10% [5]. In Latin America, four species of trypanosomes are of medical and economic importance, or can interfere with the diagnosis of livestock hemoparasitoses: *Trypanosoma equiperdum, Trypanosoma vivax, Trypanosoma evansi*, and *Trypanosoma cruzi*. Only *T. cruzi*, in the Stercoraria group, is Native American, while the other three salivarian species were imported by humans together with their main domestic hosts [5].

Trypanosoma evansi is endemic in Asia and produces a high impact in the economy of livestocks, especially to smallholders in the Southeast countries [6]. *Trypanosoma equiperdum* has also been described in Asia [7].

Most of the control and eradication programs against animal trypanosomes carried out in the infected areas are based on therapeutic and prophylactic measures, using trypanocidal drugs or combining both measures. However, only three compounds are available in the market (isometamidium chloride, homidium –bromide and chloride- and diminazene aceturate) and all of these drugs have been on the market for over 40 years. One of the most important risks for the

future use of these existing trypanocides is the development and dissemination of resistances and, for that, new drugs have been developed in the recent past and are available in the market to treat *T. evansi* (melarsomine) and, on the other hand, new anti-trypanosomes candidates are being developed and tested at clinical trial level presently.

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2. Trypanosomes section Stercoraria. Currenty status on trypanocides

2.1. *Trypanosoma theileri*

Trypanosoma theileri, that can be considered as a type-species of the subgenus *Megatrypanum*, has a wide distribution and presents a high incidence in all continents excluding Antarctica [8]. *Megatrypanum* spp. is cyclically transmitted by hematophagous insects and the infection to new hosts is by contamination route. Tabanids are the most important vector implied in the transmission of *T. theileri* [9]. The pathogenicity of *T. theileri* within the mammalian host is not well understood. *Trypanosoma theileri* may persist in cattle for many years without any evidence of clinical disease. In most of the infected animals, parasite cannot be detected in blood smears. It is thought to be of very low pathogenicity [10]. Serological tools have not yet been described for *T. theileri*. However, this species can be detected by means of PCR-amplified spliced leader transcript [11] and single PCR based on internal transcribed spacer 1 of rDNA [12].

Treatment: Specific treatment against *T. theileri* infection has not been developed.

2.2. *Trypanosoma cruzi*

Most of the literature concerning *T. cruzi* infection (Chagas's disease) is focused on human infections; however, this protozoon presents a wide host range. Many species of mammals, including bats, have been reported to be infected with *T. cruzi*; thus, all mammals are considered to be susceptible. Chagas' disease affects exclusively Latin American continent where most of the countries have reported this disease. *Trypanosoma cruzi* is not a livestock trypanosome as such, but it is reported sporadically in domestic ruminants and horses [13]. The transmission is essentially cyclical through bugs that belong to the *Reduviidae* family: triatomines, or reduviid bugs, of the genera *Rhodnius, Panstrongylus* and *Triatoma*. The metacyclic trypomastigote infective form (metatrypanosome) is present in the excreta of the bugs that contaminate bite wounds or the mucous membranes, particularly the eye. Bugs may be both reservoir and vector for *T. cruzi*. Transmission of the infective forms found in the faeces of the bugs can also take the oral route. Ingestion of infected bugs is also thought to be the cause of contamination of dogs and cats and possibly livestock [5]. The current knowledge about the pathogenicity of *T. cruzi* in livestock is scarce, but it appears to be fairly low according to the few experimental findings available [14]; however, *T. cruzi* is markedly pathogenic in dogs and produces cardiac signs with a potentially fatal outcome [5]. Diagnosis in acute phase, when parasitemia is normally high, is based on conventional parasitological tests including stained smears examination and micro-hematocrit centrifugation techniques. In chronic phases, indirect immunofluorescence assay (IFA), indirect hemagglutination assay (IHA) and enzyme-linked immunosorbent assay (ELISA) have been used.

Treatment: A nitroimidazole compound, benznidazole (Rochagan®, Radanil®, Roche) and a nitrofuran compound, nifurtimox (Lampit®, Bayer) are currently used to cure human infections. However, differences in drug susceptibility of *T. cruzi* strains have been described and even existence of naturally resistant strains to both drugs. These drugs have also been also administered to infected dogs with unsatisfactory results [15].

3. Section Salivaria: Current status on trypanocides

3.1. Tsetse Transmitted Animal Trypanosomes: *Trypanosoma vivax, Trypanosoma congolense, Trypanosoma brucei brucei*

Trypanosoma vivax, T. congolense (including several types) and *T. brucei brucei*, constitute a tsetse-transmitted group that causes a disease complex known as "nagana". It is extended over 10 million km^2 and affects 37 countries, mostly in Africa. Nagana is a very important disease in cattle, producing enormous economic losses. The disease affects also camels and poses a natural barrier avoiding the introduction of these animals into the southern Sahel area. Equines are also considered highly sensitive and it is generally believed that the historical Arabian invasion was stopped by tsetse flies because of their impact on the Arabian horses. Human infection caused by animal species of *Trypanosomes*, notably *T. vivax* and *T. congolense*, have rarely been observed [2, 16]. However, tsetse-transmitted trypanosomes can also infect humans, in particular *T. brucei gambiense* or *T. brucei rhodesiense*, the causal agents of sleeping sickness. There is a large range of domestic and wild animals which can act as reservoirs of these human trypanosomes [17].

Clinical features: Clinical signs of nagana would include intermittent fever, anemia, edema, decreased fertility and abortions, loss of milk and meat production and work capacities and emaciation. Anemia is a typical finding in infected animals and is commonly followed by weight loss, decreased productivity and mortality [17].

Diagnosis is based on the direct detection of trypanosomes by microscopic visualization or by means of polymerase chain reaction (PCR) for which positive results means active infection, or can be indirectly diagnosed by serological techniques [17].

Treatment: Treatment against tsetse-transmitted trypanosomes is based on several drugs. Currently available trypanocides are the followings [Based on 18]:

- Diminazene aceturate (Berenil® and others), has been recommended as therapeutic agent at a dose rate 3.5 to 7 mg/kg, by intramuscular route. At 3.5 mg/kg has also been recommended against *T. congolense* in cattle, *T. vivax* in large and small ruminants and at 7 mg/kg against *T. brucei* in livestock and dogs. Diminazene aceturate cannot be used in camels due to its high toxicity [18]. Nowadays, it seems that the theoretical dose of 3.5 mg/kg is able to get rid of the clinical signs, at least temporarily, but is most often unable to cure the infection [5]. Diminazene has also been used in dogs infected with *T. brucei brucei* at 7 mg/kg, although relapses occurred [20]; used at 3.5 mg/kg alone or in combination with α-difluoromethylornithine resulted in relapses at one month post-treatment [21]. In cats infected with *T. evansi* diminazene aceturate has also been investigated at dose of 3.5 mg/kg for 5 consecutive days showing an efficacy of 85.7% [22].
- Homidium chloride (Novidium®, Homidium bromide Ethidium®), used as preventive and therapeutic agent, at the dose rate of 1 mg/kg by IM route is effective against *T. congolense* in cattle and *T. vivax* in pigs, small ruminants and horses; however it is not advised to use it because of the well know carcinogenic activity of ethidium bromide [23, 5].
- Isometamidium chloride can be used at dose rate of 0.25 to 0.5 mg/kg (Samorin®) or 0.5 to 1 mg/kg (Trypamidium®), by intramuscular route as preventive and therapeutic agent for *T. vivax* and *T. congolense* in cattle and small ruminants and *T. brucei* in equines.
- Quinapyramine dimethylsulphate (Trypacide sulphate®) at dose rate of 3 to 5 mg/kg by subcutaneous route is recommended as therapeutic treatment against *T. congolense* in camels.
- Quinapyramine dimethylsulphate/chloride (Trypacide pro-Salt®), at dose rate of 3 to 5 mg/kg by subcutaneous route is suggested as prophylactic use for *T. vivax* in equines and *T. brucei* in pigs

3.2. Non Tsetse Transmitted Animal Trypanosomes

3.2.1. *Trypanosoma evansi*

Trypanosoma evansi, which causes a disease known as surra, is widely distributed and affects domestic livestock and wildlife in Africa, Asia and Latin America [24], although recent outbreaks have also been described in Europe [25]. The presence of the parasite causes an important economic impact in the endemic areas. The transmission of *T. evansi* is mechanical by biting flies particularly belonging to genus *Tabanus*, *Stomoxys* and *Lyperosia*. Vampire bats are biological vectors in South America and, since they can be infected and transmit to other bats and other hosts, they are considered as host, reservoir and vector of the parasite [26]. The disease is normally manifested by pyrexia, directly associated with parasitemia, progressive anemia, weight loss and lassitude. Such recurrent episodes lead to parasitemia and intermittent fever during the course of the disease. Edema, particularly affecting the lower parts of the body, rough coat in camels, urticarial plaques or petechial hemorrhages of the serous membranes are commonly detected. In advanced cases, parasites invade the central nervous system (CNS), which can lead to nervous signs (progressive paralysis of the hind quarters and exceptionally paraplegia), especially in horses, but also in other host species before complete recumbence and death. Abortions and immunodeficiency associated to *T. evansi* infection have also been reported, notably in bovines and pigs [26]. For definitive diagnosis, identification of the agent is needed, which can be reached by direct, but also by indirect parasitological methods since *T. evansi* can grow in laboratory rodents. Within serological tests, enzyme-linked immunosorbent assay (ELISA), card agglutination tests (CATT/*T. evansi*), indirect immunofluorescent antibody test (IFAT) and immune trypanolysis tests are recommended [27].

Treatment: Current available drugs against *T. evansi* are the followings [Based on 18]:

- Diminazene aceturate (Berenil® and others), at dose rate of 7 mg/kg by intramuscular route is recommended as therapeutic in equines.
- Isometamidium chloride (Trypamidium®), at dose rate of 0.5 to 1 mg/kg by intramuscular route is indicated as prophylactic in camels.
- Quinapyramine dimethylsulphate/chloride (Trypacide pro-Salt®), at dose rate of 3 to 5 mg/kg by subcutaneous route is indicated as prophylactic in dogs.
- Suramine (Naganol®), at dose rate of 7 to 10 g per animal by intravenous route is recommended as a therapeutic and prophylactic agent in camels and equines.

- Melarsomine (Cymelarsan®), at dose rate of 0.25 mg/kg by intramuscular or subcutaneous route is used as therapeutic in camels, but also in horses. Although melarsomine has been scarcely used in other animal species, some studies have demonstrated its efficacy but only with higher doses (0.5-0.75mg/kg) in goats, pigs, cattle and buffalos [28, 29, 30].

Treatment of Surra is currently dependent on four drugs: Polysulphonated naphtyl urea (Suramin), Diminazene aceturate (Berenil®), quinapyramine (Triquin®) and Melarsomine (Cymelarsan®), which are relatively expensive and not widely available. While the first three have been utilised for more than 50 years, Cymelarsan®, belonging to the family of melaminophenyl arsenicals, was developed about 20 years ago. Of the drugs available only melarsomine hydrochloride (Cymelarsan®) and diminazene aceturate (Berenil®, Surraplex®, Trypan®) are considered safe for use in all animal species. Unfortunately, with the appearance of resistance to these drugs [31, 32] their effective use is threatened and it is necessary to look for new drugs.

3.2.2. *Trypanosoma equiperdum*

Trypanosoma equiperdum produces a disease called "Dourine", an acute or more frequently chronic contagious disease of equines that is transmitted directly by coital contact during mating, and possibly from the mare to the foal by eye or nose mucous membrane [33]. Infected equids are the natural reservoir of the parasite, which is not transmitted by invertebrate vectors. This trypanosome is considered primarily a tissue parasite and infrequently invades the blood [27]. The natural host of *Trypanosoma equiperdum* is equines, no other animal species is affected. In horses, the disease is chronic and persists for 1-2 years. Clinically, dourine is categorized into three phases, although the evolution of the disease can vary depending on certain conditions. The first phase occurs in 1 to 2 weeks after the infection and is characterized by tumefaction, edema and effects on the genitalia. The second phase is clearly pathognomonic for dourine. Typical cutaneous plaques or skin thicknesses can be observed, which can range from very small to hand sized; the name of the disease originates from the shape of this skin lesion similar to the coin named "duro" (ancient Spanish money). The third stage is recognized by anemia, impairments of the nervous system, in particular paraplegia and paralysis of the hind legs and finally death [34]. Concerning diagnosis, typical clinical signs of the disease can be useful but clinical diagnosis needs to be confirmed by laboratory methods. Given the extreme difficulty to discover the protozoa in the body fluids of affected horses, diagnosis of dourine is based on serological tests. The antibody and antigen-ELISAs have been developed for *T. equiperdum* but complement-fixation test (CFT) is the test internationally recommended by the World Animal Health Organization (WAHO/OIE). However, this test does not distinguish among *T. evansi, T. equiperdum* and *T. b. brucei* [34].

Treatment: There is not any available drug for dourine. The disease is considered to be incurable and, for that, seropositive horses should be removed or euthanized [27]. However, in vitro sensitivity of different *T. equiperdum* strains to suramin, diminazene, quinapyramine and melarsomine has been reported [35, 36]. Some authors suggest , avoiding to euthanize the infected horses in endemic areas, where the disease is extended and horses play an important role, propose a revised strategy including treatment with Cymelarsan® [37], but results are inconclusives and additional studies are needed before this proposal can be considered.

4. Mechanisms of action and resistance

4.1. Suramin (Polysulphonated naphtyl urea)

Ehrlich described the efficacy of a series of dye molecules including trypan blue and trypan red to eliminate trypanosome infections in mice. The molecular structures of the dyes provided a starting point for the synthesis of suramin, which has been used as a trypanocidal drug since 1916 and is still in clinical use although unfortunately its large scale production for animal use was stopped int he middle of 90's. Suramin do not cross the Blood Brain Barrier. Inhibition of several enzymes by Suramin (among others, dihydrofolate reductase, fumarase, glycerol-3-phosphate dehydrogenase, hexokinase, reverse transcriptase, RNA polymerase and kinases) has been described [38]. Deprivation from cholesterol and phospholipids by inhibition of the uptake of low density lipoproteins has been proposed as the main mechanism of action for this drug.

Resistance to Suramin can be easily developed in the laboratory, by long-term exposure to subclinical concentration [39]. The resistance phenotype is fairly stable [40]. No cross-resistance with other common trypanocidal drugs has been observed in laboratory or field cases. Due to the multifactorial mode of action of this compound, resistance by single target mutation is improbable. Reduction of drug uptake and increased drug efflux have been proposed as resistance mechanism.

It has been demonstrated that suramine binds in the position of ADP/ATP at the active sites of the pyruvate kinases [41]. Affinity values for suramin inhibition are very different for human and parasite PYKs. Such differences on affinities could be useful to design and develop inhibitors. The efficacy of inhibitors could be enhanced by the concomitant use of PYK activators.

4.2. Diminazene aceturate (Berenil®)

Diamidines are dicationic molecules, which bind to the minor groove of DNA at AT-rich sites. They exert their biological activity by primarily binding to DNA and then inhibiting one or more of the DNA dependent enzymes (such as topoisomerases or nucleases) or by directly impeding the transcription process. Berenil is the only comercial diamidine for treatment of animal trypanosomosis at this moment, but new diamidines are being investigated as future therapeutic options. Two diamidine compounds (DB 75 and DB 867) present comparable efficacy at lower doses than the standard drug diminazene and could be considered as potential clinical candidates against *T. evansi* infection [42]. The selectivity of diamidines is primarily due to the selective accumulation by the pathogen rather than by host cells. Diminazene aceturate do not cross the blood–brain barrier. The new diamidine DB75 (furamidine) does not have sufficient penetration into the Central Nervous System, but a related compound (DB820, azafuramidine) seems to completely cure a murine late-stage model of sleeping sickness [43].

Diamidines are actively taken up by transporters; alterations of the transporters can cause development of drug resistance [44]. There are numerous reports of resistance to Berenil in different countries and in several *Trypanosoma* species. In any case, resistance seems to be limited to highly endemic areas where the use of this drug is very common. Barret et al. [45] demonstrated that resistance to diminazene aceturate in *T. equiperdum* was due to lack of activity of P2 aminopurine transporter, required to translocate the drug across the cell membrane. The role of the P2-type purine transporter in the uptake of arsenical diamidines, pentamidine and DA by *T. brucei*, *T. evansi* and *T. equiperdum*, and the consequences of inhibition, knocking down or silencing this gene have been extensively described and reviewed in the literature [46].

Cross resistance to diminazene aceturate with isometamidium and pentamidine have been reported [47, 48]. Loss of function of the P2 nucleoside transporter in trypanosomes has subsequently been associated with resistance to diamidines and arsenicals [49].

Drug resistance to diminazene aceturate is less widespread than isometamidium, but increasingly there are reports of multiple drug resistance.

Chemotherapeutic efficacy of diminazene aceturate and pentamidine isethionate (PMI) -a human trypanocide- was compared in dogs experimentally infected with T. brucei brucei. PMI given at 4 mg/kg i/m at days 14, 16, 18, 20, 22, 24, and 26 PI constituted a safe and efficient trypanocide and exhibited a superior trypanocidal action than diminazene aceturate in *T. brucei brucei* infected dogs [49].

4.3. Melarsomine hydrochloride (Cymelarsan®)

Melarsoprol is an arsenical compound that contains the trivalent arsenic element with a markedly reactive arsenoxide group. The presence of arsenoxide confers the physiochemical ability of lipid solubility that allows passage across the blood brain barrier. The veterinary arsenical trypanocide melarsamine hydrochloride (Cymelarsan®) is a conjugation of melarsen oxide and two equivalents of cysteamine. It is used mainly to treat *T. evansi* infections in domestic animals.

Melarsoprol resistance was first linked to the absence of the P2 aminopurine transporter [50]. Since then, these transporters have been cloned and expressed in yeasts to demonstrate their role in resistance [51, 52]. But P2 is not the only significant transporter for melaminophenyl arsenicals expressed in bloodstream trypanosomes. Some theories of heavy metal resistance in protozoa proposed the implication of aquaglyceroporins [53], thiamine transporters [54] or high affinity pentamidine transporter (HAPT1) [51], but it seems to be that these molecules do not play an important role *in vivo*.

In *T. brucei*, three ABC transporter genes have been identified and overexpression of one of them (TbMRPA) resulted in a tenfold increase in *in vitro* EC50 for melarsoprol, but had no effect on sensitivity to suramin or diamidines [55]. But MRPA overexpression could not be demonstrated in four melarsoprol-resistant field isolates so it is presently unclear whether this mechanism is relevant to melarsoprol treatment failure. Overexpression of MRPA does not engender melarsoprol resistance in vivo, though it does *in vitro* [56].

4.4. Isometamidium and Homidium

Isometamidium chloride (Samorin®, Trypamidium®, Veridium®) has been prophylactically or therapeutically used in the field for livestock suffering from trypanosomosis for several decades.

It was first synthesized by coupling homidium (Ethidium®) with *p*-aminophenyldiazonium chloride, this is, by coupling homidium with a part of the diminazene aceturate molecule. It has been proposed that the main mechanism of action of isometamidium is the cleavage of kDNA-topoisomerase complexes, causing the desegregation of the minicircle network within the kinetoplast [57]. But Kaminsky et al. [58] showed that dyskinetoplastic trypanosomes are at least as sensitive to isometamidium as kinetoplastic lines.

Resistance to isometamidium is mostly associated with cross-resistance to homidium, probably because these structurally related compounds might share the same uptake mechanism. Isometamidium is still effective in the field even though it was first marketed more than fifty years ago, so induction of resistance to isometamidium seems not to be easy. More and more cases of therapeutic failure are now being reported [59, 60, 61, 62]. The authenticity of the resistance phenotype in some of these field isolates of trypanosomes has been confirmed by in vivo testing of individual

clones derived from the isolates [60, 63]. Resistance occurs where a large proportion of the trypanosome population is exposed frequently to the drug, for instance in commercial ranches, or after government policies based on large scale block treatments [60] or where frequent underdosing of the trypanocide occurs [64].

It has been suggested that pentamidine, diminazene aceturate and isometamidium have an action within the kinetoplast of *Trypanosoma equiperdum* that is characteristic of type II topoisomerase inhibitors and mimics the effects of the antitumor compound etoposide (a specific inhibitor of mitochondrial topoisomerase enzymes) [57]. The silencing of the mitochondrial topoisomerase gene by RNA interference or by the use of specific topoisomerase II inhibitors induces the progressive shrinking and disappearance of the kinetoplast DNA network [65].

New molecular markers are being developed to determine trypanocides resistance and even when they still need to be validated there are good prospects that they will enable faster detection of trypanocidal drug resistance (a few days instead of two to three months) [66]. The molecular diagnosis of diminazene aceturate resistance in trypanosomes is facilitated by the specificity of the transport mechanism for diminazene aceturate. The molecular diagnosis of isometamidium resistance seems to be more complicated because the transport of this hybrid molecule through biological membranes seems less specific than the transport of DA. Several pathways are probably implicated in the process and several diagnostic tests will have to be performed depending on the number of importers or extruders that are potentially involved.

This will allow to investigate the current prevalence and the spread of resistance genes in trypanosome field populations in wide areas. Understanding the molecular mechanism of action and the processes that contribute to the development of resistance will lead to the development of better strategies to improve the fight against trypanosomosis [66].

5. Future perspectives

Trypanosomosis is controlled either by controlling the vector or by controlling the parasite, or a combination of both. Over the years, a large arsenal of vector-control tools has been developed. Nevertheless, the control of animal trypanosomosis in often poor rural communities has and will continue to rely heavily on the use of trypanocidal drugs. Trypanocidal drugs resistance has been reported in 17 African countries. In addition to this, some authors discuss the possible role of using trypanocides in livestock in the development of resistance observed in human cases of trypanosomosis [67].

There is an urgent need for new drugs for the chemotherapy of trypanosomosis. Progress has been made in the identification and characterization of novel drug targets for rational chemotherapy and inhibitors of trypanosomatid glycosomal enzymes, trypanothione reductase, ornithine decarboxylase, S-adenosylmethionine decarboxylase, cysteine proteases and of the purine and sterol biosynthetic pathways.

Trypanosomes have the capacity for antigenic variation, which is the basis of their ability to escape the host immune response, and because of this, prospects for the development of a vaccine against trypanosomosis have been considered poor. However, the most effective and sustainable way of controlling trypanosomosis should be a safe and cost-effective vaccine. Therefore, it is necessary to develop a vaccine against trypanosomes based on other potential target proteins of these organisms, as the proteins of the tubulin family [68, 69].

Beta-tubulin protein has only a single isoform in *Trypanosomatidae*. It is distributed beneath the surface membrane, in the flagellum, paraflagellar rod and the mitotic spindle apparatus of the dividing nuclei in trypanosomes. Beta-tubulin is considered essential because it provides structural stability to the cell in addition to other physical and biochemical functions, making it vital for the biology of organism. Besides being drug targets, have thus also been considered prospective targets for developing vaccines [68].

Immunization with recombinant beta-tubulin from *T. evansi* induces protection against *T. evansi, T. equiperdum* and *T. b. brucei* infection in mice [70]. The beta-tubulin gene of *Trypanosoma evansi* (STIB 806) was cloned and expressed in *Escherichia coli*. Mice immunized with the renatured recombinant beta-tubulin were protected from lethal challenge with *T. evansi* STIB 806, *T. equiperdum* STIB 818 and T. *b. brucei* STIB940, showing 83.3%, 70% and 76.7% protection, respectively.

Kurup and Tewari [69] have developed DNA vaccines that protect mice against *T. evansi*. Mice were immunized with a naked DNA construct encoding *T. evansi* β-tubulin gene and they were better protected against a lethal dose of *T. evansi*, likely aided by the strong anti-tubulin antibody response generated as well as the Th1 polarized serum cytokine profile.

Pallavi et al.[70] investigated the potential of Heat shock protein 90 inhibitors as drugs for the treatment of Trypanosoma infection in animals. This protein regulates cell cycle progression and signal transduction. The cellular substrates modulated by Hsp90 include AKT, p53, telomerase, heat shock factor, and other transcription factors involved in cell signalling events. This chaperone has therefore been implicated in supporting important cellular events including cell growth, signalling and development. Using geldanamycin (GA), a specific inhibitor of Hsp90 function, they were able to demonstrate specific binding of GA to purified *T. evansi* Hsp90 (TeHsp90) in whole cell lysate as well as in its purified form.

The same authors found a GA derivative, 17-(allylamino)-17-demethoxygeldanamycin (17AAG), that was able to inhibit *T. evansi* growth and cure mice infected with *T. evansi*. Their studies support the potential of PfHsp90 and TeHsp90 as drug targets and also suggest the possibility of targeting Hsp90 of protozoan parasites for the treatment of a variety of human and animal infections.

The search for new active drugs against *T. equiperdum*, considered as a non-curative disease, is urgent. Megazol (1-methyl-2-(5-amino-1,3,4-thiadiazole)-5-nitroimidazole) and 13 of its analogues have been assayed by in vitro tests against *T. equiperdum*. The *in vitro* results for the 14 compounds under study showed that six out of the 14 compounds are active against *T. equiperdum*. Structural molecular modifications of a compound with known biological activity resulting in a series of analogue compounds are an advantageous process for the introduction of new therapeutic drugs, since the process supplies data to a structure–activity relationship (SAR) study. Rosselli et al. [71] investigate megazol derivatives with activity against *T. equiperdum* and showed that the descriptors molecular electronic energy (Eelet), charge on the first nitrogen at substituent 2 (qN), volume of substituent at C5 position (V-S5), dihedral angle (D3) and bond length between atom C4 and its substituents (L4) are responsible for the separation between active and inactive compounds against *T. equiperdum*.

Tonin *et al.* [72] assessed the use of diminazene aceturate in association with vitamin E and sodium selenite in rats, concluding that results in terms of longevity, hematocrit reduction, leucocytes and lymphocytes number and lipid peroxidation were improved using this combinative therapy compared to the use of single diminazene aceturate, although sodium selenite showed better protective action than vitamin E. There is a progressive interest in the utilization of antioxidants in the prevention and treatment of this disease. *T. evansi* pathogenic mechanisms include oxidation of the erythrocytes inducing oxidative stress due to free radical generation [73]. Ranjithkumar et al [74] reported increase in oxidant parameters and decrease in antioxidant enzymes in infected horses, indicating the disturbance of oxidant/antioxidant indices. After therapy with trypanocides, there was a significant increase in haematological values, while the oxidant/antioxidant indices changed insignificantly, so these authors suggested that antioxidants might be supplemented in the therapeutic regimen.

Habila *et al.* [75] examined the trypanocidal potentials of *Azadirachta indica* seeds methanolic extract (NSME) against *Trypanosoma evansi*. In vitro studies with the NSME 100 mg/ml, 50 mg/ml and 25 mg/ml immobilized the parasites within 3 min, 8 min and 14 min respectively. It appears that NSME possesses trypanoprophylactic potency more than trypanocidal.

Dogs experimentally infected with *Trypanosoma cruzi* have been effectively treated with benznidazole. In the early stage of Chagas's disease benznidazole reduces the parasitemia and parasite kDNA detection in blood and also lead to a significant reduction in the frequency and severity of the parasite-induced cardiac disease, even if parasite are not completely eliminated [76, 77]. Borges-Argáez *et al.* [78], tested in vitro isocordoin and 2',4'-dihydroxy-3'-(gamma,gamma-dimethylallyl)-dihydrochalcone, chalcones isolated from the root of *Lonchocarpus xuul*, together with six analogues against epimastigotes of *T. cruzi*. 2',4'-dimethoxy-3'-(3-methylbut-2-enyl)-chalcone showing lower cytotoxicity in comparison with isocordoin and had the strongest trypanocidal activity with IC(50) values lower than those of nifurtimox and benznidazole, the common drugs used against these parasites. The selectivity index calculated (SI 109.3) confirms the selective trypanocidal activity of this metabolite. Cruzain is an essential cysteine protease of *T. cruzi* and a therapeutic target for Chagas' disease. Eight dogs were infected with *T. cruzi*; three were treated with an inhibitor of cruzain, K777, for 14 days. Treatment with K777 abrogated myocardial damage by *T. cruzi*, as documented by histopathological lesion scores and serum troponin I levels [79].

Albaconazole is an experimental triazole derivative with potent and broad-spectrum antifungal activity and a remarkably long half-life in dogs, monkeys, and humans. Da Matta Guedes *et al*. [80] demonstrated that albaconazole is very effective in suppressing the proliferation of the parasite and preventing the death of infected dogs; although natural resistance to this compound was also indicated.

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