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Antiprotozoal compounds: state of the art and new developments

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ABSTRACT

Protozoa can cause severe diseases, including malaria, leishmaniasis, Chagas disease, sleeping sickness and amoebiasis, all being responsible for morbidity and mortality particularly in tropical countries. To date there are no protective vaccines against any of these diseases, and many of the available drugs are old or elicit serious adverse reactions. Moreover, parasite resistance to existing drugs has become a serious problem. Owing to lack of financial returns, research in this field is of limited interest to pharmaceutical companies and largely depends on funding by public authorities. This article aims to provide a concise overview of the state-of-the-art treatment for the most important tropical protozoal infections as well as new approaches.

1. Malaria

Approximately 3.3 billion people live in 108 malarious countries and are seasonally at risk of infection with *Plasmodium* spp. For 2008, the World Health Organization (WHO) estimated that there were 243 million cases of malaria worldwide, accounting for an estimated 863 000 deaths [1]. The vast majority of cases (85%) occurred in Africa, followed by 10% in Southeast Asia and 4% in Eastern Mediterranean regions.

Traditionally, four *Plasmodium* spp. are recognised as human pathogens, namely *Plasmodium falciparum* (the causative agent of severe tropical malaria), *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium ovale*. However, an increasing number of human infections with simian malaria parasites, mainly *Plasmodium cynomolgi* [2] and *Plasmodium knowlesi* [3], have been reported in recent years.

Attempts to control malaria include vector control, chemotherapy and development of vaccines. When the insecticidal activity of DDT was discovered it was thought that forceful vector control combined with chloroquine (CQ) treatment of patients could lead to eradication of malaria [4]. Indeed, DDT spraying resulted in a decrease and even eradication of malaria in many areas [5], but use of DDT in the environment was abandoned in 1969 owing to ecological and public health concerns and the development of mosquito resistance. DDT was replaced by insecticidal pyrethroids and new control programmes were started. Insecticide-treated bed nets lead to reduced child mortality in several African countries [6], however only 1 in 50 children are currently protected by such nets in endemic countries in Africa [7]. Only 11 of 1223 new molecular entities authorised between 1975 and 1996 were designated for tropical diseases, including the four antimalarials mefloquine (1987), halofantrine

(1989), atovaquone/proguanil (1997) and artemether (1997) [8]. Despite all efforts, no effective vaccine for malaria prophylaxis has been developed so far [9]. A vaccine developed by GlaxoSmithKline GmbH & Co. KG is currently being evaluated in a phase III clinical study and is planned to be launched on the market in 2012.

Currently, malaria control relies on a limited number of tools, in particular malaria treatment with artemisinin derivatives and so-called artemisinin-based combination therapy (ACT) as well as vector control with insecticidal pyrethroids, but both could be lost to resistance at any time [10–12]. Sustainability of effective programmes through training of clinical employees and institution strengthening of malaria clinics as well as improved surveillance and drug development are necessary for malaria eradication [13].

Uncomplicated *P. falciparum* malaria that is treated appropriately and promptly has a mortality rate of 0.1%, but mortality of untreated severe malaria, particularly cerebral malaria (CM), is almost 100%, and despite treatment mortality in CM still ranges from 10% to 50% [14]. In the treatment of severe malaria, the main focus is to prevent death; secondary objectives are prevention of disabilities and the prevention of recrudescence [15].

1.1. Treatment of benign Plasmodium falciparum infections

Known CQ-sensitive strains of *P. falciparum* should be treated with chloroquine phosphate (Aralen[®] and generics) or hydroxychloroquine (Plaquenil[®] and generics) given orally in doses of 10 mg/kg body weight immediately, followed by 5 mg base/kg after 6, 24 and 48 h [16]. The same treatment course is recommended for pregnant

women. A fixed-dosed combination of azithromycin (Zithromax[®]) and CQ is currently under investigation by Pfizer Inc. (Groton, CT) in a large pivotal phase III clinical study in several East and Southern African countries [17]. Azithromycin and CQ have demonstrated safety in children and during pregnancy over a number of years [18]. Azithromycin and CQ intermittent preventative treatment in pregnant women (IPTp) is compared with sulfadoxine/pyrimethamine in terms of reducing the incidence of adverse pregnancy outcomes [19].

Multidrug resistance to antimalarial drugs has increased in frequency and distribution. CQ resistance was first reported at the Thai–Cambodian border [20], followed by drug resistance throughout the tropical world. In addition, sulfadoxine/pyrimethamine and mefloquine resistance [21] have also been reported. More recently, artemisinin resistance [10] and ACT resistance for artesunate/mefloquine [22] were reported. To avoid further drug resistance against artemisinin and its derivatives, there are efforts to ban oral artemisinin-based monotherapies from the market and to replace them with ACTs [15]. Nevertheless, 37 countries still allow the use of oral artemisinin-based monotherapies, mostly in Africa.

According to the WHO, ACT combining artesunate and mefloquine continues to yield satisfactory cure rates. Artesunate is given in oral doses of 4 mg/kg/day for 3 days combined with mefloquine in doses of 25 mg/kg in either 8 mg/kg daily for 3 days or 15 mg/kg on Day 2 and then 10 mg/kg on Day 3. Artemether and lumefantrine (Coartem[®]) is administered orally twice daily in doses of 1.5 mg artemether/9 mg lumefantrine per kg for 3 days [16]. The drug should be taken with food. If the patient

vomits within 30 min of taking a dose, then the dose should be repeated. Another recommended ACT is orally administered artesunate in doses of 4 mg/kg for 3 days combined with sulfadoxine (25 mg/kg) and pyrimethamine (1.25 mg/kg) as a single dose. Alternatively, 4 mg of orally administered artesunate/kg daily for 3 days plus amodiaquine in doses of 10 mg of base/kg/day for 3 days can be used in the treatment of *P. falciparum* malaria. ACTs are generally well tolerated, with the exception of mefloquine, which is associated with vomiting and dizziness [23]. New developments are a fixed-dose combination of dihydroartemisinin and piperazine (Eurartesim[®]), as well as Pyramax[®], the fixed-dose combination of pyronaridine and artesunate, was launched on the market as a once-daily 3-day treatment for uncomplicated *P. falciparum* and blood-stage *P. vivax* malaria in infants, children and adults. Dihydroartemisinin and piperazine are currently under registration by the European Medicines Agency (EMA) under Orphan Drug legislation. A programme to develop a paediatric formulation has been initiated. Piperazine is a 4-aminoquinoline with a relatively long half-life compared with other drugs used in combination with artemisinins. Therefore, combination therapy is effective in malaria therapy and provides longer protection from re-infection than other ACTs.

Alternatively, the highly effective combination atovaquone/proguanil (Malarone[®]), which is also in use for prophylactic purposes, can be used for the treatment of uncomplicated *P. falciparum* infections. Atovaquone/proguanil is available in tablets for adults (250 mg atovaquone/100 mg proguanil) and children (62.5 mg atovaquone/25 mg proguanil) [16]. Atovaquone/proguanil can also be used for second-line treatment, for treatment of imported malaria, or prophylaxis. Other effective second-line treatments for recrudescence following first-line therapy are a 7-

day course of quinine or artesunate combined with a 7-day course of any of the following antibiotics: oral tetracycline (250 mg four times daily); oral doxycycline (100 mg twice a day); or oral clindamycin (10 mg/kg twice a day) [16,24]. Tetracycline and doxycycline cannot be administered during pregnancy and cannot be given to children under 8 years of age. Atovaquone/proguanil and artemether/lumefantrine are also not recommended during pregnancy. However, they might be used for the treatment of uncomplicated malaria caused by CQ-resistant *P. falciparum* strains if no other treatment options are available, but only if the potential benefit is judged to outweigh the potential risks. Mefloquine has been reported to be safe for children and for pregnant women in the second and third trimester [25], but is associated with an increase of stillbirths in the first trimester [26] and is thus not recommended during pregnancy [16].

Tafenoquine, an 8-aminoquinoline antimalarial, is currently being investigated by GlaxoSmithKline [27]. The main advantage of tafenoquine is that it has a longer half-life than its relative primaquine; however, like primaquine, it causes haemolysis in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Tafenoquine was proven to be a highly efficacious and safe drug with good tolerability for malaria prophylaxis in a first clinical phase III trial, randomised, double-blinded study [28].

The spirotetrahydro β -carboline, or spiroindolones, are potent drugs that kill the blood stages of *P. falciparum* and *P. vivax* clinical isolates at low nanomolar concentrations [29]. NITD 609, developed by Novartis Institute for Tropical Diseases (Singapore) in co-operation with the Medicines for Malaria Venture, is a novel synthetic antimalarial molecule of the spiroindolone class [30]. NITD 609 has

pharmacokinetic (PK) properties compatible with once-daily oral dosing and showed single-dose efficacy in a rodent malaria model. A clinical phase I study was initiated in December 2010 with three doses having been successfully administered to humans with no safety concerns [31]. The study is planned to be finished in 2011; if the tolerability and PK profile is approved, NITD 609 will enter a proof-of-concept study. Interestingly, NITD 609 would be the first antimalarial drug entering clinical efficacy studies that does not belong to either the artemisinins or the peroxide class.

1.2. Treatment of malaria caused by Plasmodium vivax, Plasmodium ovale or Plasmodium malariae

In the case of *P. vivax* and *P. ovale* infections, CQ should be applied where it is effective [15]. CQ is given orally at an initial dose of 10 mg base/kg followed by 10 mg/kg on the second day and 5 mg/kg on the third day. ACTs should be applied in areas where *P. vivax* and *P. ovale* is resistant to CQ, with the exception of artesunate plus sulfadoxine/pyrimethamine.

Plasmodium vivax, *P. ovale* and *P. cynomolgi* are known to form hypnozoites [32–34], which are able to remain in the liver for weeks up to 5 years until they re-enter the cell cycle, a phenomenon termed ‘relapse’. Therefore, treatment of *P. vivax*, *P. ovale* and *P. cynomolgi* has to be combined with 14 days of primaquine to prevent relapse. The adult oral dose of primaquine is 0.25 mg/kg/day, but in Southeast Asia, particularly Indonesia, and in Oceania 0.5 mg base/kg/day are required because of increasing resistance. To avoid abdominal discomfort, primaquine should be taken with food. Further reported adverse reactions are mild nausea and vomiting. Primaquine can cause oxidant haemolysis in G6PD-deficient individuals, therefore

the dose of primaquine should be reduced to 0.75 mg/kg administered once a week for 6 weeks in moderately affected individuals.

Further monotherapies that have been used effectively for the treatment of CQ-resistant *P. vivax* infections are, for example, amodiaquine and mefloquine. Amodiaquine has been reported to be well tolerated [35]. It is given orally in doses of 25–30 mg base/kg over 3 days [15]. Mefloquine is given in a single dose of 15 mg/kg. A treatment success of 100% has been reported [36]. Doxycycline monotherapy applied in 100 mg oral doses twice a day for 7 days shows poor cure rates in *P. vivax* infections [37], however quinine given in doses of 10 mg salt/kg three times a day for 7 days is also effective against CQ-resistant *P. vivax* strains [38]. Nevertheless, quinine is not considered an ideal treatment owing to its toxicity. Furthermore, it was found that treatment of *P. vivax* infections with quinine might lead to early relapses owing to the short half-life of quinine [36].

Plasmodium malariae should be treated with the standard regimen of CQ, as for *P. vivax* malaria, but it does not require radical cure with primaquine because no hypnozoites are formed during *P. malariae* infection [15].

Tafenoquine is the only new drug that also demonstrated activity against the hypnozoites of *P. vivax* in vitro and in patients [39].

1.3. Treatment of severe malaria

In severe malaria, immediate and effective parenteral or rectal antimalarial treatment is required [15]. Cinchona alkaloids (quinine and quinidine) or artemisinin derivatives,

e.g. artesunate, artemether and artemotil, can be used. Due to widespread CQ resistance of *P. falciparum* and the fear of quinine resistance, quinine monotherapy is today largely replaced by artemisinin derivatives used in combination with other antimalarial compounds [40]. Artesunate is administered intravenously or intramuscularly at doses of 2.4 mg/kg. The treatment has to be repeated after 12 h and 24 h, followed by once a day treatments. In contrast to quinine, artesunate does not require rate-controlled infusion or cardiac monitoring.

Quinine should be administered as 20 mg quinine dihydrochloride/kg every 8 h, with doses not exceeding 5 mg salt/kg/h by rate-controlled infusion. Alternatively, it can be administered intramuscularly. The first dose has to be divided into 10 mg/kg injections into each thigh (instead of the buttock) to avoid sciatic nerve injury. Also, it is recommended to dilute quinine dihydrochloride to concentrations of 60–100 mg/mL for intramuscular (i.m.) injection to avoid pain due to low pH. Gluconate salts are less acidic and better tolerated quinine formulations than dihydrochloride salts.

Children are treated according to the same guidelines. Artemether 3.2 mg/kg i.m. as an alternative should only be used if artesunate or quinine is not available. Parenteral antimalarials have to be administered for a minimum of 24 h in the treatment of severe malaria in children and adults. Finally, the treatment has to be completed by an entire course of the following ACTs: artemether and lumefantrine; artesunate and amodiaquine; artesunate and sulfadoxine/pyrimethamine; dihydroartemisinin and piperaquine; artesunate and pyronaridine; and artesunate and clindamycin or doxycycline. Alternatively, quinine and clindamycin or doxycycline can be used.

2. Leishmaniases

Protozoan parasites of the genera *Leishmania* and *Viannia* are the causative agents of different forms of visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) in humans. VL and CL are important public health problems in endemic geographic regions in 88 countries worldwide, with around 12 million infected people.

Therapeutic problems are toxicity and teratogenicity of the available drugs, low response in human immunodeficiency virus (HIV)/*Leishmania* co-infections and upcoming resistances. The pentavalent antimony (Sb^V) compounds sodium stibogluconate (Pentostam[®]) with 100 mg antimony/mL and meglumine antimoniate (Glucantime[®]) with 85 mg antimony/mL are still the drugs of choice in most endemic countries. Nevertheless, resistance is increasing, particularly in the region of Bihar (India) [41], where 50–65% of patients do not respond to the treatment. Resistance might be explained by reduced accumulation of the drug [42], loss of reduction of the metal [43] and gene amplification [44], but it has not yet been entirely elucidated as the mode of action of Sb^V compounds is still not clear. It is suggested that the pentavalent form of the compound has to be reduced into a trivalent form in the parasite in order to become active [43]. The biocidal effect is based on the blocking of glycolysis and degradation of fatty acids. In addition to increasing resistance, severe side effects have been reported for antimonials, especially high rates of cardiotoxicity [45] occurring more frequently with generics [46]. Pancreatitis is frequent in HIV/*Leishmania* co-infections [47]. Other side effects are the metallic taste, anaphylaxis, anaemia, leukopenia, thrombocytopenia, rash, headache, abdominal pain, nausea, vomiting, arthralgia and myalgia. Sb^V compounds are administered on the basis of their antimony content: the recommended dosage is 20

mg/kg per day for 20 days in CL and 28 days in VL and mucocutaneous leishmaniasis [48]. Drugs are injected by the i.m. or intravenous (i.v.) route.

Pentamidine (Pentacarinat[®]), an aromatic diamidine, is used as alternative drug in Sb^v-resistant cases, but side effects such as vomiting, tachycardia, pruritus, urticaria, phlebitis and syncope during injection have also been reported. Toxic side effects on blood cell lines, kidney and pancreas have been demonstrated in vitro and in experimental animals [49,50]. Use of pentamidine is nowadays restricted to CL owing to the arising toxicity occurring in the required long-term treatment of VL [48]; 2–3 mg/kg are injected i.m. or i.v. daily or every second day in four to seven doses.

Amphotericin B (AmB) is used as a second-line antileishmanial drug in developing countries and as a first-line drug in industrialised countries. It is a polyene antibiotic and was initially investigated for systemic fungal infections [51]. AmB binds to ergosterol-like sterols in the plasma membrane, which are similar in fungi and *Leishmania* spp., and forms pores causing leakage of ions. Moreover, AmB stimulates cytokine production and therefore enhances the phagocytic activity of macrophages. Therefore, AmB became valuable in the treatment of severe leishmaniasis and in cases of antimony resistance. Cure rates of up to 90% are achieved, but AmB resistance in HIV/ *Leishmania infantum* co-infection has been reported [52]. Serious acute side effects following infusion have been reported, e.g. high fever, chills, hypotension, anorexia, dyspnoea, tachypnoea and, exceptionally, anaphylactic shock or cardiogenic shock. AmB is toxic for haematological cell lines and for renal function. The drug is administered as an i.v. infusion formulated as AmB deoxycholate colloidal suspension (Fungizone[®]). In adults as well as in children, 0.5–

1 mg/kg dissolved in 500 mL of 5% dextrose solution are administered daily or every second day up to 8 weeks [48].

Alternatively, liposomal amphotericin B (L-AmB) can be used. Three similar liposomal formulations are available. AmBisome[®], the only approved formulation for VL, is a unilamellar bilayer liposome made of phospholipids with AmB incorporated into the membrane [53]. Abelcet[®], a phospholipid AmB complex, and Amphocil[®], a cholesterol dispersion, are not yet licensed for VL. L-AmB leads to greater drug accumulation in infected cells, thereby increasing the therapeutic index and becoming more efficient both in immunocompetent and immunocompromised patients [54]. L-AmB is less toxic than conventional AmB, but in rare cases anaphylaxis has also been reported here. However, liposomal applications are prohibitively expensive and it is therefore difficult to increase their use in developing countries. L-AmB treatment is largely based on clinical trials of patients infected with *L. infantum* [55]. The recommended short-course treatment includes five daily injections of 3 mg/kg followed by two single further injections on Days 14 and 21 for adults and children. For immunocompromised patients, five daily injections of 4 mg/kg are followed by further injections on Days 10, 17, 24, 31 and 38, but relapse rates are high.

Since 2002, the oral antineoplastic agent miltefosine (Impavido[®]), an alkylphosphocholine, has been available on the market for the treatment of leishmaniasis [56]. Cure rates of up to 94% have been documented [57], which are the highest for any available antileishmanial drug. Nevertheless, there are concerns about developing resistance, as in vitro resistant strains can be established easily

[58]. Miltefosine resistance occurs due to point mutations in the plasma membrane that lead to deficient drug uptake [59]. Miltefosine is assumed to be teratogenic, however this has never clearly been demonstrated. The digestive system is frequently affected, resulting in, for example, mild vomiting, diarrhoea and motion sickness [48,60]. According to the US Centers for Disease Control and Prevention (CDC), miltefosine should be administered in oral dosages of 2.5 mg/kg/day (max. 150 mg/day) for 28 days.

Immunotherapy can be administered in combination with Sb^V compounds, especially in immunosuppressed patients. Interferon-gamma ($IFN\gamma$) is used to trigger the phagocytic activity of macrophages in order to kill amastigotes that settle in them. However, this treatment is cost intensive and due to lack of clinical trials it remains limited today [61].

The triazole antimycotic posaconazole, which is currently being investigated by the Drugs for Neglected Diseases initiative (DNDi) against Chagas disease, was shown to be active against *Leishmania amazonensis* and *Leishmania donovani* in rodent models [62]. Further compounds investigated by the DNDi are oxaborole molecules from Anacor Pharmaceuticals [63] and nitroimidazole compounds from TB Alliance. Both showed in vivo efficacy. PK trials are currently underway in order to find pre-clinical drug candidates.

Interestingly, the antimalarial compound tafenoquine was reported to be highly active against *Leishmania* in vitro and in a rodent model [64]. Another antimalarial, orally

administered artemisinin, was shown to effectively reduce the parasite burden in a BALB/c model of VL [65].

3. Chagas disease

Chagas disease, caused by *Trypanosoma cruzi*, represents an important public health problem in endemic geographic regions in Middle and South America, affecting 15 million infected people. Treatment options are limited by toxicity of available drugs, parasite resistance and poor drug activity during the chronic phase of the disease. Clinically, the pathogenesis of the disease can be divided into three phases, namely a short acute phase, a long-lasting latent phase, and a chronic phase emerging in 10–30% of patients.

The drugs of choice for treatment of Chagas disease are benznidazole (Rochagan[®]; Roche) and nifurtimox (Lampit[®]; Bayer) [66], but resistances are increasing and therapeutic success is phase-dependent. Both substances are effective in the acute and initial chronic phases of the infection but not in the late chronic phase. Therefore, the dose and duration of treatment depend on the stage of the disease and on the natural susceptibility of *T. cruzi* isolates. According to the CDC [48], benznidazole should be administered in three to four per oral (p.o.) doses of 8–10 mg/kg/day for adults. The paediatric dosage for benznidazole is 15–20 mg/kg/day in four p.o. doses for children aged 1–10 years and 12.5–15 mg/kg/day in four p.o. doses for children aged 11–16 years. Treatment should be applied for 90–120 days in the adult and paediatric dosage. The adult dosage for nifurtimox is 5–7 mg/kg/day in two p.o. doses and the applied paediatric dosage is 10 mg/kg/day in two p.o. doses in children until 12 years. Treatment with nifurtimox should last 30–90 days.

Severe side effects have been reported for both drugs. Adverse reactions of benznidazole can be classified into three groups [66]: (i) hypersensibility, dermatitis with cutaneous eruptions (usually appearing between the 7th and 10th day of treatment), fever, lymphadenopathy, and articular and muscular pain; (ii) functional insufficiency of bone marrow, thrombocytopenic purpura and agranulocytosis; and (iii) polyneuropathy, paraesthesia and polyneuritis of peripheral nerves. During treatment with nifurtimox, adverse effects such as anorexia, loss of weight, psychotic alterations, excitability or sleepiness, nausea, vomiting, intestinal colic and diarrhoea were observed. Although both drugs are potentially contraindicated during pregnancy, treatment of pregnant women with benznidazole or nifurtimox is recommended in any stage of *T. cruzi* infection and for newborns [67,68] since approximately 40% of all untreated cases are fatal [69].

There are no drugs available for the chronic phase of the disease. Therefore, supportive chemotherapy with benznidazole is suggested in the treatment of heart failures due to Chagas disease [70]. Furthermore, sodium intake, diuretics and vasodilators are restricted, and digitalis might be recommended in patients suffering from heart failure. Acute meningoencephalitis can be managed with anticonvulsants, sedatives and i.v. mannitol. Pacemakers might be used if bradycardia does not respond to atropine or for atrial fibrillation; in arrhythmias, amiodarone is the most effective drug. Heart transplantation is the last resort. Dietary and surgical steps are a vital part of the treatment of chronic Chagas disease in megaesophagus and megacolon.

Alternatively, the antileishmanial drug AmB can be used as a second-line treatment [71,72], which is however rather expensive in its less toxic liposomal formulation. BioDelivery Sciences International Inc. is currently developing Bioral[®] amphotericin B, a new L-AmB drug against Chagas disease, and has successfully finished clinical phase I trials [73].

Azole derivatives were reported to show synergistic antitrypanosomal effects in *T. cruzi* in vitro and in in vivo experimental models, with benznidazole and other compounds involved in the sterol biosynthesis pathway. Pre-clinical studies by the DNDi demonstrated clear synergistic effects for benznidazole and nifurtimox in combination with posaconazole, with reduction of mortality and parasitaemia suppression observed in animals. Further studies are planned to evaluate the potential clinical use of the azole class in combination with both antitrypanosomal drugs. Posaconazole was furthermore reported to show beneficial effects in a case report study [74]. E-1224, a new-generation triazole compound, is currently being investigated as a new agent for the treatment of Chagas disease in a phase II clinical study by the DNDi and Eisai Co., Ltd. [75].

4. Sleeping sickness

Human African trypanosomiasis (HAT), also known as sleeping sickness, is restricted to 36 sub-Saharan Africa countries. Following continued control efforts, the number of cases reported in 2009 by the WHO has dropped below 10 000 for first time in 50 years, however the estimated number of actual cases is currently 30 000.

Trypanosoma brucei gambiense, found in West and Central Africa, is responsible for 95% of all reported cases, whereas *Trypanosoma brucei rhodesiense*, which causes

a more acute and virulent infection, is restricted to rural areas in Eastern and Southern Africa. Both forms begin with a haemolymphatic stage and become fatal when the trypanosomes invade the central nervous system (CNS). HAT is one of the very few infectious diseases with a mortality rate of 100% if left untreated.

Four drugs are registered for the treatment of sleeping sickness and are provided free of charge to endemic countries through a WHO private partnership with Sanofi-Aventis (pentamidine, melarsoprol and eflornithine) and Bayer AG (suramin).

During the first stage, pentamidine is used for *T. b. gambiense* infections; it is also effective against the haemolymphatic stage of *T. b. rhodesiense*, but suramin shows higher efficacy [48]. The adult and paediatric dose for pentamidine is 4 mg/kg i.m. or i.v. for 7–10 days. Despite a few undesirable effects, the drug is usually well tolerated. Suramin should be given in a 2 mg test dose for children and a 100 mg test dose for adults prior to the first dose to monitor the patient's hemodynamic stability. Afterwards, 20 mg/kg suramin i.v. is given on Days 1, 3, 5, 14 and 21 for children and 1 g suramin i.v. is administered on Days 1, 3, 5, 14 and 21 for adults. Suramin can cause allergic reactions and side effects in the urinary tract.

Second-stage treatment melarsoprol, which is used for both forms of HAT, is an arsenic compound and has severe side effects, including reactive encephalopathy that can be fatal in up to 10% of cases. Moreover, upcoming resistance has been reported, particularly from Central Africa. Melarsoprol 2–3.6 mg/kg i.v. is administered for 3 days. A second and third series of 3.6 mg/kg i.v. are administered for 3 days after 7 days from the prior course of therapy. The dosage regimen is the

same for children and adults. Alternatively, eflornithine can be used, which is less toxic than melarsoprol but is only effective against *T. b. gambiense*. However, the regimen is rather difficult to apply: 400 mg/kg/day in four doses for 14 days.

A combination treatment of nifurtimox and eflornithine (NECT) was introduced in 2009. This mode of administration simplifies the use of eflornithine. Moreover, nifurtimox is not effective for *T. b. rhodesiense* and it is only registered for the treatment of Chagas disease. Nevertheless, after safety and efficacy approval of NECT in clinical trials, the drug was listed as an 'Essential Medicine' by the WHO. Furthermore, NECT is provided free for the treatment of HAT by the WHO.

Fexinidazole is a 2-substituted 5-nitroimidazole that was rediscovered by the DNDi after screening more than 700 new and existing nitroheterocycles [76]. Fexinidazole is the first new clinical drug candidate in 30 years with the potential to treat advanced-stage sleeping sickness. After promising pre-clinical pharmacological and safety studies, fexinidazole as oral treatment has entered first-in-human phase I studies in September 2009.

Oxaboroles, also investigated for their use in VL, showed activity in animal models of *T. brucei* infection [63]. Some compounds, in particular SCYX-6759, cured murine CNS infection, however they were actively transported from the brain and had to be administered at high doses. New compounds that are not effluxed from the brain were identified, for example SCYX-7158 advanced became a pre-clinical candidate at the end of 2009.

5. Amoebiasis, giardiasis and trichomoniasis

Entamoeba histolytica is the causative agents of amoebic colitis and liver abscess, with around 40 million infections per year and up to 100 000 deaths. *Giardia duodenalis* causes around 280 million symptomatic infections every year and has a high morbidity rate, particularly among children, due to persistent diarrhoea. The drug of choice for treatment of both parasites is metronidazole, which has been used successfully for more than four decades. Reported side effects are anorexia, nausea, vomiting, malaise, metallic taste and potential teratogenicity. Metronidazole is administered at 15 mg/kg p.o. in three divided doses for 7 days, 20 mg/kg/day for 5 days and 30 mg/kg twice daily for 7 days [77].

Metronidazole is also the drug of choice for *Trichomonas vaginalis* infection, which is the most prevalent non-viral sexually transmitted disease with more than 170 million new cases every year. However, resistance in *T. vaginalis* is emerging and there is no well established alternative treatment available. Pentamycin, a polyene macrolide used for the treatment of candidiasis, might constitute a potential new drug for the treatment of trichomoniasis [78], however further studies have to be performed.

Diloxanide furoate, which is given orally at 25 mg/kg for 10 days, is a safe and effective alternative treatment for *E. histolytica* infections. Reported adverse reactions are flatulence, dizziness, diarrhoea or cramping, nausea and headache.

Tinidazole and ornidazole are further alternatives to metronidazole in the treatment of amoebiasis, giardiasis and trichomoniasis [79–82]. Tinidazole is administered in a 2 g dose taken daily over 3 days for intestinal amoebiasis in adults, and in children at 50

mg/kg/day (up to 2 g per day) doses taken over 3 days. Amoebic liver abscess is treated with tinidazole in a 2 g dose per day for 3–5 days. The paediatric dosage is 50 mg/kg/day for 3–5 days with food. Tinidazole is given in single 2 g doses for adults and in a single 50 mg/kg dose for children in giardiasis and trichomoniasis. Reported side effects are upset stomach, bitter taste, itchiness, headache, physical fatigue and dizziness. The reported side effects of ornidazole are similar to metronidazole and tinidazole and include metallic taste, diarrhoea, drowsiness, nausea, vomiting, headache and sleep disturbance. Cross-resistances of tinidazole and ornidazole to other 5-nitroimidazole drugs such as metronidazole are a significant problem [80,83].

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None declared.

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