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Drugs used in the treatment of sleeping sickness (human African trypanosomiasis: HAT)

M. Wéry*

Institute of Tropical Medicine, 155 Nationalestraat B-2000 Antwerp, Belgium

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Abstract

From the first decade of this century arsenicals have been the most universal and most effective drugs for all cases of sleeping sickness. Melarsoprol, introduced in the 1940s, remains the most universal of these compounds. However, resistance of trypanosomes and toxicity that may be fatal for the patient are two major shortcomings. Pentamidine, suramin and Berenil® are active only in the first stage of the disease, when the parasites are confined to blood and lymph. Nifurtimox taken orally for 1 to 2 months and alpha-difluoro-methylornithine (α-DFMO) with an administration scheme spread over 5 weeks including 14 days of intravenous injections, provide interesting alternatives for all cases, since they reach the central nervous system. However, DFMO is known to be less active against *T. rhodesiense*. Imidazoles, new arsenical derivatives and antimitotics have been successfully tested in experimental models. Combinations of drugs with additive or potentiating effects mainly based on inhibition of decarboxylase enzymes or exposure to oxidative stress appear promising.

Key words: Human African trypanosomiasis; Sleeping sickness, treatment; Drugs development

1. Introduction

Sleeping sickness, or human African trypanosomiasis (HAT), occurs in Africa south of the Sahara and is caused by man-adapted subspecies of *Trypanosoma brucei* (*T. b. gambiense* and *T. b. rhodesiense*) and is transmitted by blood-sucking flies (tse-tse flies, *Glossina*). The treatment of patients is difficult for two reasons: (i) the disease develops through two stages, haemolymphatic and nervous; this requires that different treatment modalities need to be used and directed against both phases of the disease; (ii) 2 years of post-treatment follow-up of patients by means of periodic monitoring of the cerebrospinal fluid (CSF) for inflammatory characters is necessary to substantiate the suppression of the infection.

After inoculation by the bite of a glossina, the trypanosomes multiply in the blood and lymph. They elude the immune response of the patient by changing their variable surface antigen. The haemolymphatic stage consists of successive peaks of parasitaemia that end in massive immune lysis and spread of antigen–antibody complexes. Plasma, trypanosomes and leukocytes progressively escape from the vascular compartment through the damaged endothelial layer of small vessels and capillaries. They invade the extracellular compartment in all organs, including the central nervous system (CNS) and the cerebrospinal fluid producing the so-called 'nervous stage' of the disease. Hence the importance of drugs that reach all compartments of the body and pass the blood– brain barrier.

The nature and the properties of this barrier are, however, ill defined: its selective permeability usually prevents large molecules from reaching the cerebral tissue but the permeability might be altered in an unpredictable way by the presence of trypanosomes [1]. It is impossible to know precisely when parasites first escape from the blood and lymph compartment, invade extravascular spaces and reach the CNS: it happens probably long before any change is noticed in the CSF.

* Corresponding author. Tel. (+32-3) 2476355; Fax (+32-3) 2476362.

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The two subspecies of *Trypanosoma brucei* are among at least four groups of parasites identified as zymodemes [2] that differ by specific DNA sequences [3,4], and in geographical distribution, vector species, vertebrate host diversity (infectivity for man), drug susceptibility and virulence. However, all representatives of the species share similar behaviour and metabolic activities throughout their cycle in vertebrate and invertebrate hosts.

In less than 3 months after inoculation of *T. b. rhodesiense* by the infected fly, high grade parasitaemia and the presence of parasites in the CSF may be found. In some cases, this can occur as early as 8 days [5]. The epidemiological reservoir of parasites is in both wild and domestic naturally infected animals.

The less virulent *T. b. gambiense* gives a lower grade parasitaemia, with mild symptoms during the first months and an insidious evolution towards the nervous stage [6]. The reservoir of the infection is infected persons, who remain infective for the vector during the very long incubation period.

One major approach to the control of the disease is the reduction of the parasite human reservoir through early diagnosis and treatment of infected persons. Thus, drugs are applied in two different situations: patients calling at the hospital with symptoms and mass treatment of asymptomatic persons by survey teams. The CNS involvement necessitates the use of toxic drugs of low molecular weight (arsenical compounds) with a risk of lethal toxicity during treatment through a poorly understood reactive arsenal-induced encephalopathy (RAE) [7].

Over half a dozen other species and subspecies of trypanosomes infect animals only, which may have important economic consequences. Drugs are available for treatment and prevention of animal trypanosomiases but these are, as a rule, not used against sleeping sickness.

2. Development of drugs active against sleeping sickness

Medical intervention in the course of infectious diseases has always relied mainly on the administration of drugs (chemotherapy). Arsenic, a trypanocide component still in use and among the most efficient, is one of the oldest chemotherapeutic agents.

In history, drugs have been classified into three groups:

— natural products from the '*Materia medica*' of the Greek Dioscorides (100 AD) including '*Simplicia*', simple elements such as mercury, iron, iodine, arsenic, antimony, sulphur, present in nature as minerals, and a wide variety of extracts, isolated and purified from various parts of plants;

— chemical compounds resulting from the combination of elements by chemical reactions;

— metabolites of living organisms (antibiotics).

Ibn al Baikar (1197–1248) followed the model of Dioscorides for the description of more than 2000 drugs including cashew-nut, amber, ammonia, nux vomica...

Paracelsus (1493–1541), lecturer in chemistry and son of a medical practitioner, promoted the use of alchemy for the development of medicines rather than the transmutation of base materials into gold. Back to the simple elements, the goal of pharmacy should be, he said, to isolate the most sublime extractive, the 'Quinta Essentia' which represents the effective part of every substance. Distinction was made between volatile (Hg), combustible (S) and fire-resistant (NaCl) elements. He promoted the use of mercury for the recently recognized syphilis, of antimony as an emetic agent, and of iron, arsenic and zinc for dermatological and other disorders.

During the 16th and 17th centuries, alchemy developed into chemistry, with the use of the 'great' acids (sulphuric and hydrochloric) under the influence of Van Helmont. The discovery of the new world brought prominent new plants into use in medicine: Peru balsam, jalap, cascara and, of course, cinchona bark as an antipyretic.

During the 19th century, refined chemical techniques made possible both the extraction of pure components from plants and chemical synthesis. Isolation of alkaloids by Stertüner, of quinine by Caventou and Pelletier (1820) and the synthesis of pyramidon by Filehme are a few major steps in this revolution. The work of Ehrlich (1854–1915) started from the observation that synthetic dyes could attach to structures of living cells. The selective fixing of certain agents to tissues, cells or even organelles leads to damaged stained structures. The cornerstone of this approach may well be the dye methylene blue synthesised by Caro in 1876 and the observation that methylene blue is taken up by leukocyte granules as demonstrated by Ehrlich in 1881. In 1891 Ehrlich and Guttmann suggested its therapeutic use on the base of the elective uptake of the dye by the newly discovered malaria parasite. The theory arose that every toxin molecule possesses a haptophore group whose role is to attach to a cell structure ('corpora non agunt nisi fixata') and a toxophore group possessing a destructive potential. Ehrlich was looking for molecules that could attach to bacteria or parasites and not to host cells. Trypan red, trypan blue and afridol violet were synthesised starting from benzidine.

The observation that arsenic could protect against snake bites initiated its use against the effects of the tse-tse fly bite and gave it, as early as 1856, under the influence of Livingstone [8], the reputation of killing 'germs' inoculated into animals or humans by the bite of tse-tse flies. However, it was only after the discovery of the trypanosome cycle in 'nagana' (*T. b. brucei* infection in cattle) by Bruce in 1896 [9], and the setting up of laboratory infection models or in vitro cultivation for animal and human trypanosomes, that experimental treatment of trypanosomiasis started around 1904.

Inorganic molecules, like arsenic trioxide (As_2O_3) (Fowler's solution) [10], sodium arsenate (Na_2HAsO_4) and arsenic sulphur (Orpiment) [11,12], were tried without real success on the recently discovered (1901) human trypanosomiasis and were subsequently abandoned because of their toxicity.

Two families of organic arsenical compounds were investigated: pentavalent with the general formula [R-AsO(OH)$_2$] and trivalent with the formula [R-AsO]. Sodium arsanilate (Atoxyl) had been prepared in 1863 by Bechamp by adding aniline to sodium arsenate and was described by Ehrlich in 1907 [13] as a pentavalent compound. Thomas and Breindl [14] introduced it in 1903 in the treatment of the human trypanosome infections [15] while its activity was compared to that of antimony potassium tartrate (Emetic tartar) by Broden and Rodhain [16]. The drug had marked toxic effects on the optic nerve and numbers (3 to 5%) of treated patients became blinded. Moreover, according to Broden and Rhodhain, it was not active in cases of CNS involvement [16,17].

In the same period, salvarsan (arsphenamine) and neosalvarsan, trivalent arsenical compounds used against treponematoses, were shown to be active upon peripheral trypanosomes but they were never used routinely. Thereafter the family of phenylarsanilic acids remained a stepping stone for future investigation and identification of potential trypanocides.

Meanwhile, Ehrlich's dyes were promoted for their trypanocide action: trypan blue and afridol violet were used with some success in bovine trypanosomiasis and trypan blue was also active against piroplasmoses. The staining of muscle of treated cattle had, however, an important negative economic side effect. Therefore, modifications of the molecules to preserve the toxic effect without staining were promoted. Trials with trypan red, trypan blue, or afridol violet carried out on sleeping sickness patients (Mesnil and Nicolle 1906, Broden and Rodhain [16]) did not give satisfactory results; the parasites were not even eliminated from the blood.

Afridol (trypan) violet, containing an urea link, deserves special mention, since it was the origin of the synthesis of sodium suramin by Heymann and Roehl (1916), through replacement of azo by amido groups. Heymann (1911) from Bayer Industries, prepared a diphenyl urea, a colourless analogue of trypan red, by introduction of a p-aminobenzoyl moiety. From this precursor, the synthesis of Bayer 205 [18] was accomplished probably about 1916 but its structure and testing were kept secret owing to war-time economic strategies. The drug was tested with great success in patients during an expedition in Africa in 1921 [19] and as a result thereof Germanin® was launched in 1923 and received enthusiastic attention under the names of suramin, Germanin®, antrypol, naganol, naphuride. It is noteworthy that French authors somehow became aware of the successful formula and commercialised around the same period the same molecule under the trade names 'Fourneau 309', Moranyl®. Excellent results of curative treatments were reported from various places in Africa and Europe in patients without involvement of the CSF [20–23]. The use in prophylaxis was less successful [24]. Recent progress in the knowledge of the pharmacokinetic properties of suramin is mainly due to its use as inhibitor of reverse transcriptase of RNA tumour viruses and HIV [25]. The same molecule is also used in the treatment of onchocerciasis for its macrofilaricidal effect.

From 1919 until 1960 the pentavalent sodium salt of N-phenylglycine-amide-p-arsanilic acid (Glyphenarsyl, Tryponarsyl®) was the drug of choice (i.v., 20% solution) for all stages of human trypanosomiasis [26–28] together with the sodium 4-acetamido-2-hydroxyphenyl-arsanilate (Orsanine, '270 Fourneau'), isomer of stovarsol, another pentavalent arsenical introduced in 1923. To some extent, these drugs penetrate the blood–brain barrier because it was shown that after administration of high doses, arsenic was found in the CNS where it appears to destroy the parasites. The results were in general excellent, with a cure rate of 85, but toxic effects on the gastrointestinal tract (nausea, vomiting, diarrhoea and abdominal pain), skin (dermatitis), eye (atrophy of the optic nerve) were regularly observed.

Meanwhile, the first experiments which later brought the discovery of pentamidine and the other diamidines relied on the observation that the trypanosome needs great amounts of glucose for its energetic metabolism. The history of the guanidine and diamidine derivatives goes back to 1926 when Franke, Northmann and Wagner introduced decamethylene diguanidine (Synthalin®) as a substitute for insulin, to lower blood sugar concentrations in diabetes patients. Ten years later Von Jancso and Von Jancso [29], showed that Synthalin® could cure *T. brucei* infections in mice. The idea of interfering with the nutrition of the parasite was the incentive to further testing of the activity of a large series of guanidines, amidines and isothioureas on trypanosomes.

Lourie and Yorke [30] successively introduced stilbamidine, propamidine and pentamidine. The first successful clinical trials were inaugurated by McLetchie [31] in Nigeria (1940) in first stage *T. gambiense* infections. Patients with nervous involvement did not react to treatment.

Van Hoof in 1942 in the former Belgian Congo was among the first to inaugurate chemoprophylaxis in the Kwango area and to support evidence that a single 5 mg/kg injection of pentamidine protected the individual against *T. gambiense* for as long as 6 months [32,33].

Isethionate and dihydroxymethanesulfonate, respectively available under the trade names Pentacarinate® (M&B 800) and Lomidine® (251 2 RP), were used for intramuscular injections.

Recent progress has been achieved in the pharmacokinetics of pentamidine salts since they are currently used

in the treatment of *Pneumocystis carinii* infections, an opportunistic protozoan in patients with immunodeficiency syndrome [34].

Diminazene is an aromatic diamidine resulting indirectly from the investigations of related trypanocidal 4,6-diaminoquinaldine derivatives. The most interesting are congasin (or Surfen C), a highly potent trypanocide for animal trypanosomiases, and a substituted malonamide named Bayer 7602, the first active compound against acute *T. cruzi* infection.

In the form of a diaceturate salt (Trypazen®, Berenil®) the compound was found to have a marked activity against a variety of trypanosome infections (except *T. cruzi*) [35] and also against babesiasis, an important veterinary parasitic infection.

Neujean and Evens [6], Hutchinson and Watson [36], and Ruppol and Burke [37] demonstrated the activity of Berenil® in clinical trials performed in the 1960s on *T. b. gambiense* and *rhodesiense* infections provided they were in the early stage without any signs of nervous system involvement. Although it remained officially a drug for veterinary use and was never released for treatment of human patients, the relative toxicity of arsenical compounds and the growing resistance of trypanosomes to the available drugs enforced the use of Berenil® in many countries of Central Africa where trypanosomes are endemic.

After World War II resistance of trypanosomes to Tryponarsyl® and Orsanine® became more and more evident and both drugs were abandoned when the more active trivalent melamine derivatives took over and are still in use at the present time.

Friedheim took advantage of the development of melamine chemistry in the plastics industry and initiated the synthesis of a line of triazinylarsonic acid derivatives and their antimony analogues. Melarsen was prepared by interaction of 2-chloro-6-diamino-1,3,5-triazine with sodium arsanilate (Atoxyl®).

In 1940, Friedheim [38] had synthesised melarsenoxide by reduction of melarsen sodium, in support of Ehrlich's argumentation that aromatic pentavalent arsenical compounds become actively trypanocidal only after they have been reduced in vivo into trivalent derivatives. Friedheim argued that chemosynthesis could spare the patient metabolic effort by operating the reduction in vitro. The resulting molecule underwent various subsequent transformations: combination with dimercaprol [39] (BAL) in order to reduce the toxicity of the arsenoxides resulted in melarsoprol (Mel B, Arsobal®) [38,40]; and transformation into a potassium salt of the insoluble melarsenoxyde (Trimelarsan®, Mel W). The latter was introduced in 1959 [41–43], and has been used instead of Mel B when intravenous injections were not possible.

A new water-soluble salt, the melaminylthioarsenite Cymelarsan®. Mel Cy [44], showed better trypanocidal activity than Mel W in recent trials.

Advocated by Daniels [45], combinations of drugs like Atoxyl® plus Emetic tartar with or without Salvarsan were used to increase the success rates.

Nitrofurazone was found in 1951 [46] to be curative for *T. equiperdum*, a parasite of horses as well as in experimental infections in mice. Packchanian [47] first drew attention to the activity of the nitrofurans in experimental infections with *T. cruzi*. As a result, Chagas' disease became the main investigation and trial line for the newly established nitrofurans. However, the results of clinical trials with nitrofurazone in Chagas patients were disappointing while the same author [47] demonstrated in 1955 the curative effects of Furacim® (Eaton Laboratories) against *T. b. gambiense* and *T. b. rhodesiense* infections in mice. The new drug was used for the first time [48] in 1957 in 32 patients in various stages of *T. b. gambiense* infection, with encouraging therapeutic results. Levofuraltadone (Nf 902), made available by Eaton Laboratories, was given extensive clinical trials in Zaire [37] and showed remarkable activity, giving permanent cure of patients in all stages of the disease. However, the occurrence of relapses as long as 2 years after the apparently successful treatment remained unexplained and the drug soon became unavailable.

Nifurtimox (Bayer 2502, Lampit®), produced in 1967, was the first treatment for the etiology of Chagas' disease [49], especially active in acute infections in children. However, prolonged treatment is necessary to increase the probability of cure in the chronic stage. Initially, this drug was officially reserved by the manufacturer for Chagas' disease. It is now released in Europe and in Africa and is largely used with some success in western and central Africa, for compassionate treatment of otherwise untreatable *T. b. gambiense* sleeping sickness patients resistant to melarsoprol.

Alpha-difluoromethylornithine (α-DFMO) is an inhibitor of ornithine decarboxylase, active against the multiplication of cancer cells [50]. Its activity on trypanosomes was first demonstrated in 1979 [51] and studied by Bacchi [52] and McCann [53] in the early 1980s. Clinical trials with DFMO monotherapy started in 1982, in patients mostly infected with *T. b. gambiense* [54,55] and refractory to melarsoprol.

3. Drugs in current use

The few drugs currently used for the treatment of HAT are divided into two categories based on their ability to cross the blood–brain barrier.

In *T. b. gambiense* infections the list of drugs suitable for the first stage (blood and lymph infections) includes pentamidine (Lomidine®, Pentacarinate®), diminazene aceturate (Berenil®) and suramin (Moranyl®, Bayer 205, Germanin®) while for patients with nervous involvement (second stage) melarsoprol (Mel B, Arsobal®), nifurti-

mox (Lampit®) and alpha-difluoromethylornithine (α-DFMO, eflornithine, Ornidyl®), are the choice because they reach the CNS compartment.

In *T. b. rhodesiense* infections, the choice is still more restricted: Bayer 205 in the first stage and melarsoprol in the second are the only active compounds.

Combinations of drugs are of growing interest [56]. This approach is possible because of a better understanding of metabolic pathways of trypanosomes.

3.1. Arsenical drugs

Melarsoprol is a combination of melarsenoxyde with a chelator agent, dimercaptopropanol (British Anti Lewisite — BAL). Arsobal® (Specia) is presented as a 3.6% propylene glycol solution in 5 ml vials (36 mg/ml) for strictly intravenous injections. It is active against both *T. b. gambiense* and *T. b. rhodesiense*, in the blood, lymph and extravascular spaces, and easily crosses the blood– brain barrier. The administration scheme of Neujean [57] consists of 2 to 4 series of 3 daily injections of 3.6 mg/kg (1 ml per 10 kg) according to the degree of involvement of CNS, with a maximum of 200 mg per injection. In the early 1970s an increased rate of relapses was observed in Zaire among correctly treated *T. gambiense* patients (Reports of the 'Bureau Central de la Trypanosomiase', Kinshasa).

Wellde et al. [58] reported 92.1% cure in a group of 269 patients infected with *T. b. rhodesiense* 3 years after melarsoprol treatment using doses of more than 30 ml (1.08 g). Failures included 1.4% relapses and 6.5% deaths within the 3-year follow-up period (of which 5.2% during administration). Treatment of relapsing *T. b. rhodesiense* patients after unsuccessful melarsoprol therapy remains a challenge, as neither α-DFMO nor nifurtimox is reliable in the case of *T. b. rhodesiense* [59].

It is usually recommended that the trypanosomes should first be removed from blood and lymph by the use of suramin or pentamidine and subsequently from extravascular spaces, CNS and CSF, with melarsoprol. Arroz [60] did not observe any difference, however, in the number of reactive arsenical encephalopathy and fatal outcomes between a group of *T. b. rhodesiense* patients treated with a series of four melarsoprol injections only and another group given suramin pretreatment followed by progressive doses of melarsoprol and corticosteroids. The frequency of accidents did not seem to be related to the degree of CNS involvement either, as judged by CSF examination. Moreover, drug-induced immunosuppression in mice has been shown to cause more frequent relapses after melarsoprol treatment, and selection of arsenic-resistant trypanosomes occurs more rapidly [61].

On the contrary, in *T. b. gambiense* infections, Pepin et al. [62] confirmed that RAE was more frequent in patients with inflammatory CSF (white blood cell counts higher than 100/μl). Prednisolone treatment reduced the incidence of RAE to 4.14% in a group of 290 as compared with the 11.36% RAE observed in a group of 308 treated with melarsoprol alone.

Toxicity remains a cause of concern [63]. Within the first hours or days, a number of intestinal, renal, cutaneous, rheumatoid, cardiac or general alarm signs can be a reason for stopping administration. The end of the first week is the most critical period, when often fatal severe reactive encephalopathy occurs, characterised by nervous, digestive, psychiatric and vascular signs leading to hyperthermic coma and death within 48 h. Administration of BAL does not bring much improvement in releasing the toxic manifestations, but the use of corticoids may help. It is commonly believed that encephalopathy is a consequence of massive destruction of trypanosomes in the nervous system, and that its incidence and severity are related to the stage at which the disease is treated and hence to the number of parasites present in the nervous tissues at the time of treatment. Encephalitis after suramin treatment is indeed not observed in clinical practice while RAE occurs frequently (7.8%) in patients in the late stage of *T. b. gambiense* treated with melarsoprol in Zaire [64].

Hunter et al. [65] advocated the use of higher doses because it was observed in mice infected with *T. brucei* that more aggressive treatment giving a complete clearance of parasites did not cause reactive arsenical encephalopathy while subcurative dosages caused a higher frequency of encephalitis and of course led to higher incidence of relapses.

The 'chelation hypothesis' to explain the toxicity of arsenical treatments was recently proposed by Golden [66]. Organic arsenicals chelate elements of the selenium/sulphur group, whose concentrations are already low in patients in bad physical conditions (malnutrition etc.). According to this author, encephalitis only occurs in patients previously depleted of those elements, particularly selenium which has a protective effect against arsenic-induced chromosomal damage in mammalian cells in vitro [67].

With a view to reducing doses of melarsoprol, its combination with other drugs has been tested (see under 'combination of drugs' in this revue). Pharmacokinetic studies are in progress [68] using Burri's in vitro bioassay [69] performed on cloned populations of *T. b. rhodesiense* cultured in wells of a microtiter plate. The sensitivity of the proposed assay is in the order of 10 ng/ml.

3.2. Suramin

This compound (Bayer 205, Germanin®, Moranyl®) is available in vials containing 0.5 g to 5.0 g of white powder. A 10% solution in distilled water should be made for immediate intravenous injection. The single dose is 20 mg/kg body weight and the total curative dose is 200 mg/kg body weight (6 to 9 injections of 1 to 1.5 g for

an adult patient). Recommended intervals between injections vary greatly but weekly administrations are preferred.

Its pharmacokinetic profiles vary from one individual to another [70]. Serum concentrations are usually high immediately after intravenous injection but fall rapidly within the first few hours and then more slowly over ensuing days. Low concentrations are maintained for as long as 2 or 3 months [71]. Excretion is assumed to occur mainly by the renal route. Refined techniques have been set up recently for the determination of suramin in the body fluids [72,73].

The drug has marked activity against all trypanosome species except *T. cruzi* but does not reach the CNS.

Temporary reactions (nausea, photophobia, syncope and albuminuria) [74] should not be considered as a contra-indication for continuing the treatment. Systemic toxic action on renal and adrenal glands is mentioned [36].

Infections by *T. b. rhodesiense* are especially suited for treatment with this drug. Even after only one injection, trypanosomes are cleared very rapidly from the blood, lymph and peripheral tissues. When involvement of the CNS calls for the use of other drugs (especially arsenical compounds), treatment is often initiated with one injection of suramin to eliminate blood parasites [64]. This should also decrease the probability of shock when treatment with arsenic is started.

3.3. Diamidines

Pentamidine® (M&B 800) — Lomidine® (2512 RP)

Among the different salts improving the solubility of pentamidine, a stable aqueous solution of methanesulfonate which contains 4% Lomidine base has been marketed by Specia (Lomidine®), and isethionate in powder by May and Baker. The usual treatment scheme consists of one intramuscular injection of 3 mg base per kg of body weight, daily for at least 10 consecutive days. Pentamidine resistance is rather common, due to its former extensive use in prophylaxis [75] and therefore combined treatments should be recommended.

Adverse reactions such as a fall in blood pressure with syncope, breathlessness, tachycardia, nausea and vomiting may be dramatic but are usually of no great consequence [76].

Sensitive methods for measuring the concentrations of diamidines in the body fluids have recently been made available [77]. Pentamidine® is stored in the tissues where it acts as a repository drug but does not reach the CNS (0.8% of the plasma concentration) [78]. In a series of ten intramuscular injections of 3.5 to 4.5 mg/kg of body weight given on alternate days, the maximal plasma concentration was reached 12 to 24 h after the injection and the median half-life was 22.4 h after the first dose and 47.1 h after the tenth injection [79]. Prophylactic use of pentamidine has therefore been advocated [41]. The drug has been used with success, together with other measures, in mass campaigns aiming at the interruption of transmission of the infection. This approach is, however, not devoid of difficulties due to side-effects (including abortion in pregnant women!) and to the masking of symptoms by the low dosage used in the schedule: infected persons may thus remain undetected and only emerge later with severe involvement of the nervous system. The prophylactic use of pentamidine for tourists should therefore be discouraged.

Diminazene aceturate (Berenil®Hoechst, Trypazen®)

The activity of Berenil® was demonstrated in clinical trials on *T. b. gambiense* and *rhodesiense* infections in the early stage [44,80]. The drug does not reach the CNS. The mild toxic effects are nervous and digestive. There is as yet no uniformly accepted treatment protocol as the drug is marketed only for veterinary use [81]. It is accepted that *T. brucei* infections respond successfully to a total dose of 15 to 20 mg/kg, injected intramuscularly. The single dose should not exceed 7 mg/kg.

3.4. Furans

Nifurtimox

It is a 5-nitrofurane compound that has been used for years in the treatment of Chagas' disease and was shown to be very efficient against *T. b. gambiense* and *T. b. rhodesiense* in experimental infections [82]. The drug readily crosses the blood–brain barrier. The recommended dosage is 10 to 20 mg/kg daily in three divided doses for 60 to 120 days. Tablets of 30 mg (for children) and 120 mg are available.

Concentrations of 100, 10 and 1 μg/ml killed cultured *T. b. brucei* in 12 to 48 h [83], but trypanosomes after contact with 0.1 μg/ml were shown to be viable. In *T. b. brucei*-infected mice, radical cure was only possible using toxic doses of 200 mg/kg per day or higher.

Clinical trials on *T. b. gambiense* patients were performed in three different places. As a rule, trypanosomes disappeared, WBC counts decreased sharply in the CSF and the clinical picture improved markedly during treatment. In Zaire [84], 80% of 15 patients were cured in Bwamanda with 12.5–15 mg/kg per day of nifurtimox monotherapy during 60 days. On the other hand, only 37% of 25 melarsoprol-resistant patients in Nioki treated with 12–17 mg/kg per day of nifurtimox as sole drug during 60 days were cured and 63% relapsed 1 to 9 months after the end of the treatment [85]. The bad results of the Nioki study might be due to poor compliance.

Van Nieuwenhove [86,87] treated late stage patients in Sudan (Equatoria) with nifurtimox: 85% of a first series of 20 patients receiving a single injection of suramin (20 mg/kg of body weight in children, 1.5 g in adults) or Berenil® (7 mg/kg body weight) before the 15 to 45 days of oral nifurtimox (15 mg/kg per day) were completely

cured. In a second series of 75 patients, 72% were completely cured after nifurtimox monotherapy. Of these 75 patients, 17% were lost for complete follow-up but biologically negative and clinically improved by the end of the observation period, 7% relapsed with trypanosomes found in the CSF, and 6% died, two fatal cases being related to drug toxicity. The author concluded that nifurtimox, having cured nearly 89% of these patients, is at present routinely used as alternative treatment in arsenic refractory cases in Sudan.

Recently, Pepin et al. resumed a series of treatments in Zaire [88], using higher doses (30 mg/kg per day during 30 days). The important adverse reactions included confusion, tremor, vertigo, anorexia and weight loss. And yet, with this toxic dosage, 9 out of 30 patients (30%) relapsed within 18 months of follow-up.

From the previous observations, the sensitivity of the *T. b. gambiense* stocks seems to vary from one place to another in Central Africa. There are no records available of *T. b. rhodesiense* patients treated with nifurtimox. However, experimental acute infections with *T. brucei* in mice are difficult to cure with this drug [83].

3.5. Inhibitors of decarboxylases

Clinical trials continue with eflornithine (α-DFMO, Ornidyl®) alone (inhibitor of ornithine decarboxylase), mainly on *T. b. gambiense* patients who relapsed after melarsoprol treatment. Because of the very rapid elimination of the drug from the body, the treatment scheme giving the best results is a series of intravenous injections or infusions of 100 mg/kg four times daily for 14 days, followed by oral administration of 75 mg/kg four times daily for 21 to 30 days.

In the past ten years or so, 771 patients have been treated in Africa, Europe and the USA [52,53,87,89–91]. The general observation is a rapid clinical improvement, accompanied by the disappearance of trypanosomes from both blood and CSF, and a marked improvement of the inflammatory character of the CSF.

Side effects observed by most authors are anaemia, diarrhoea, leucopenia, hair loss, vomiting, abdominal pain, seizures, dizziness. Death during treatment is rare, occurring in some patients in very bad physical condition (severe anaemia, cachexia, etc.). Diarrhoea was more frequent during oral administration. Relapses were more frequent (14%) with the sole oral regimen or 12 hourly i.v. scheme (11%) than with 6 hourly i.v. regimen (3%). Children must receive higher doses per kg of body weight.

In this series, of 711 patients [87], 38 (5.3%) relapsed, 49 (6.9%) died during or shortly after treatment and 6 (0.8%) did not improve clinically. Thus 678 (87.9%) patients were 'cured', but 270 of these were lost before 12 months and 157 between 12 and 24 months of follow-up. Only 191 patients were cured according to the required standards of the *T. gambiense* post-treatment controls.

It seems well established [92] that *T. b. rhodesiense* is less sensitive to DFMO than *T. b. gambiense* and that among stocks of *T. b. brucei* there is a broad spectrum of susceptibilities, the 50% inhibition concentration (IC_{50}) varying between 81 and 691 μmol [93].

4. Future prospects

From the better knowledge of parasite metabolism, new approaches to attacking the trypanosomes [94] have been suggested, but so far none of them has provided valuable replacement for the existing therapeutic schemes. Two important targets are the glycosome, an organelle, and trypanothione, a metabolite.

As source of energy, trypanosomes are mainly dependent on the transformation of glucose to pyruvate. Enzymes responsible for these metabolic steps are in part, in trypanosomes, contained in an original intracytoplasmic organelle, the glycosome, which has been studied in detail [95].

Trypanothione (a polyamine-containing glutathione) is a unique metabolite of trypanosomes. It provides protection against free radicals, hydrogen peroxide and the maintenance of the sulphite balance [96,97].

4.1. New molecules

Arsenicals

Spiroarsorane (octamethyl tetraoxa arsa anilino spiro nonane), a low toxicity pentavalent molecule, cured cerebral infections in sheep experimentally infected with *T. brucei*, provided multiple doses of 30 mg/kg were given [98].

Cymelarsan®, a water-soluble trivalent arsenical on the same model as Mel W [27], was tried with success in different animal trypanosomes.

The release of these drugs for human medicine is, however, not yet planned.

Azole derivatives

2-substituted 5-nitroimidazoles were found in 1983 to be curative in chronic mice infections with CNS involvement produced by *T. b. brucei*, the mode of action being probably an increased oxidative stress [99]. They were thereafter used with success in various combinations.

4.2. Combinations of drugs

Experimentally, some drug combinations show unusual performances [56], but none of the following has been ever used for human cases:

— in animal models, doses of melarsoprol may be reduced by five to ten times if dispensed following a 14-day

DFMO treatment [100]: melarsoprol acts by binding to trypanothione making it unable to play its role in oxidation–reduction processes whereas DFMO causes trypanothione depletion necessitating less melarsoprol to reach the goal;
— DFMO, through the depletion in trypanothione, was shown to act synergistically with nitroimidazoles that increase the need for protection of trypanosomes to oxidative stress [101] and with diminazene aceturate [102], curing easily *T. b. brucei* infections with CNS involvement;
— the combination of nitrocompounds and arsenicals (Mel W) takes advantage of the oxidative stress produced by nitroimidazole together with the blockage of trypanothione by arsenicals, and cures mice infected with *T. brucei* after a 5-day treatment [103] instead of several weeks as for nifurtimox alone;
— observations that incomplete treatment with diminazene regularly causes a post-treatment encephalopathy, while complete parasite clearance obtained using nitrocompounds and Mel Cy is never accompanied by encepalopathy [104], favour the hypothesis that the trypanosomes remaining during the treatment and at the end of it should be the target of a restored full capacity of the immune system, ending in production of cytokines and particularly TNFα and of some interleukines by activated astrocytes and macrophages, responsible for perivascular cuffing, meningitis and encephalitis [105];
— the addition of suramin to the previous combination (Mel Cy and nitroimidazole) gives further potentiation [106] and produces a complete clearance of parasites from the tissues after a 2- or even 1-day treatment according to the dose [107];
— non-steroidal anti-inflammatory drugs [108] help Berenil® to cure *T. brucei* in mice with CNS involvement.

5. Conclusions

In 1994 the physician's choice is restricted to five drugs more or less officially accepted. 'Optimization of current drug treatment regimens for African trypanosomiasis remains a priority topic for TDR funded applied field research', a statement in the October 1993 issue of TDR news (no. 43) shows clearly that we are still far from the ideal drug.

The actualised treatment schemes using pentamidine, suramin and melarsoprol were described recently in a WHO document [109,110]. New schemes using DFMO or nifurtimox were extensively tried by Van Nieuwenhove [85] in southern Sudan and by Pepin [88] in Zaire.

Sleeping sickness patients are classified in four groups based on the etiological agent (*T. gambiense* or *T. rhodesiense*) and on the stage of the disease (with or without nervous involvement). The recommended treatment schemes are as follows:

T. gambiense, CSF normal: schemes 1, 2, 6 or 7
T. gambiense, CSF altered: schemes 3, 4, 5, 6 or 7
T. rhodesiense, CSF normal: scheme 2
T. rhodesiense, CSF altered: scheme 4.

Scheme No. 1
Pentamidine monotherapy (intramuscular injections [109,110]

Timing (days)	Dose (mg/kg)	Amount injected (of either...	or...)	Secondary effects
		methane-sulphonate (40 mg/ml)	isethionate (100 mg/ml)	
1	4	5 ml	2 ml	*Local*
2	4	5 ml	2 ml	pain, induration,
3	4	5 ml	2 ml	sterile abscess
4	4	5 ml	2 ml	*General*
5	4	5 ml	2 ml	vomiting, abdominal pain,
6	4	5 ml	2 ml	hypotension,
7	4	5 ml	2 ml	nevritis, confusion, hypoglycemia

Scheme No. 2
Suramine monotherapy (intravenous injections) [109,110]

Timing (days)	Dose (mg/kg)	Amount injected (100 mg/ml)	Secondary effects
1	5	2.5 ml	*Hypersensitivity:*
3	10	5 ml	fever, joint pains, sole pains,
5	20	10 ml	skin eruption, desquamation
11	20	10 ml	*Renal*
17	20	10 ml	kidney tubules deposit;
23	20	10 ml	if increasing proteinuria
30	20	10 ml	stop treatment

Scheme No. 3
(as used in Côte d'Ivoire for *T. gambiense* infections): Melarsoprol with pentamidine pretreatment [109,110]

Timing (day)	Drug	Dose (mg/kg)	Amount injected	Secondary effects
1	pentamidine	4 mg/kg	5 ml*	see scheme No. 1
2	pentamidine	4 mg/kg	5 ml*	
4	melarsoprol	1.2 mg/kg	1.7 ml**	*Reactive encephalopathy*
5	melarsoprol	2.4 mg/kg	3.3 ml**	fever, headache, shivering,
6	melarsoprol	3.6 mg/kg	5 ml**	seizures, coma
17	melarsoprol	1.2 mg/kg	1.7 ml**	
18	melarsoprol	2.4 mg/kg	3.3 ml**	
19	melarsoprol	3.6 mg/kg	5 ml**	
20	melarsoprol	3.6 mg/kg	5 ml**	
30	melarsoprol	1.2 mg/kg	1.7 ml**	
31	melarsoprol	2.4 mg/kg	3.3 ml**	
32	melarsoprol	3.6 mg/kg	5 ml**	
33	melarsoprol	3.6 mg/kg	5 ml**	

*Intramuscular injection. **Intravenous injection.

Scheme No. 4
(as used in Kenya and Zambia for *T. rhodesiense* injections): Melarsoprol, increasing doses, with suramin pretreatment (intravenous route) [109,110]

Timing (days)	Drug	Dose (mg/kg)	Amount injected (36 mg/ml)	Secondary effects
1	suramine	5	2.5 ml	see scheme No. 2
3	suramine	10	5 ml	
5	suramine	20	10 ml	
7	melarsoprol	0.36	0.5 ml	*Reactive encephalopathy*
8	melarsoprol	0.72	1 ml	fever, headache, shivering,
9	melarsoprol	1.1	1.5 ml	seizures, coma
16	melarsoprol	1.4	2 ml	
17	melarsoprol	1.8	2.5 ml	
18	melarsoprol	1.8	2.5 ml	
25	melarsoprol	2.2	3 ml	
26	melarsoprol	2.9	4 ml	
27	melarsoprol	3.6	5 ml	
34	melarsoprol	3.6	5 ml	
35	melarsoprol	3.6	5 ml	
36	melarsoprol	3.6	5 ml	

Scheme No. 5
Melarsoprol, steady doses (Neujean modified), with suramin pretreatment (intravenous injections) [57,110]

Timing (day)	Drug	Dose (mg/kg)	Amount injected (36 mg/ml)	Secondary effects
1	suramine	5	2.5 ml	see scheme No. 2
3	suramine	10	5 ml	
5	suramine	20	10 ml	
	for CSF < 20 leucocytes/μl	continue	up to day 10	
7	melarsoprol	3.6 mg/kg	5 ml	see scheme No. 3
8	melarsoprol	3.6 mg/kg	5 ml	
9	melarsoprol	3.6 mg/kg	5 ml	
10	melarsoprol	3.6 mg/kg	5 ml	
	if CSF > 20 < 100 leucocytes/μl	continue	up to day 24	
21	melarsoprol	3.6 mg/kg	5 ml	
22	melarsoprol	3.6 mg/kg	5 ml	
23	melarsoprol	3.6 mg/kg	5 ml	
24	melarsoprol	3.6 mg/kg	5 ml	
	if CSF > 100 leucocytes/μl	continue	up to day 38	
35	melarsoprol	3.6 mg/kg	5 ml	
36	melarsoprol	3.6 mg/kg	5 ml	
37	melarsoprol	3.6 mg/kg	5 ml	
38	melarsoprol	3.6 mg/kg	5 ml	

Scheme No. 6
Eflornithine monotherapy (intravenous injections) [87]

Timing (days)	Times per day	Dose (mg/kg)	Amount given	Secondary effects
1 to 14	4	100	250 ml i.v. drip	*Blood*: anaemia; leucopaenia; thrombocytopaenia *Digestive tract*: diarrheoa; vomiting; abdominal pain; anorexia *Nervous system*: convulsions; fever; dizziness

Scheme No. 7
Nifurtimox monotherapy (oral administration) [87,88]

Timing (days)	Times per day	Dose (mg/kg)	Amount given	Secondary effects
1 to 14	3	5	tablets	*Nervous system*: dizziness, convulsions, psychotic reactions, headache, peripheral polyneuropathy; arthralgesia
Children 1 to 21	3	6.5	tablets	*Digestive tract*: intestinal discomfort *Other*: skin rashes

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