Review

Human African trypanosomiasis: current situation of a still neglected disease

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ABSTRACT

Trypanosomes inoculated by tsetse flies provoke human African trypanosomiasis (HAT), also called sleeping sickness. HAT exists in two forms: a chronic one caused by *Trypanosoma brucei gambiense* (g-HAT), in western/central Africa, and an acute form caused by *T. b. rhodesiense* (r-HAT), in eastern/southern Africa. HAT develops throughout two stages: (1) the haemolymphatic stage 1 comprising of irregular fever, chronic fatigue and lymphadenopathy; (2) the meningoencephalitic stage 2 with trypanosomes entering in brain comprises neurological signs, neuropsychiatric and sleep disorders. At the end of stage 2, a comatose state takes place prior to death. HAT diagnosis is based on serology and trypanosome search. A staging based on cerebrospinal fluid analysis may be performed. A dysregulation of cytokine networks and production is a HAT characteristic. Regarding nitric oxide (NO), trypanosomes deploy two main processes. In the periphery (1), they influence the competition between arginase/NO synthase (NOS) for their common substrate L-arginine; by inducing arginase, they favor polyamine production which is essential for their growth. They also decrease the trypanocidal NO production. In brain (2), an increase in the NO content has been evidenced together with the inducible NOS (iNOS) expression in glial cells. The enhanced brain NO production may protect against trypanosomes but also lead to the production of deleterious peroxynitrites. Four drugs administered parenterally have been employed: pentamidine and suramin for stage 1, and difluoromethylornithine (eflornithine) and melarsoprol for stage 2. All these drugs are endowed of toxicity. Moreover, to treat stage 2 g-HAT, nifurtimox can also be administered with eflornithine. Since 2018, however, fexinidazole, active orally in stage 1 and the early stage 2 of g-HAT, has greatly facilitated patient care. Preliminary results obtained with a single oral dose of acoziborole active on both stages are promising. Finally, for r-HAT stage 2, the toxic melarsoprol remains the only rescue.

1. Introduction

The sleeping sickness, also named human African trypanosomiasis (HAT), is caused by trypanosome parasites that are transmitted by the bite of infected tsetse flies. Uniquely found in sub-Saharan Africa, this disease is provoked by two subspecies of *Trypanosoma brucei* (*T. b.*), i.e., *T. b. gambiense* (g-HAT) in West and Central Africa, and *T. b. rhodesiense* (r-HAT) in East Africa. This life-threatening disease particularly affects poor rural populations, causing significant damages. It also

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has to be considered for the travellers who cross the endemic areas, since the HAT transmission requires the interaction between human and the tsetse flies infected by the parasite's reservoirs (human or animals) [1-4]. Here, we must thank the sustained efforts granted over the past 15 years since, according to the WHO, the number of reported cases has fallen to a very low level, i.e., fewer than 1,000 cases for 2018. Despite these successes, the disease is still endemic in sub-Saharan countries, notably in central Africa. The present favorable situation should not involve, however, a drop out of the controls since the existence of parasite reservoirs may lead to a rapid re-emergence of the disease [5]. In the lack of a vaccine possibility, the disease control still depends on vector control, patient detection and conventional treatments [2].

In the present report, we describe the current situation for HAT, the mechanisms deployed by trypanosomes for entering the organism together with those of the host defences against the parasite invasion and, finally, the efficient drugs that are now available for treating the disease.

2. Clinical forms

At the beginning of the 20th century, trypanosomes were discovered in blood and cerebrospinal fluid (CSF) of HAT patients. Shortly after, the vector role played by tsetse flies was highlighted. In humans, after the tsetse bite, the disease progresses through two stages, a haemolymphatic stage 1 followed by a meningoencephalitic stage 2. This later stage appears when trypanosomes cross the blood-brain barrier (BBB) and invade the central nervous system (CNS) [6]. The g-HAT form accounts for 97% of the reported cases and is a chronic infection, lasting sometimes for years without major clinical signs. The r-HAT form, however, represents less than 3% of reported cases and causes an acute infection [1, 2, 4].

2.1. Haemolymphatic stage 1

At the site of the tsetse bite, a 3-4 cm dermal reaction called chancre appears within 2-3 days. This chancre is formed by a local hot hardened erythema where trypanosomes multiply. It disappears in about two weeks without any remaining trace. This reaction, rarely seen in g-HAT, is more often observed in r-HAT. The stage 1 of the disease begins one to three weeks after the bite; however, it is sometimes asymptomatic for months or years in g-HAT. Fever, lymphadenopathy, splenomegaly, hepatomegaly, fleeting skin redness, pruritus, joint pains, headaches and chronic fatigue are broadly present signs. Fever, which is severe at the onset, persists throughout the time course of the disease as an interspersed fever exhibiting some thermal peaks. Swollen, firm, elastic, mobile and painless, lymph nodes sit in any place of the body, but, preferably along the posterior cervical lymph node chain (Winterbottom sign). Oedema, which is often in suborbital position, may occur. Tachycardia, but also pericarditis or myocarditis may take place [7]. In this respect, lengthening of the QT interval (i.e. lengthening of the time between the Q point and the top of the T wave on the electrocardiogram) carries a risk of arrhythmia that can be fatal. These heart disorders are mostly seen in r-HAT [8]. Neurological disturbances, including sleep disorder, are typical of the second-stage disease; however, most signs and symptoms are common to both stages.

2.2. Meningoencephalitic stage 2

The stage 2 begins weeks to months later. It is determined by the presence of trypanosomes and/or an elevation of white blood cells (WBC) in the CSF (more than 5 WBC.μl^{-1}) [9]. It appears slowly and insidiously in g-HAT with a low-intensity of the neurological disorders (Fig. 1) [10]. In r-HAT, however, the evolution towards this stage can be faster, without any real temporal demarcation with stage 1. The behavior of patients often tends to change, altering family and professional relationships.

The endocrine abnormalities are characterized by continuous reluctance, a disturbance of sexual functions (impotence or amenorrhea) and a disruption of hunger and thirst feelings (bulimia or anorexia, polydipsia or adipsia) [1]. The neurological symptoms include headache, attention deficit and difficulty for gesture coordination, sensory disorders, tremors and ataxia. Psychiatric manifestations of varying intensity are common, ranging from simple changes in mood to psychotic conditions that may lead to criminal acts [11]. A variety of muscle disorders including tremors and disturbances

disruptions in the 24 h plasma hormonal profiles (cortisol, prolactin, growth hormone) [14].

By using T2-weighted magnetic resonance imaging (MRI), hyper-intensities in cortical and periventricular areas, basal ganglia and cerebellar cortex have also been evidenced. In these areas, micro-hemorrhages can also be present [15]. Through a positron emission tomography (PET) scan, sparse areas exhibiting an increase in the metabolic activity have been also evidenced in brain parenchyma [16]. Moreover, during stage 2, infiltration of mononuclear cells takes place into the CNS together with Mott cells in leptomeninges and perivascular spaces. Lastly, a strong astrocytosis also comprising proliferative microglial cells, has been also observed [17].

3. Diagnosis

Diagnosis of HAT is a multistep procedure, comprising mass screening, clinical suspicion, screening test followed by parasitological confirmation and, finally, stage determination [18]. In mass screening, palpation at the neck level checks the existence of lymphadenopathy. Blood is also punctured at the pulp of a finger for serological and parasitological analysis.

3.1. Serological screening

Only g-HAT detection, due to a relative antigenic stability of *T. b. gambiense* in endemic areas, can benefit from the card agglutination trypanosomiasis test (CATT) [19]. Presently, immunochromatographic-based rapid diagnostic tests (RDTs), exhibiting a correct sensitivity and specificity, are available in the fields [20]. Their use, without power supply, is easier and safer since it limits handling blood samples. However, the existence of both false positive and false negative tests, together with the necessity to avoid an unnecessary difficult and toxic therapy, requires a parasitological confirmation. The highly specific and sensitive serum trypanolysis test is a reference technique employed in few laboratories only [21].

3.2 Parasitological confirmation

The existence of lymphadenopathy allows the collection of lymph node juice and parasite search under the microscope.

Fig. 1. A young patient (D.A.), from Republic of Congo, Ngabé, 2017, suffering from g-HAT at the meningoencephalitic stage 2. The patient is sleepy all day long and remains indifferent to the environment.

of speech, gait, and reflexes can be present. Pain, profound hyperpathy, paralysis, and seizures may also occur [1]. Intellectual functions are far conserved and dementia appears only in the terminal phase of the disease. Deep somnolence appears late and is progressively more and more difficult to overcome. Cachexia and malnutrition are on the rise. Alteration of the general condition gradually brings the patient to a prostrate or comatose state before death [11].

Regarding sleep disorders, primarily based on the disease designation as a "sleeping sickness", it must be specified that the patients exhibit a dysregulation of the sleep circadian rhythmicity rather than a hypersomnia [12, 13]. This dysregulation is proportional to the degree of severity of the clinical and biological symptoms. Sleep onset in rapid eye movement (SOREM) also occurs. These SOREM events are more frequent in severely sick patients, who also show major

In g-HAT, the trypanosome load in blood is often scarce and the trypanosome search by blood centrifugation in capillary tubes (procedure that concentrates trypanosomes) has a limited sensitivity [22]. Presently, the minicolumn method or miniature anion exchange/centrifugation technique (mAECT) is more sensitive and represents the reference technique for blood trypanosomes search [23, 24].

In r-HAT, trypanosomes are often in large number in blood and the thick blood smear stained with May-Grünwald-Giemsa can lead to a satisfactory diagnosis.

Here, it is to be remarked that in specialized laboratories, molecular biology techniques, polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), for a similar sensitivity *vs* minicolumns, require more sophisticated equipment and expertise [25]. To date, the reverse transcriptase-polymerase chain reaction (RT-PCR) provides a good sensitivity and appears as a more promising technique [26, 27].

3.3. Stage diagnosis

If trypanosomes are present in blood and/or lymph node juice, the subject should be classified as stage 1. For stage 2 definition, a lumbar puncture is necessary. By definition, stage 2 corresponds to at least the presence of trypanosomes and/or more than 5 WBC.μl^{-1} in the CSF [2]. Further, a dramatic increase in IgM in CSF, as in blood, is a very sensitive indicator of the meningoencephalitic phase and reflects the major modifications taking place in the immune system. In practice, the IgM assay is rarely achieved in the field.

4. HAT immunopathology

4.1. Immune response in HAT

This aspect is important, but to avoid too long a development, we report here the main features of this field. In the extracellular bloodstream, African trypanosomes are exposed to immune cells and humoral factors. The innate and adaptive immune systems play crucial roles against trypanosome infection. Trypanosomes, however, can escape and invade host tissues, since a variant surface glycoprotein (VSG), the predominant surface antigen, covers their membrane. The ever-changing ability

of this VSG is an elegant and powerful means to escape the adaptive immune response [28]. Although VSGs are highly antigenic and induce the production of specific IgG antibodies, antigenic variation renders the host's response ineffective in controlling the parasite invasion [29].

Macrophages contribute to the innate immunity in modulating the adaptive response of various immune molecules, i.e., pro-inflammatory cytokines and nitric oxide (NO). They also contribute to the clearing of the trypanosome load by phagocytosis [29]. An early switch from classically activated macrophages to an alternatively activated phenotype also avoids excessive amounts of proinflammatory cytokines and further tissue damages [30]. Biological changes and tissue damages are associated with the harmful over-release of NO and tumor necrosis factor (TNF)-α by activated macrophages [31, 32].

The trypanosome antigen initiates B cell activation. Such cells proliferate intensively in secondary lymphoid organs leading to the production of various antibodies belonging mostly to the IgM class. Some other antibodies like IgG, specific or not for trypanosomes, may act as autoantibodies (anti-tryptophan, anti-galactocerebrosides, anti-neurofilament, etc.) [33, 34]. To date, their specific role is not fully understood [29].

The primary response to infection thus appears to result in a "pro-inflammatory mediators" production including TNF-α, interleukin (IL)-1, IL-6, and NO. These molecules are trypanostatic or trypanocidal, but, their uncontrolled production may also exert deleterious effects. Other mediators like IL-10 and interferon (IFN)-γ may act as "Janus" mediators bringing inflammatory or an anti-inflammatory potential capable of modulating the excessive parasite growth [29].

4.2. Peripheral and brain impairments in HAT

Under this subheading we considered, with a special focus on NO, the impairments produced either in the periphery or in brain, by inoculation, to animal models, of the protozoan parasites belonging to the genus *Trypanosoma*. Mainly by use of rats or mice infected with *T. b. brucei*, it is established that the mechanisms involved in the periphery or in brain are different and tightly linked with the L-arginine/NO metabolism [35-37].

Before detailing these mechanisms and to facilitate their understanding, we first recall the basic knowledge available for NO.

4.2.1. Basic knowledge of nitric oxide

The discovery of the endothelium-derived relaxing factor initiated studies that ultimately led to the discovery of the biological paracrine messenger identified as NO [38, 39]. This messenger, highly diffusible and strongly reactive, is synthesized from L-arginine through a two-step oxidative reaction catalyzed with four distinct NO-synthases (NOSs) that are either constitutive (neuronal, nNOS; endothelial, eNOS; mitochondrial, mNOS) or inducible (iNOS) in nature [40]. All NOS isoforms are homodimeric proteins in their active form with an N-terminal sequence specific to the isoform [40]. Calcium is required for nNOS and eNOS activities while its presence is not essential for that of iNOS since calmodulin bind sufficiently tightly to it. In aerobic conditions, NOSs catalyze the oxidation of L-arginine, the end products being NO and L-citrulline [40]. Nitric oxide is now accepted as a potent messenger insuring regulatory processes in the immune, vascular and nervous systems with pleiotropic functions related to its cellular rate of production. Three main processes regulate NO/NOSs output, i.e., the L-arginine/arginase substrate-competing system, the L-citrulline/arginosuccinate-recycling system and the asymmetric dimethyl-/monomethyl-L-arginine-inhibiting system (Fig. 2) [40].
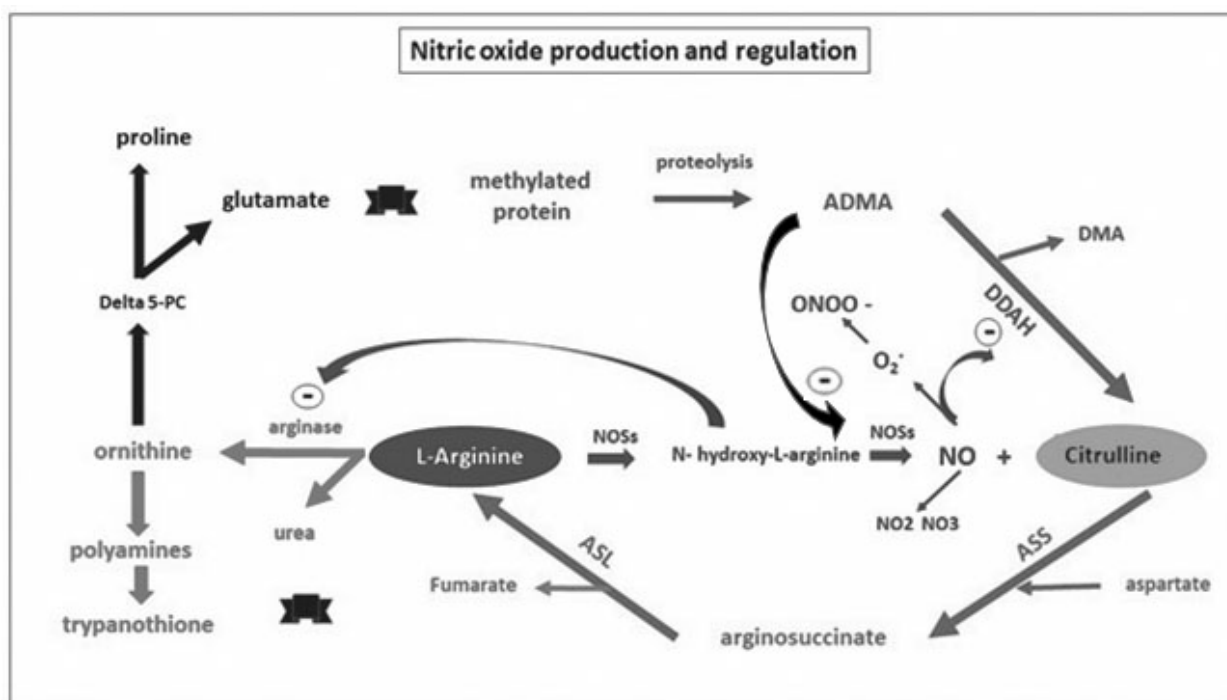

Fig. 2. Nitric oxide (NO) production and regulation. From L-arginine, the L-arginine/NOS pathway ensures equimolar production of NO and L-citrulline. In pathological conditions, an over production of NO by way of the inducible NO-synthase (iNOS) expression, conduces to the production of deleterious species (peroxinitrites, ONOO⁻). Still with L-arginine as a precursor, the L-arginine/arginase pathway contributes to production of L-ornithine, trypanothione and urea. ADMA, produced *via* proteolysis, also contributes to L-citrulline production through the action of the ADMA/DDAH pathway. *Via* the successive activities of ASS and ASL, the L-citrulline/L-arginine cycle contributes to production of L-arginine besides that obtained by uptake from blood. Finally, ADMA actively inhibits NOSs whereas NO inhibits DDAH. Abbreviations: ADMA: asymmetric dimethyl arginine; ASL: arginosuccinate lyase; ASS: arginosuccinate synthetase; DDAH: dimethylarginine dimethylaminohydrolase; DMA: dimethylamine; NOSs: nitric oxide synthases; d5-PC: d5-pyrroline carboxylate.

4.2.2. Impairments induced in the periphery

In several parasitic diseases, NO, synthesized in large quantity by the iNOS in activated macrophages, exerts a parasiticidal activity either directly or indirectly after cross-reactivity with oxygen species [41]. In the experimental animal model employing rodents infected with *T. b. brucei*, NO production has been determined to be decreased in the periphery (macrophages and blood) [36]. This early peripheral decrease, solely linked with the iNOS enzyme [37], is enabled by the secretome of trypanosomes to reduce the trypanocidal pressure exerted by NO. Such a secretome contains, indeed, extremely active peptidases capable of inducing a rapid accumulation of asymmetric dimethyl arginine, a potent iNOS inhibitor [36, 42]. Moreover, in the cascade of events triggered by the *T. b. brucei* secretome, an orphan kinesin has been reported to shift the host arginine/NO metabolism in macrophages from trypanocidal production of NO to that of arginase-dependent L-ornithine and derivatives [43]. These derivatives are essential for trypanosome growth through the trypanothione synthesis [35, 44]. At this step, the peripheral immune system, deployed by the host to face the parasite (macrophages, B and T lymphocytes, NK cells, cytokines and NO) [29], remains inefficient to counteract brain penetration by trypanosomes.

4.2.3. Impairments induced in the brain

In the central compartment, the situation does not appear to be favourable for trypanosomes multiplication and spread since an increase in the trypanocidal NO production has been reported in animal models [45]. This increase is mainly due to the expression of dimethyl arginine dimethylaminohydrolase-2 and to its ability to reduce the level of the asymmetric dimethyl arginine [37]. Moreover, still in brain, the arginase activity directly involved in the production of polyamines (compounds necessary for trypanosome growth) remains unchanged throughout the infectious process (Fig. 2) [37]. It appears therefore that, in brain, the mechanisms deployed to counteract trypanosome invasion are more efficient than those observed in the periphery. It is likely that the parasites may pass through the blood-brain barrier (BBB) or between the endothelial cells and the vessel basement membranes [37]. Here, it

must be further mentioned that the highly significant rise in the NO production resulting from the enhanced iNOS activity of astrocytes and microglial cells [31, 36] can also be deleterious. The overproduction of NO can lead to reactivity with the superoxide anions to form peroxynitrites ($ONOO^-$) that are capable of damaging proteins, lipids and deoxyribonucleic acid [46]. In consequence, the inflammatory form and response deployed by microglial cells and astrocytes [47] to protect the infected brain may also create conditions for neuronal vulnerability [48, 49]. Despite the possibility that neuronal iNOS contributes to the deleterious oxidative stress, its implication remains to be further investigated. It may be involved in neuronal differentiation [47, 50], tissue reparation [51] or the maintenance of basic functions such as the sleep-wake alternation in the aging process [52].

5. Treatment of HAT

5.1. Available drugs

Separation into stage 1 and stage 2, by examination of the CSF, was for a long time the basis for the HAT therapeutic approach. In such a situation, the therapeutic choice relied then on five drugs, all endowed with greater or lesser toxicity. Four drugs were administered parenterally, i.e., pentamidine, suramin, difluoromethylornithine (DFMO, also called eflornithine) and melarsoprol. By oral way, nifurtimox is still given with DFMO. All drugs involve long treatment regimens [53, 54].

In 2018, the marketing authorization for fexinidazole (active orally) led to profound changes in the therapeutic approach [55-58]. Indeed, fexinidazole must be administered orally during a food intake since its activity depends on specific nitroreductases that are promoted when ingested with a meal [59]. To date, fexinidazole is the recommended first-line treatment in g-HAT for 10 days [54]. Moreover, a promising new drug i.e., acoziborole or SCYX-7158, is currently undergoing a clinical development [60]. This drug is orally active and capable of curing, with a single-dose treatment, both stages of g-HAT. Generally, all drugs against HAT are donated to the WHO, which is responsible for their distribution (see Table 1).

Table 1. Treatment regimens for drugs against HAT.

Drugs	Disease	Dosage	Side events
Pentamidine isethionate	g-HAT stage 1	4 mg/kg per day IM (if IV: dilution in 0.9% NaCl, infusion for 2 hours) for 7 consecutive days	Painful IM injection; lying rest for 30 minutes to 1 hour after each injection to avoid syncope; nausea and vomiting; frequent but reversible renal toxicity
Suramin	r-HAT stage 1	One test dose IV of 4 or 5 mg/kg, then 20 mg/kg IV per week for 5 consecutive weeks (maximum dose per injection: 1 g)	Renal toxicity (proteinuria assay after administration of suramin)
Fexinidazole (*)	g-HAT stage 1 and stage 2	PO, once daily for 10 days, with a loading dose for the first 4 days and a maintenance dose for the last 6 days (#)	Headache; vomiting; nausea; insomnia; decreased appetite; asthenia; tremors and dizziness. Fexinidazole should be used with caution in patients with psychiatric disorders (neuropsychiatric adverse reactions are more frequent with fexinidazole than with NECT)
NECT	g-HAT stage 2	200 mg/kg of eflornithine in IV infusion for 2 hours every 12 hours for 7 consecutive days, and 5 mg/kg of nifurtimox PO every 8 hours for 10 consecutive days	Seizures; nausea and vomiting. Better tolerated in children than fexinidazole. Side events are less intense when eflornithine or nifurtimox are taken alone
NECT-long (rescue)	g-HAT stage 2	200 mg/kg of eflornithine in IV infusion for 2 hours every 12 hours for 14 consecutive days, and 5 mg/kg of nifurtimox PO every 8 hours for 10 consecutive days	Seizures; nausea and vomiting
Eflornithine (rescue)	g-HAT stage 2	100 mg/kg slow IV infusion every 6 hours for 14 consecutive days	Anemia, diarrhea, seizures, vomiting, abdominal pain, headache, alopecia (most seizure episodes occur within the first week of treatment)
Nifurtimox	g-HAT stage 2	Only used in NECT and NECT-long combinations	Convulsions, weight loss, vomiting and diarrhea. Reversible cerebellar syndrome (ataxia, nystagmus, tremors and vertigo) may occur at a high dose of 30 mg/kg daily for 30 consecutive days.
Melarsoprol	r-HAT stage 2	2.2 mg/kg per day IV for 10 consecutive days	Painful IV injection; risk of necrosis at the injection site (toxicity of propylene glycol used as a solvent for melarsene oxide); peripheral polyneuropathy; headaches; fever; nausea; myalgia; rashes; cardiac, renal and hepatic toxicity; agranulocytosis; especially unpredictable risk of reactive arsenical encephalopathy (5 to 18% including 10 to 70% fatal)

Abbreviations and legend: kg, kilogram; mg, milligram; IM, intramuscular route; IV, intravenous route; PO, per os; NECT, Nifurtimox Eflornithine Combination Therapy. (*). Fexinidazole is only recommended when the post-treatment follow-up is properly insured. (#), all tablets contain 600 mg of fexinidazole. The tablets should be taken with food, preferably at the same time each day. Fexinidazole should be taken once a day for 10 consecutive days; the dosage for the first 4 days is reduced for the next 6 days. The dosage in patients aged ≥6 years and weighing ≥20 kg comprises two categories separated by a threshold set at 35 kg: range from 20 to 34 kg, 1800 mg per day for 4 days, then after 1200 mg per day for 6 days. From 35 kg, 1200 mg per day for 4 days, then after 600 mg per day for 6 days.

5.2. Therapeutics schemes

Recommended treatments are detailed in Tables 1 and 2. Drugs that do not cross the BBB in sufficient quantities are specific for stage 1: pentamidine for g-HAT, and suramin for r-HAT. Melarsoprol, eflornithine and nifurtimox are reserved for stage 2. These last drugs are not employed for patients in stage 1 since they are more toxic and more complex to administer. Eflornithine and nifurtimox are now used only in a combination called nifurtimox eflornithine combination therapy (NECT) for g-HAT [54, 55, 61]. Melarsoprol is no longer employed except for stage 2 of r-HAT, for which it remains the only rescue [62].

Fexinidazole is active at the same dosage in both stage 1 and early stage 2 of g-HAT because it crosses the BBB. Fexinidazole has two major advantages versus other therapies: it is orally active and, often, lumbar puncture seems to be avoidable. These two advantages allow the recommendation of this treatment under an adequate clinical expertise [54, 55, 63]. This treatment, however, does not show advantages only: (1) it requires a rigorous clinical examination, which is difficult to achieve in poorly equipped health centers; (2) it deprives of the initial CSF cell count benefice, which is necessary to evaluate a relapse; (3) it necessitates a real possibility of a post-treatment follow-up [5, 64]. Clinical trials to judge the efficacy of fexinidazole on r-HAT are currently underway [65].

5.3. Resistance to treatment

The resistance mechanisms of *T. b. gambiense* to the five above-mentioned molecules have been studied and the probability of the emergence of resistance to the treatments, except for melarsoprol, remains low [66-69]. The probability for the emergence of resistance will have to be evaluated for fexinidazole throughout the extension of its use.

5.4. Special cases: children and pregnant women

Oral fexinidazole is a safe and effective first-line treatment option in pediatric patients [70]. However, fexinidazole is not recommended for children under 6 years of age or weighing less than 20 kg. The use of pentamidine isethionate for stage 1 and NECT for stage 2 can be continued. Besides, literature data are very scarce regarding the use of these drugs in pregnant women. They are theoretically

contraindicated. The action to be taken must be studied case-by-case, specifically in agreement with the clinical conditions and the term of the women pregnancies. It is also necessary to avoid a possible transmission of the trypanosomes to the foetus. These considerations are irrelevant in the case of r-HAT given the rapidity of development and the severity of the infection: immediate treatment during diagnosis is essential [62].

5.5. Cure and follow-up

In the majority of the cases, after treatment, the patient's condition improves. A good response to the treatment results in the disappearance of the parasites in blood and CSF together with the disappearance of the clinical signs. However, treated patients must benefit from a long post-therapeutic follow-up of 24 months including clinical and biological examinations (lumbar puncture at 6-month intervals). The real effectiveness of this post-therapeutic scheme is poorly known given the difficulty of carrying it out in the field. The sequelae are rare in patients treated at stage 1, but they are frequent, particularly neurological, at stage 2 and are mainly of the psychomotor type in children [71]. The potential animal reservoir for g-HAT, and the reservoir represented by parasites hosted in the skin of individuals remains problematic and as potential sources of unexplained relapses. Moreover, no data are now available on the efficacy of the different treatments on the forms of *T. b. gambiense* in the dermis [72, 73].

Conclusion

The WHO aims to eliminate HAT as a public health problem by 2030 since the number of reported cases has fallen under 1,000 cases in 2018. However, despite this success, the disease is still endemic in parts of sub-Saharan Africa, notably in central Africa. The present favorable situation should not lead to a drop out of the controls since the existence of parasite reservoirs may drive a rapid re-emergence of the disease. Moreover, it must be remembered that there are asymptomatic carriers capable of harboring the parasite for more than 20 years. The discontinuation of the stakeholder funding can jeopardize the laborious and costly search for isolated cases. Teams that no longer see HAT cases also lose their diagnostic

Table 2. Recommended treatment for stage 1 and stage 2 g-HAT and r-HAT.

Disease	Stage	Patients	Treatment	Relapse treatment	Recurrent relapse treatment
g-HAT	No clinical signs of disease severity (staging not needed, no lumbar puncture)	Adults Pregnant women after the first trimester Children ≥6 years and ≥20 kg	Fexinidazole	NECT	NECT-long Eflornithine Melarsoprol if no other possibility (rescue treatment of recurrent relapses)
g-HAT	Stage 1	All patients Pregnant women in the first trimester Recommended for children if <6 years or <20 kg	Pentamidine isethionate	Fexinidazole (not recommended for pregnant women in the first trimester and for children if <6 years or <20 kg) NECT	NECT-long Eflornithine Melarsoprol if no other possibility (rescue treatment of recurrent relapses)
g-HAT	Stage 2	All patients Pregnant women in the first trimester Recommended for children <6 years or <20 kg Instead of fexinidazole if presence of clinical signs of severity and CSF cell count ≥ 100 WBC/µl or not known.	NECT	NECT-long	Eflornithine Melarsoprol if no other possibility (rescue treatment of recurrent relapses)
r-HAT	Stage 1	All patients including pregnant women and children	Suramin	Melarsoprol	
r-HAT	Stage 2	All patients including pregnant women and children	Melarsoprol	Melarsoprol (no other rescue treatment)	

Abbreviations: CSF, cerebrospinal fluid; WBC, white blood cell; µl, microliter; kg, kilogram; NECT, nifurtimox eflornithine combination therapy.

skills. Without vaccine possibility, the disease control still depends on vector control, detection and conventional treatments.

CONFLICT OF INTEREST STATEMENT

None to declare.

REFERENCES

1. Buguet, A., Cespuglio, R. and Bouteille, B. 2015, Sleep Medicine. A comprehensive guide to its development, clinical milestones, and advances in treatment, S. Chokroverty and M. Billiard (Eds), Springer-Verlag, New York, USA, 159.
2. Büscher, P., Cecchi, G., Jamonneau, V. and Priotto, G. 2017, Lancet, 390, 2397.
3. Kennedy, G. E. and Rodgers, J. 2019, Front. Immunol., 10, 39.
4. Bottieau, E. and Clerinx, J. 2019, Infect. Dis. Clin. North. Am., 33, 61.
5. Franco, J. R., Cecchi, G., Paone, M., Diarra, A., Grout, L., Kadima Ebeja, A., Simarro, P. P., Diarra, A., Sankara, D., Zhao, W. and Dagne, D. A. 2022, PLoS Negl. Trop. Dis., 16, e0010047.
6. Steverding, D. 2008, Parasit. Vectors, 1, 3.
7. Blum, J. A., Zellweger, M. J., Burri, C. and Hatz, C. 2008, Lancet Infect. Dis., 8, 631.
8. Blum, J. A., Schmid, C., Burri, C., Hatz, C., Olson, C., Fungula, B. Kazumba, L., Mangoni, P., Mbo, F., Deo, K., Mpanya, A., Dala, A., Franco, J. R., Pohlig, G. and Zellweger, M. J. 2009, PLoS Negl. Trop. Dis., 3, e383.
9. Masocha, W., Robertson, B., Rottenberg, M. E., Mhlanga, J., Sorokin, L. and Kristensson, K. 2004, J. Clin. Invest., 114, 689.
10. Jamonneau, V., Ilboudo, H., Kaboré, J., Kaba, D., Koffi, M. and Solano, P. 2012, PLoS Negl. Trop. Dis., 6, e1691.
11. Cox, F. E. G. 2004, Infect. Dis. Clin. N. Am., 18, 231.
12. Buguet, A., Gati, R., Sèvre, J. P., Develoux, M., Bogui, P. and Lonsdorfer, J. 1989, Electroencephalogr. Clin. Neurophysiol., 72, 471.
13. Buguet, A., Bourdon, L., Bouteille, B., Cespuglio, R., Vincendeau, P., Radomski, M. W. and Dumas, M. 2001, Sleep Med. Rev., 5, 139.
14. Buguet, A. 1999, Chronobiol. Int., 16, 477.
15. Patel, N. K., Clegg, A., Brown, M. and Hyare, H. 2018, BJR Case Rep., 4, 20180039.
16. Luna, L. P., Meleki, Z., Shanechi, A. M., Yedavalli, V. and Civelek, A. C. 2022, Clin. Nucl. Med., 47, e26.
17. Poltera, A. A. 1985, Brit. Med. Bull., 41, 169.
18. Van Meirvenne, N. and Le Ray, D. 1985, Brit. Med. Bull., 41, 156.
19. Magnus, E., Vervoort, T. and Van Meirvenne, N. 1978, Ann. Soc. Belg. Med. Trop., 58, 169.
20. Jamonneau, V., Camara, O., Ilboudo, H., Peylhard, M., Koffi, M., Sakande, H. N'Dri, L, Sanou, D., Dama, E., Camara, M. and Lejon, V. 2015, PLoS Negl. Trop. Dis., 9, e0003480.
21. Dama, E., Camara, O., Kaba, D., Koffi, M., Camara, M., Compaoré, C., Ilboudo H., Courtin, F., Kaboré, J., N'Gouan, E. K., Büscher, P., Lejon, V., Bucheton, B. and Jamonneau, V. 2019, Parasite, 26, 68.
22. Woo, P. T. 1970, Acta Trop., 27, 384.
23. Büscher, P., Ngoyi, D. M., Kaboré, J., Lejon, V., Robays, J., Jamonneau, V., Bebronne, N., Van der Veken, W. and Biéler, S. 2009, PLoS Negl. Trop. Dis., 3, e471.
24. Lejon, V., Büscher, P., Nzoumbou-Boko, R., Bossard, G., Jamonneau, V., Bucheton, B., Truc, P., Lemesre, J. L., Solano, P. and Vincendeau, P. 2019, PLoS Negl. Trop. Dis., 13, e0007051.
25. Mumba Ngoyi, D., Ali Ekangu, R., Mumvemba Kodi, M. F., Pyana, P. P., Balharbi, F., Decq, M., Kande Betu, V., Van der Veken, W., Sese, C., Menten, J., Büscher, P. and Lejon, V. 2014, PLoS Negl. Trop. Dis., 8, e2954.
26. Ilboudo, H., Camara, O., Ravel, S., Bucheton, B., Lejon, V., Camara, M., Kaboré, J., Jamonneau, V. and Deborggraeve, S. 2015, J. Infect. Dis., 212, 1996.

27. Ngay Lukusa, I., Van Reet, N., Mumba Ngoyi, D., Miaka, E. M., Masumu, J., Patient Pyana, P., Mutombo, W., Ngolo, D., Kobo, V., Akwaso, F., Ilunga, M., Kaninda, L., Mutanda, S., Muamba, D. M., Valverde Mordt, O., Tarral, A., Rembry, S., Büscher, P. and Lejon, V. 2021, PLoS Negl. Trop. Dis., 15, e0009739.

28. Dubois, M. E., Demick, K. P. and Mansfield, J. M. 2005, Infect. Immun., 73, 2690.

29. Vincendeau, P. and Bouteille, B. 2005, An. Acad. Bras. Cienc., 78, 645.

30. De Baetselier, P., Namangala, B., Noel, W., Brys, L., Pays, E. and Beschin, A. 2001, Int. J. Parasitol., 31, 575.

31. Keita, M., Vincendeau, P., Buguet, A., Cespuglio, R., Vallat, J. M., Dumas, M. and Bouteille, B. 2000, Exp. Parasitol., 95, 19.

32. Daulouède, S., Bouteille, B., Moynet, D., De Baetselier, P., Courtois, P., Lemesre, J. L., Buguet, A., Cespuglio, R. and Vincendeau, P. 2001, J. Infect. Dis., 183, 988.

33. Semballa, S., Okomo-Assoumou, M. C., Holzmuller, P., Büscher, P., Magez, S., Lemesre, J. L., Daulouède, S., Courtois, P., Geffard, M. and Vincendeau, P. 2007, Exp. Parasitol., 115, 173.

34. Courtioux, B., Bisser, S., M'belesso, P., Ngoungou, E., Girard, M., Nangouma, A., Josenando, T., Jauberteau-Marchan, M.O. and Bouteille, B. 2005, J. Clin. Microbiol., 43, 4789.

35. Gobert, A. P., Daulouède, S., Lepoivre, M., Boucher, J. L., Bouteille, B., Buguet, A., Cespuglio, R., Veyret, B. and Vincendeau, P. 2000, Infect. Immun., 68, 4653.

36. Amrouni, D., Gautier-Sauvigné, S., Meiller, A., Vincendeau, P., Bouteille, B., Buguet, A. and Cespuglio, R. 2010, PLoS ONE, 5, e9211.

37. Amrouni, A., Meiller, A., Gautier-Sauvigné, S., Piraud, M., Bouteille, B., Vincendeau, P., Buguet, A. and Cespuglio, R. 2011, PLoS ONE, 6, e16891.

38. Hibbs, J. B. Jr., Taintor, R. R. and Vavrin, Z. 1987, Science, 235, 473.

39. Ignarro, L., Byrns, R. and Wood, K. S. 1988, Vasodilatation, Vascular Smooth Muscle, Peptide, Autonomic Nerves and Endothelium, P. M. Vanhoutte (Ed.), Raven Press, New York, USA., 427.

40. Gautier-Sauvigné, S., Colas, D., Parmantier, P., Clément, P., Gharib, A., Sarda, N. and Cespuglio, R. 2005, Sleep Med. Rev., 9, 101.

41. Mossalayi, M. D., Arock, M., Mazier, D., Vincendeau, P. and Vouldoukis, I. 1999, Parasitol. Today, 15, 342.

42. Böger, R. H. 2004, J. Nutr., 134, 2842S.

43. Vincendeau, P., Gobert, A. P., Daulouède, S., Moynet, D. and Mossalayi, M. D. 2003, Trends Parasitol., 19, 9.

44. De Muylder, G., Daulouède, S., Lecordier, L., Uzureau, P., Morias, Y., Van Den Abbeele, J., Caljon, G., Hérin, M., Holzmuller, P., Semballa, S., Courtois, P., Vanhamme, L., Stijlemans, B., De Baetselier, P., Barrett, M. P., Barlow, J. L., McKenzie, A. N., Barron, L., Wynn, T. A., Beschin, A., Vincendeau, P. and Pays, E. 2013, PLoS Pathog., 9, e1003731.

45. Cespuglio, R., Amrouni, D., Raymond, E. F., Bouteille, B. and Buguet, A. 2019, PLoS ONE, 14, e0215070.

46. Pacher, P., Beckman, J. S. and Liaudet, L. 2007, Physiol. Rev., 87, 315.

47. Heneka, M. T. and Feinstein, D. L. 2001, J. Neuroimmunol., 114, 8.

48. Murphy, S. 2000, Glia, 29, 1.

49. Wyss-Coray, T. and Mucke, L. 2002, Neuron., 35, 419.

50. Heneka, M. T., Klockgether, T. and Feinstein, D. L. 2000, J. Neurosci., 20, 6862.

51. Luo, C. X., Zhu, X. J., Zhou, Q. G., Wang, B., Wang, W., Cai, H. H., Sun, Y. J., Hu, M., Jiang, J., Hua, Y., Han, X. and Zhu, D. Y. 2007, J. Neurochem., 103, 1872.

52. Cespuglio, R., Amrouni, D., Meiller, A., Buguet, A. and Gautier-Sauvigné, S. 2012, Sleep Med. Rev., 16, 265.

53. Bouteille, B., Oukem, O., Bisser, S. and Dumas, M. 2003, Fundam. Clin. Pharmacol., 17, 171.

54. World Health Organisation. 2019, WHO interim guidelines for the treatment of gambiense human African trypanosomiasis, Geneva.

55. Lindner, A. K., Lejon, V., Chappuis, F., Seixas, J., Kazumba, L., Barrett, M. P., Mwamba, E., Erphas, O., Akl, E. A., Villanueva, G., Bergman, H., Simarro, P., Kadima Ebeja, A., Priotto, G. and Franco, J. R. 2020, Lancet Infect. Dis., 20, e38.

56. Pelfrene, E., Harvey Allchurch, M., Ntamabyaliro, N., Nambasa, V., Ventura, F. V., Nagercoil, N. and Cavaleri, M. 2019, PLoS Negl. Trop. Dis., 13, e0007381.

57. Kande Betu Ku Mesu, V., Mutombo Kalonji, W., Bardonneau, C., Valverde Mordt, O., Ngolo Tete, D., Blesson, S., Simon, F., Delhomme, S., Bernhard, S., Mahenzi Mbembo, H., Mpia Moke, C., Lumeya Vuvu, S., Mudji E'kitiak, J., Akwaso Masa, F., Mukendi Ilunga, M., Mpoyi Muamba Nzambi, D., Mayala Malu, T., Kapongo Tshilumbwa, S., Botalema Bolengi, F., Nkieri Matsho, M., Lumbala, C., Scherrer, B., Strub-Wourgaft, N. and Tarral, A. 2021, Lancet Glob. Health, 9, e999.

58. Valverde Mordt, O., Tarral, A. and Strub-Wourgaft, N. 2022, Am. J. Trop. Med. Hyg., 106(5 suppl.), 61.

59. Wilkinson, S. R., Taylor, M. C., Horn, D., Kelly, J. M. and Cheeseman, I. 2008, Proc. Natl. Acad. Sci. USA, 105, 5022.

60. Betu Kumeso, V. K., Kalonji, W. M., Rembry, S., Valverde Mordt, O., Ngolo Tete, D., Prêtre, A., Delhomme, S., Ilunga Wa Kyhi, M., Camara, M., Catusse, J., Schneitter, S., Nusbaumer, M., Mwamba Miaka, E., Mahenzi Mbembo, H., Makaya Mayawula, J., Layba Camara, M., Akwaso Massa, F., Kaninda Badibabi, L., Kasongo Bonama, A., Kavunga Lukula, P., Mutanda Kalonji, S., Mariero Philemon, P., Mokilifi Nganyonyi, R., Embana Mankiara, H., Asuka Akongo Nguba, A., Kobo Muanza, V., Mulenge Nasandhel, E., Fifi Nzeza Bambuwu, A., Scherrer, B., Strub-Wourgaft, N. and Tarral, A. 2022, Lancet Infect. Dis., S1473-3099(22)00660-0 (correction in: 2023, Lancet Infect. Dis., 23, e47).

61. Priotto, G., Kasparian, S., Mutombo, W., Ngouama, D., Ghorashian, S., Arnold U., Ghabri, S., Baudin, E., Buard, V., Kazadi-Kyanza, S., Ilunga, M., Mutangala, W., Pohlig, G., Schmid, C., Karunakara, U., Torreele, E. and Kande, V. 2009, Lancet, 374, 56.

62. Kuepfer, I., Schmid, C., Allan, M., Edielu, A., Haary, E. M., Kakembo, A., Kibona, S., Blum, J. and Burri, C. 2012, PLoS Negl. Trop. Dis., 6, e1695.

63. Chappuis, F. 2018, Lancet, 391, 100.

64. Das, A. M., Chitnis, N., Burri, C., Paris, D. H., Patel, S., Spencer, S. E. F., Miaka, E. M. and Castaño, M. S. 2021, PLoS Negl. Trop. Dis., 15, e0009992.

65. Lee, S. J., Apio, R. J. and Palmer, J. J. 2020, Trop. Med. Infect. Dis., 5, 16.

66. Graf, F. E., Ludin, P., Wenzler, T., Kaiser, M., Brun, R., Pyana, P. P., Büscher, P., de Koning, H. P., Horn, D. and Mäser, P. 2013, PLoS Negl. Trop. Dis., 7, e2475.

67. Fairlamb, A. H. and Horn, D. 2018, Trends Parasitol., 34, 481.

68. de Koning, H. P. 2020, Trop. Med. Infect. Dis., 5, 14.

69. Kasozi, K. I., MacLeod, E. T. and Welburn, S. C. 2022, Pathogens, 11, 1100.

70. Kande Betu Kumesu, V., Mutombo Kalonji, W., Bardonneau, C., Valverde Mordt, O., Ngolo Tete, D., Blesson, S., Simon, F., Delhomme, S., Bernhard, S., Nganzobo Ngima, P., Mahenzi Mbembo, H., Fina Lubaki, J. P., Lumeya Vuvu, S., Kuziena Mindele, W., Ilunga Wa Kyhi, M., Mandula Mokenge, G., Kaninda Badibabi, L., Kasongo Bonama, A., Kavunga Lukula, P., Lumbala, C., Scherrer, B., Strub-Wourgaft, N. and Tarral, A. 2022, Lancet Glob. Health, 10, e1665-74.

71. Mudji, Jr., Blum, A., Grize, L., Wampfler, R., Ruf, M. T., Cnops, L., Nickel, B., Burri, C. and Blum, J. 2020, Trop. Med. Infect. Dis., 5, 10.

72. Capewell, P., Cren-Travaillé, C., Marchesi, F., Johnston, P., Clucas, C., Benson, R. A., Gorman, T. A., Calvo-Alvarez, E., Crouzols, A., Jouvion, G., Jamonneau, V., Weir, W., Stevenson, M. L., O'Neill, K., Cooper, A., Swar, N. K., Bucheton, B., Ngoyi, D. M., Garside, P., Rotureau B. and MacLeod, A. 2016, eLife, 5, e17716.

73. Camara, M., Soumah, A. M., Ilboudo, H., Travaillé, C., Clucas, C., Cooper, A., Kuispond Swar, N. R., Camara, O., Sadissou, I., Calvo Alvarez, E., Crouzols, A., Bart, J. M., Jamonneau, V., Camara, M., MacLeod, A., Bucheton, B. and Rotureau, B. 2021, Clin. Infect. Dis., 73, 12.