REVIEW

Sleeping sickness

– human African
trypanosomiasis

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INTRODUCTION

One of the great advantages of working with veterinary colleagues is the insight they often bring to the pathogenesis of human disease through their studies of natural animal diseases and animal models. In 1988 Professor Max Murray of the University of Glasgow Veterinary School introduced me to the problem of African trypanosomiasis in animals, its close relationship to its human counterpart that is known as sleeping sickness, and the unsolved problems of neurological involvement in that condition. We have now been investigating the neuropathogenesis of human African trypanosomiasis (HAT) in the laboratory and the African field for the last 16 years (Fig. 1). Neurologists will realize of course that HAT is not to be confused with the *other* 'sleeping sickness' known as encephalitis lethargica, famous for the pandemic during the first quarter of the 20th century, and which still occurs sporadically. While the cause of the latter disease has yet to be discovered (although many suspect a virus as the culprit), the cause of HAT has been known for over a century. In 1899, Sir David Bruce identified a protozoan parasite known as *Trypanosoma brucei* as the cause of the disease in cattle in Zululand. The trypanosome is transmitted by the bite of the tsetse fly of the *Glossina* species. Within 10 years of that discovery, the trypanosomes causing human disease in Africa were identified with the seminal descriptions of trypanosomes in the blood and cerebrospinal fluid (CSF) by Aldo Castellani in 1903 (Williams

1996). Infected animals, usually wild and domestic cattle, are the reservoirs for the parasites causing HAT

EPIDEMIOLOGY

The global importance of HAT is not generally well known, but it is in fact considerable. Indeed, we coined the term 'Cinderella disease' to highlight the relative lack of research interest and funding for HAT despite the fact that it is a major threat to human health, and invariably fatal if untreated (Kennedy *et al.* 2002).

The disease occurs in 36 countries in sub-Saharan Africa, and about 60 million people are at risk of developing it world wide (WHO 1986). The incidence is difficult to determine precisely, but a likely figure is at least 300 000 cases annually, and probably more. About one-third of the entire African land mass is infested with the tsetse fly, which makes farming in these areas impossible.

There are two types of HAT – West African sleeping sickness caused by *Trypanosoma brucei* (*T.b*) *gambiense*, and East African sleeping sickness caused by *T.b.rhodesiense*. The geographical distribution differs considerably (Fig. 2). The tempo of the two forms of the disease is different too. *Rhodesiense* infection is acute with early CNS involvement 3–4 weeks after the initial bite, with death in weeks to months, whereas *gambiense* is slower with later involvement of the CNS and a chronic course of months to years until death (Atouguia & Kennedy 2000).

Figure 1 The author and his colleague Dr Joseph Sulo at the Alupe Sleeping Sickness Treatment Centre in the Busia district of North-western Kenya.

Figure 2 The distribution of the two types of human African trypanosomiasis (reproduced with permission from Atouguia & Kennedy 2000).

The incidence and mortality from HAT have varied considerably over the last 100 years. The disease was nearly brought under control in the 1950s, and had almost disappeared by 1962, but since then the incidence and mortality have steadily increased. There are many possible reasons for this increase, and the periodic epidemics and very localized outbreaks in various parts of Africa. A prominent factor for the periodic re-emergences has been a breakdown of disease surveillance, especially during wars when public health systems become disrupted, even though during these periods one might expect *less* reporting of the disease. Other factors have also played a part including changes in host susceptibility, changes in parasite virulence and drug resistance, variations in climate and vegetation, and geographical changes in animal reservoirs (Kuzoe 1993).

SUMMARY OF THE RELEVANT PARASITE BIOLOGY

For a detailed account of the general and molecular biology of HAT the reader is referred to our recent reviews (Atouguia & Kennedy 2000; Kennedy 2004). In brief, after the tsetse fly feeds from an infected animal, the trypanosomes undergo a complex series of structural and biochemical changes in the fly's midgut. Once the parasites reach the fly's salivary glands they are infective, about 21 days after the 'blood

meal'. They are transmitted to the human host through biting (Fig. 3). About 5–15 days after the bite, a painful circumscribed primary skin lesion known as a trypanosomal chancre usually develops following which the parasites spread through the bloodstream to the lymph nodes and other organs such as spleen, liver, heart, endocrine system and eye (Duggan & Hutchinson 1966; Atouguia & Kennedy 2000). Untreated, the parasites then cross the blood–brain barrier and invade the CNS. Without treatment an infected individual will invariably die.

A critical feature of the trypanosomes is their remarkable ability to undergo antigenic variation and thereby to constantly evade the immune response of the host resulting in waves of parasitaemia (parasites in the blood). The molecular basis of this phenomenon is essentially due to a constant variable surface glycoprotein (VSG) gene switching process, which causes rapid changes of the VSGs distributed over the surface of the parasite, making it impossible to develop an effective vaccine (Barry 1997; Donelson 2002).

CLINICAL FEATURES

There are two stages – the early or haemolymphatic stage, and the late or encephalitic stage. The range of systemic features that may be seen in the early stage is summarized in Table 1. The first symptoms, which are more non-specific

Figure 3 Tsetse fly biting a human arm.

Table 1 Non-neurological features of human African trypanosomiasis (modified from Atouguia & Kennedy (2000) with permission)

Systemic features
Fever
Debility
Headache
Facial oedema
Anaemia (mainly haemolytic)
Lymph node enlargement
Skin
Rash
Chancre
Pruritis
Gastro-intestinal features
Hepatic dysfunction and hepatomegaly
Splenomegaly
Heart
Early tachycardia
Myocarditis
Congestive cardiac failure
Pericarditis
Endocrine dysfunction
Menstrual disorders, sterility and abortion
Premature still births
Loss of skin hair
Loss of libido and impotence
Gynaeacomastia
Testicular atrophy and orchitis
Eye
Iritis
Iridocyclitis
Keratitis
Conjunctivitis
Choroidal atrophy

such as malaise, fever and fatigue (Apted 1970), occur 7–21 days after the initial bite in *rhodesiense* infection, and usually some weeks longer than that in *gambiense* infection (J. Atouguia, personal communication). Episodes of fever, and also generalized lymphadenopathy, are typical and the fever may be associated with headache, rigors and vomiting. The patient may feel relatively well between these febrile episodes, which not surprisingly are often misdiagnosed as malaria. Splenomegaly and liver dysfunction may be apparent and a variety of skin symptoms may occur such as rashes. In particular, an evanescent, circinate and macular skin rash has been described over the trunk, shoulders and thighs and is thought to be a specific early sign in European patients (Duggan & Hutchinson 1966). Pruri-

tis and facial oedema in addition to the primary chancre also occur. Cardiac involvement may be indicated by tachycardia and a variety of cardiac abnormalities. A range of endocrine disturbances has also been described (Atouguia & Kennedy 2000). Eye involvement such as iritis, keratitis and conjunctivitis is well recognized (Atouguia & Kennedy 2000). Other visual disturbances occur due to CNS involvement (see below).

The late stage features of HAT can be conveniently grouped into psychiatric, sleep disorders, motor, sensory, abnormal reflexes and ophthalmic. As can be seen from Table 2, a wide range of psychiatric symptoms, some of them quite non-specific, have been described and early symptoms may be subtle making the diagnosis very difficult. The CNS symptoms usually emerge within a few weeks of infection in *rhodesiense* disease but over the course of several months

Table 2 Neurological features of human African trypanosomiasis (modified, with permission, from Atouguia & Kennedy (2000))

Psychiatric and mental disturbances
Lassitude and indifference
Anxiety and irritability
Mania and agitation
Violent and suicidal behaviour
Uncontrolled sexual impulses
Hallucinations and delirium
Sleep disturbances
Daytime somnolence
Nocturnal insomnia
Uncontrollable urges to sleep
Motor disturbances
Pyramidal signs
Extrapyramidal – abnormal movements and tremors
Muscle fasciculation
Slurred speech
Cerebellar ataxia
Neuritis and polyneuritis
Sensory disturbances
Pruritis
Paraesthesia, hyperaesthesia, anaesthesia
Abnormal reflexes
Pout reflex
Palmo-mental reflex
Visual involvement
Double vision
Optic neuritis
Optic atrophy
Papilloedema

The patient may feel relatively well between these febrile episodes, which not surprisingly are often misdiagnosed as malaria.

after infection in *gambiense* disease. The transition from early to late-stage disease is not always distinct, especially in *rhodesiense* infection. Of course, the characteristic sleep disturbances have given the disease its name of 'sleeping sickness'. Typically the patient shows loss of attention and distractibility and has an uncontrollable urge to sleep with daytime somnolence often alternating with nocturnal insomnia. In the final stages of the disease the uncontrollable urge to sleep is virtually continuous. Motor system involvement may be diverse with pyramidal signs, extrapyramidal features and a variety of abnormal movements including tremor and chorea, cerebellar features and peripheral nerve involvement (Atouguia & Kennedy 2000). Sensory symptoms are common and include, especially in European patients, a type of deep hyperaesthesia (known as Kerandel's sign). Pout and palmo-mental reflexes may be present and indicate frontal lobe involvement. The visual problems that may occur as a result of CNS involvement *per se* include optic neuritis, diplopia and papilloedema. Untreated the patient progressively deteriorates with mental decline, uncontrollable urges to sleep, cerebral oedema, incontinence and finally death. This process takes a few weeks in *rhodesiense* disease and several months to even years with *gambiense* disease.

DIAGNOSIS

There is a wide differential diagnosis, but the most important one is malaria, which may well coexist with HAT in the same patient. Other conditions that may have to be excluded include tuberculosis, leishmaniasis, human immunodeficiency virus (HIV) infection, toxoplasmosis, viral encephalitis, typhoid and hookworm infestation (Kennedy 2004). There is no definite evidence that HIV infection predisposes an individual to HAT. A critical component of the diagnosis is the matching of a typical or possible clinical presentation with local geographical knowledge of where HAT is currently endemic or epidemic. But the definitive diagnosis, which can only be made by specific laboratory investigations, has been somewhat problematic for many years. There are two aspects to this – the diagnosis of HAT infection itself, and then the identification of HAT-positive patients who have definite CNS infection, i.e. late-stage disease. Accurate staging of the disease is absolutely critical because treatment of early stage disease is ineffective for late stage disease, and the drugs for late stage disease are very toxic as will be discussed below.

Demonstration of parasites

The diagnosis of HAT is certain when the parasites can be identified in the peripheral blood (Fig. 4) or lymph nodes on light microscopy. In *rhodesiense* disease identification of parasites in the blood is usually possible because of the persistent parasitaemia. If enlarged lymph nodes are present, which is typical of *gambiense* disease, then most physicians aspirate (not biopsy) them to identify parasites directly, which is particularly important if the blood is negative for parasites. In *gambiense* disease, identification of parasites in the blood is much more difficult because there is greater adaptation between the parasite and the host and the

Figure 4 Trypanosomes (*T.b. brucei*) stained on a blood slide. Four parasites can be seen in this image (courtesy of Dr Jorge Atouguia).

parasitaemia tends to be cyclical. In the latter case the mainstay of diagnosis is the card agglutination trypanosomiasis test (CATT) which can be performed easily and rapidly (Chappuis *et al.* 2005). One of the main problems, however, is how to proceed with a weakly positive and equivocal CATT result, but field algorithms have recently been suggested to deal with this uncertainty (Chappuis *et al.* 2005).

Failure to detect parasites in the blood certainly does not exclude HAT. Nonetheless, even with a positive CATT titre it is prudent to look for parasites in the blood or enlarged lymph nodes. All CATT-positive patients, and all patients who are suspected of having CNS involvement, require a CSF examination as there are no totally reliable clinical criteria for defining early stage disease only, i.e. patients who have not yet entered the late-stage (Fig. 5).

Diagnosis of CNS involvement

The definition of CNS involvement, i.e. late-stage disease, is somewhat controversial. The World Health Organization

Figure 5 Staff at the Alupe Treatment centre performing a lumbar puncture on a patient suspected of having CNS sleeping sickness.

(WHO) criteria are the presence of trypanosomes in the CSF, or a white blood cell (WBC) count in the CSF of $> 5/\mu\text{L}$ (WHO 1998). However, not everyone accepts this definition with some authorities in West Africa regarding a CSF WBC of $> 20 \text{ WBC}/\mu\text{L}$ as defining CNS disease. A very recent thoughtful review has suggested a compromise figure of $> 10 \text{ WBC}/\mu\text{L}$ (Chappuis *et al.* 2005). I personally currently favour the WHO criteria until there is a robust evidence base for some better ones. Typically the CSF protein in late stage disease is 40–200 mg/100 ml, and intrathecal IGM synthesis has also been identified as a sensitive marker for CNS involvement (Lejon *et al.* 2002).

It is hardly surprising that there are so few studies describing the neuroradiological (CT and MRI) findings in HAT because the facilities are not usually available. While there have been no detailed systematic series, several reports have documented MRI abnormalities in (Fig. 6). These have included diffuse, sometimes asymmetrical white matter changes, ventricular enlargement and diffuse basal ganglia hyperintensities.

Polysomnographic abnormalities have been documented, showing the expected abnormal sleep-wake patterns which improve with treatment.

At least three abnormal EEG patterns have been described in HAT: a sustained low voltage background correlating with early cerebral involvement, a pattern of paroxysmal waves correlating with acute cerebral involvement, and a mixture of delta wave and rapid intermittent high-voltage delta bursts interspersed between periods of low-voltage activity correlating with meningoencephalitis (Atouguia & Kennedy 2000).

Figure 6 MRI scan (proton density) of a 13-year-old patient with CNS disease for 3 years following completion of several treatments for multiple relapses. The scan shows ventricular enlargement and diffuse white matter changes especially in the right frontal (arrow) and periventricular areas (reproduced with permission from Atouguia & Kennedy 2000).

TREATMENT

Current treatment is unsatisfactory. It is based on four main drugs some of which would probably not have passed contemporary high safety standards had they been developed recently. The drugs can be divided into those that are effective for early-stage disease, and those that cross the blood brain barrier and are therefore suitable for late-stage disease. The two key problems are that none of them can be given orally, and all of them have potentially serious adverse effects:

- For *rhodesiense* infection the drug of choice is suramin given intravenously (5 injections of 20 mg/kg over about 3 weeks).
- For early stage *gambiense* disease the drug of choice is pentamidine given intramuscularly (3–4 mg/kg daily for 7–10 days).
- Prompt treatment is usually effective.
- The only drug that is effective for CNS involvement in both types of HAT is the trivalent arsenical melarsoprol (Mel B). This drug, which was first used in 1949, has usually been given as 2–4 courses of three injections. For example, in cases where there are more than 100 WBC/ μL in the CSF, typically three courses of three injections are given with a 1 week interval between each course (Atouguia & Kennedy 2000). If there are 20–100 WBC/ μL in the CSF then just two courses of three injections could be given with a 1-week interval between the courses. For repeated treatments then four courses of three injections can be given with 1-week intervals between each course. The usual dose is 3.6 mg/kg. However, alternative regimes have been used, in particular a shorter more continuous dose regime has also been devised i.e. daily doses of 1.8 mg/kg for just 10 days (Burri *et al.* 2000).
- In melarsoprol-refractory *gambiense* disease, DFMO (eflornithine) can be given as an alternative, but it is largely ineffective against *rhodesiense* infection. Although it does have potentially serious adverse effects, including bone marrow toxicity and seizures, it is much safer than melarsoprol. The history of the development of this drug is remarkable. It was first used for *gambiense* HAT in 1981 but it then became an orphan drug, unprofitable for the drug companies. But Medecins Sans Frontieres worked with the pharmaceutical industry (Aventis Pharma and Bristol-Myers) as well as WHO to make the drug available again for use in Africa (Kennedy *et al.* 2002).
- There is increasing evidence of melarsoprol resistance in Northern Uganda, which is a real concern.

Post-treatment reactive encephalopathy

The greatest problem with melarsoprol is that it is followed by a severe post-treatment reactive encephalopathy (PTRE) in about 10% of cases and in about 50% of these this complication is fatal. Thus the drug kills about 5% of all those who receive it (Pepin *et al.* 1994; Kennedy 2004). This is a remarkable figure and PTRE is a major focus of our current research. Although this drug complication rate is very high, it is of course preferable to the 100% death rate of untreated disease.

The PTRE usually occurs between the first and second course of melarsoprol treatment in the 3–4-weekly regime (Atouguia & Kennedy 2000), and between the eighth and ninth injection in

the case of the newer 10-day drug regime (J.Atouguia, personal communication). It may take the form of:

- fulminant convulsive status epilepticus and cerebral oedema;
- rapidly progressive coma developing within hours without convulsions or cerebral oedema;
- deep coma with sporadic seizures (Atouguia & Kennedy 2000).

It is the timing of one of these syndromes in relation to melarsoprol therapy, and the sudden onset and severity of the PTRE, that usually allows the distinction of the PTRE from CNS HAT *per se*.

Treatment of an established PTRE consists of general medical support, corticosteroids and control of seizures. The role of prophylactic corticosteroids to *prevent* the PTRE is controversial. Once the patient has recovered from an episode of PTRE this complication is, fortunately, unlikely to recur so the remainder of the melarsoprol course can be completed.

The existence of PTRE underpins the critical importance of accurate staging of HAT. For if one withholds melarsoprol from a patient who *does* have CNS involvement then death will inevitably ensue. But if one mistakenly gives melarsoprol to a patient who does *not* in fact have CNS disease then one is subjecting the patient unnecessarily to a 5% risk of death from the treatment.

Follow up

Follow up of all treated patients, both early and late stage, is essential but may be problematic for logistical reasons. Ideally, 6-monthly clinical and laboratory evaluations including blood and CSF analysis (for CNS cases) should be carried out for a period of 2 years. At that point the patient is considered cured if all the screening tests are negative or normal. However, one or more relapses of the disease may occur. The treatment with melarsoprol needs to be repeated if the CSF is active (by WHO criteria), even if the patient is otherwise asymptomatic.

The prognosis of patients with CNS disease is not as good as in those with only early stage disease who have been treated promptly. Unfortunately, even patients who have been treated for late-stage disease may suffer from long-term neurological impairments including psychiatric and cognitive abnormalities, personality change, alteration of libido, hemiplegia, ataxia, involuntary movements and epilepsy (Atouguia & Kennedy 2000).

There is considerable interest at present in developing more effective therapies for HAT but the only drug on the horizon, the research development of which is supported by the Bill and Melinda Gates Foundation, is DB 289 which is showing considerable promise as oral therapy for early stage disease. DB 289 is a diamidine derivative and an oral prodrug of the active form known as DB 75. A promising avenue of research for combating CNS disease is combination chemotherapy including use of the drug Nifurtimox, which might prove useful when combined with DFMO, and a field trial is ongoing. Another approach would be to modify the blood brain barrier penetration of existing drugs so as to make them more effective

in CNS disease but such developments are, frankly, a long way off and will require much more financial support than is currently available.

NEUROPATHOGENESIS OF CNS HUMAN AFRICAN TRYPANOSOMIASIS AND THE POST-TREATMENT REACTIVE ENCEPHALOPATHY

I have extensively reviewed this subject elsewhere (Kennedy 1999, 2004) and only a few key points will be made here. The essential neuropathological findings in late stage disease are those of a meningoencephalitis. Marked widespread perivascular cuffing is typical together with diffuse white matter infiltration with macrophages, lymphocytes and plasma cells (Adams *et al.* 1986). Marked astrocyte and macrophage activation is also present. The changes in PTRE are similar but more severe.

Insights into the mechanisms of the disease have come from studies in patients suffering from late stage HAT, and from animal models. A variety of cytokine alterations have been reported in the CSF of patients with CNS sleeping sickness; IL-10 and TNF-alpha levels are elevated in late stage disease (Maclean *et al.* 2001). Our current hypothesis is that the balance between pro-inflammatory cytokines, e.g. IL-1 and TNF-alpha, and counter-inflammatory cytokines, e.g. IL-10, is a major determinant of the outcome of CNS HAT. As for the cause of PTRE, there are several possible mechanisms including the effects of an acute immune response to a massive release of killed parasites following chemotherapy, subcurative chemotherapy, deposition of immune complexes in the brain, autoimmune mechanisms and arsenical toxicity (Kennedy 1999). Animal models have given insights into the role of interferon-gamma, which appears to determine the ability of trypanosomes to cross the blood brain barrier (Masocha *et al.* 2004), and the possible role of altered brain MHC 1 gene expression in determining the sleep disorders, which can also be modelled in animals (Bentivoglio *et al.* 1994).

THE FUTURE

There is unquestionably a recent resurgence of interest in HAT, and funding bodies such as the Gates Foundation have already recognized the scale and urgency of the problem. Without considerable increases in research, funding progress in this area will continue to be slow. A safe oral drug for early stage disease is likely to be in widespread use within 5 years, but the hope for more effective drugs for treating CNS HAT is poor at present. However, increasing use of DFMO may improve current prospects for late stage *gambiense* disease. Novel approaches for treating CNS disease include combination therapy and methods to increase the blood brain barrier permeability of existing drugs. Accurate staging of the disease also remains a crucial goal, at least until safe and effective CNS therapy becomes available. Better control of HAT will also require more effective and widespread surveillance and screening of at-risk humans and domestic cattle reservoirs, as well as improved methods of reducing man/tsetse fly contact through use of fly traps in infected regions and

PRACTICE POINTS

- HAT should always be considered in a patient with a fever and/or typical symptoms in an HAT-endemic area, but the most important differential diagnosis is malaria which may co-exist.
- While *rhodesiense* HAT is more acute with early CNS invasion, *gambiense* is usually slower and chronic with late CNS infection lasting months to years. But both types are ultimately fatal if untreated.
- The transition from early to late stage, i.e. CNS infection may not always be distinct, especially in *rhodesiense* infection.
- While a diagnosis of HAT can usually be made in *rhodesiense* disease by demonstration of parasites in the blood, the diagnosis is more often established by serological means in *gambiense* disease due to the cyclical nature of the parasitaemia.
- Accurate disease staging of HAT is crucial because the melarsoprol treatment of late stage, i.e. CNS disease, is very toxic.
- It is vital to make a positive diagnosis of CNS disease before starting melarsoprol treatment and this can be achieved using the WHO criteria of trypanosomes detected in the CSF and/or $> 5\text{WBC}/\mu\text{L}$ in the CSF.
- For early stage *rhodesiense* disease the drug of choice is intravenous suramin, whereas the drug of choice for early stage *gambiense* is intramuscular pentamidine.
- For both *rhodesiense* and *gambiense* the treatment of choice for CNS disease is intravenous melarsoprol. But this is followed in about 10% of cases by a severe post-treatment reactive encephalopathy (PTRE) that is fatal in about 50% of cases giving an overall mortality for melarsoprol therapy of about 5%.
- An increasingly used alternative drug for CNS *gambiense* is efflornithine (DFMO), which is less toxic than melarsoprol, but it is very expensive, not free from adverse effects and ineffective in *rhodesiense* disease.
- All patients with HAT must be followed up with regular clinical and laboratory assessments for 2 years. In CNS disease, drug therapy needs to be repeated if the CSF is active and meets the WHO criteria for CNS infection.

new means of insecticide delivery. Neuropathological insights gained from animal models of HAT have been considerable and the challenge now is to combine imaginative efforts and increased research funding to somehow translate these findings into improved therapies to improve the lot of patients suffering from this hitherto neglected killer.

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