Sleeping Sickness and the Issue with its Animal Models

Anne Rudolf, MSc. Neuroscience, TCD

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Abstract

Despite its lethality and pervasiveness, relatively little is known about the underlying pathology of Human African Trypanosomiasis (HAT), colloquially known as "sleeping sickness". This condition is a major cause of death in sub-Saharan Africa. Animal models could provide useful information pertaining to the course and underlying pathology of this disease, and may also help to elucidate the features displayed by the Trypanosoma parasite, which is the causative agent of this disease. This review aims to both describe and critique the animal models presently used in HAT research. To my knowledge, to date no review of animal models in HAT research has been published. Since many trypanosome types also affect animals other than the tse-tse fly, there also exists a small body of literature about research in that domain, e.g. cattle research. This review, however, focuses on research aiming to alleviate the danger of sleeping sickness in humans, a goal that is in urgent need of a more systematic approach.

Introduction

Sleeping sickness or human african trypanosomiasis (HAT) is a disease threatening people in 36 countries of sub-Saharan Africa, where it is one of the major causes of mortality. Overall, about 60 million people are at risk for developing it[1]. Two forms of Trypanosoma parasites can infect humans: the East African HAT caused by Trypanosoma brucei rhodesiense and the West African HAT, which is caused by Trypanosoma brucei gambiense[2]. Trypanosoma parasites, transmitted by the bite of infected tse-tse flies, cause a two-stage disease. In the early or hemolymphatic stage, the parasites are present in the blood and lymph, causing inconclusive symptoms such as joint pain, headache, itching, and fever. A challenge for detection and early intervention is comorbidity with a myriad of other diseases that present with similar symptoms. The late or meningoencephalitic stage, which occurs when the parasites have crossed the blood-brain barrier (BBB) and invade the central nervous system (CNS), is accompanied by behavioural changes, confusion, sensory disturbances, poor coordination and, not surprisingly, disturbance of the sleep cycle. It leads to death if untreated[3]. Unfortunately, late-stage treatment is ineffective and highly dangerous itself, killing 5% of people receiving it[1]. Treatment consists of melarsoprol, an arsenic compound that is also used in cancer treatment[4]. Furthermore, presumably since its epicentre is so far away from sites where money is invested into research, despite its fatality and pervasiveness, sleeping sickness is a disease still not familiar to many researchers. As a result, the pathways by which the parasites can invade the CNS are still not fully understood[2]. This is where animal models provide a novel opportunity to investigate the underlying mechanisms of Trypanosoma infections. The following models are currently used in different stages of the disease.

The HAT animal model

An animal can easily be infected with any kind of trypanosomes intraperitoneally and displays similar clinical features to those displayed in humans, including a disregulated sleep pattern and locomotor changes[5]. However some research groups use animal parasites like Trypanosoma brucei brucei, which cannot infect humans[5, 6]. Other trypanosomes that can infect animals but which cannot infect humans include Trypanosoma brucei congolense and brucei evansi. The reason researchers have chosen to use these parasites is likely due to safety considerations: no research group should be blamed for not wanting to work with lethal parasites. But the extrapolation of results obtained from these observations to human sleeping sickness should be questioned critically. After all, there is a reason behind the categorisation of different types of trypanosomes, and it is straightforward to believe that different types display different features. Already the two types that can infect humans have distinct char-

acteristics and give rise to distinct clinical manifestations[1]. Also available is the vervet monkey model of sleeping sickness, basically another HAT animal model with a different name. In that model, vervet monkeys were infected with Trypanosoma from a human patient in Uganda[7], probably mimicking HAT more closely.

Gene knockout mice models

Various knockout mice models have been used to study HAT, mostly to investigate the involvement of immune system factors. An important example is the IFN-γ-knockout mouse, which, when infected with trypanosomes, led to the discovery that IFN-γ is crucial for trypanosomes to cross the BBB[8]. Other knockout models used are B-cell-deficient and immunoglobulin M (IgM)-deficient mice and TNF-knockout mice.

Inbred mice models

tant to trypanosomes[9].

The late stage model

This model is used to investigate the course of disease during the late or meningoencephalitic stage. Animals, e.g. vervet monkeys[10], are infected with the parasite several weeks before their behaviour or their brains are assayed, or – to test the effectiveness of a new drug – before treatment begins. Twenty-eight days after the infection, a drug to clear the blood from the trypanosomes – berenil – is given. Berenil cannot cross the BBB, indicating that the parasites that already infiltrated the brain can proliferate. When trypanosomes reappear in the blood or cerebrospinal fluid (CSF) samples collected during the next weeks, this is said to be indicative for the late stage of trypanosomiasis (for Ngotho *et al*[10] this took 98 days in total). This phase is accompanied by additional signs, such as peak CSF white blood cell levels, CSF and serum IgM (anti-parasite immunoglobulin M antibodies) and CSF IgG (immunoglobulin G).

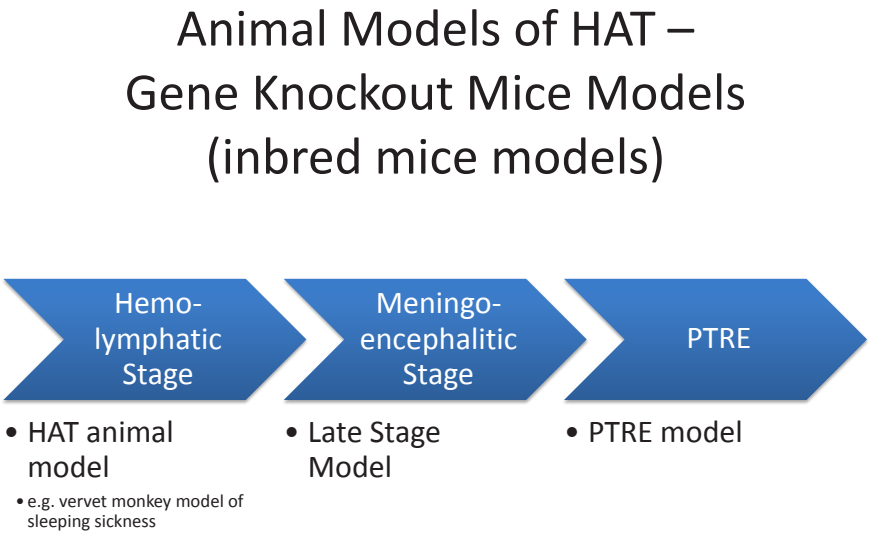

Figure 1: Relation of different animal models to stages of human African trypanosomiasis

These models enable genetic studies of phenotypic differences between different mouse strains. Different breeds show different features with respect to survival after infection and immunological control of the parasites. These parameters also vary with different trypanosome infections, which contributes to a clearer picture of what results might be transferred from animal research to HAT. Interestingly, to date no strain of mouse is considered to be resistant to trypanosomes[9].

At this point, it is important to note that no clear-cut border between early and late stage has been defined. Every research group is therefore free to decide where to draw the line. Do we call it late stage if the parasites infiltrated the brain, or only once CNS damage is visible? Does late-stage phase occur only when behavioural changes are observable? Comparisons between experimental findings therefore are difficult to draw.

The PTRE model

The post-treatment reactive encephalopathy (PTRE) model is used to investigate neurological disorders that appear not as a reaction to the disease, but as a consequence of the treatment with the arsenic compound melarsoprol. PTRE is characterised by ischaemic cell changes and fibrinoid necrosis and occurs in about 10% of treated patients[1]. It tends to be fatal in half of these[1,11]. Using mice, different severities of the PTRE can be modelled by administering melarsoprol: medium-to-severe pathogenic CNS invasion occurs after one trypanolytic treatment phase, severe PTRE after a second phase[9]. It is noteworthy that some research groups use berenil instead of melarsoprol to induce PTRE, so what is called PTRE model actually is a late stage model (compare the work of Ngotho *et al*[10] and Kennedy[1]). In other words, in many papers published to date, the CNS damage caused by the disease and the CNS damage caused by the treatment are not clearly separated. Yet another approach is to distinguish the two models by the number of berenil treatments given to mice[11].

Summary and conclusion

An overview of the animal models used in HAT research related to their specific disease state is presented in Figure 1. Having described the state-of-the-art models, specifically in the progressed state of the disease, it is quite clear that researchers in this field need to be more precise with regard to working definitions, and that we need to read HAT research results far more critically than we currently do in order to improve our understanding of this fatal disease.

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