Biotechnol Res 2020; Vol 6(3):15-20
eISSN 2395-6763

Copyright © 2020 Max et al
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORIGINAL RESEARCH

Toward a Diagnostic Test for Human African Trypanosomaisis

Travis JAZTLAU[1], Takeya SCOTT[1], Max FontusFONTUS[1]
[1]Texas Undergraduate Medical Academy, Prairie View A&M University, Prairie View, TX 77446.
*Corresponding Author email: mafontus@pvamu.edu

• Received: 3 April 2020• Accepted: 16 July 2020 • Published: 4 September 2020 •

ABSTRACT
Human African Trypanosomiasis (HAT), caused by Trypanosoma brucei, T. brucei, disproportionately affects inhabitants of Sub Saharan Africa. T. brucei gambiense and T. brucei rhodesiense are the only strains that produce illness in humans. The purpose of this project is to determine if T. brucei Superoxide Dismutases, TbSODs, can be used as a biomarker for positive diagnosis of HAT. The hypothesis to be tested is if a rat is infected with T. brucei, then it will have traces of tbSOD in its bloodstream because as the rat's immune system fights T. brucei infection some parasitic cells will lyse excreting many internal proteins including SOD into the bloodstream. Our methodology will consist of developing a modified version of the Villagran et al 2005 protocol whereby a series of blood tests will be performed on infected rats. If the blood tests positive for TbSOD then it is assumed that they have a strain of T. brucei. Finally, after confirming the presence of TbSOD, we will use ELISA to establish a correlation between TbSOD's level and the degree of progression of the infection.

KEYWORDS: *Trypanosoma brucei, trypanosomiasis, Epimastigote cultures*

INTRODUCTION

Trypanosoma brucei is a protozoan parasite that affects Sub Saharan Africa. (WHO 2014) It is transmitted through the bites of the Tsetse fly. T. brucei causes African sleeping sickness or Human African Trypanosomiasis (HAT) in humans and nagana in animals [2]. There are several strains of Trypanosoma brucei, but only two infect humans: Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense. T.b. gambiense accounts for over 95% of reported cases of HAT and causes a chronic form of the disease. This strain is considered chronic because individuals infected live for several months to a few years before succumbing to the disease.[11] T.b. rhodesiense accounts for less than 5% of reported cases [2] and causes an acute form of the disease killing the host within a few months. There are two stages of HAT: the circulatory stage [6] and the central nervous system stage[13]. The symptoms of this early phase (nausea, fever, severe headaches, irritability, fatigue, and lethargy) are nonspecific and easily confused with those of other diseases such as influenza and malaria. In the late phase, trypanosomes cross the blood-brain barrier and can be found in neural tissue and cerebrospinal fluid. Subsequent neural damage and host reaction cause the classical symptoms of sleeping sickness: disruption of biological rhythms, inappropriate and irregular sleep patterns, and loss of concentration and coordination. [4] In 2013 there were 9000 cases of HAT in Sub Saharan Africa. [2]

Table 1: Drugs for HAT and Side Effects [8]

Drugs	Stage of Disease	Strain	Additional Info	Structure
NECT nifurtimox-eflornithine combination therapy	Central Nervous System Stage	Gambiense	• Nifurtimox used in the treatment of Chagas disease	NIFURTIMOX NO_2 O N N S O O CH_3 + F F NH_2 H_2N O OH
Pentamidine	Circulatory Stage	Gambiense	• Side effect: effects on the pancreas	HN NH_2 O O NH NH_2
Eflornithine	Central Nervous System Stage	Gambiense	• Side effect: Hematologic abnormalities, hearing loss, Thrombocytopenia	F F NH_2 H_2N O OH
Melarsoprol	Central Nervous System Stage	Rhodesiense	• Arsenic derivative • Side effect: Convulsions, fever, loss of consciousness, rashes, bloody stools, nausea, and vomiting. encephalopathy	NH_2 N N N H_2N HN S As S OH
Suramin	Circulatory Stage	Gambiense and Rhodesiense	• Side effects: rashes, adrenal cortical damage, kidney damage	

Currently, three methods exist by which diagnosing HAT caused by T.b. gambiense is performed. [6] The first method is an initial serologic test, card agglutination test for trypanosomiasis (CATT). The CATT is not specific enough for confirmation of infection, however, it is helpful in identifying possible cases. T.b. rhodesiense does not have a prescreened serological test, so its diagnosis only involves the latter two methods. The second method is the parasitological test. [5] They are conducted by drawing blood, usually from lymph nodes, and the fluid is examined under a microscope for visual confirmation of live parasites. However, this test can be misleading especially in the case of T.b. gambiense due to the relatively low amounts of the parasite in the blood and lymph nodes, so serial tests are required.[7] The third method is staging the disease. If the central nervous system involvement is expected, a lumbar puncture will be performed and the cerebrospinal fluid will be checked for live parasites. [6] The diagnostic processes for this disease are inefficient and need to be

updated. Many cases of HAT are in remote rural areas that do not have access to lab facilities needed for diagnosis. The necessity of an effective serological test for quick and easy diagnosis in these remote areas which are mostly affected by HAT cannot, therefore, be overstated.

Treatment options for HAT are limited and problematic due to severe side effects, limited efficacy and cost. New treatments are hence imperative. T. brucei, HAT causative agent, is vulnerable to oxidative stress and the drugs used to treat HAT exploit this weakness in a variety of ways. Drugs like nifurtimox and benznidazole cause oxidative stress by redox cycling of the drug leading to the generation of reactive oxygen species, ROS. While for other drugs the oxidative stress stems from the reduction of the thiol pool. NECT or nifurtimox-eflornithine combination therapy is the newest treatment available. However, it's problematic as well since it requires oral nifurtimox for 10 days and intravenous eflornithine for 7 days. While this treatment is effective against T.b. gambiense and has limited side effects, the second round of therapy can be expensive and requires hospitalization; what's more, it means that NECT does not treat T.b. rhodesiense. Finally, drugs like Melarsoprol are highly toxic and have many side effects. See Table 1 for a list of drugs along with their side effects.

MATERIALS AND METHODS

Draw blood of uninfected rats and run ELISA, western blot, IFA, blood smear, and IHA. Enzyme-linked Immunosorbent Assay (ELISA) is used to detect antibodies in the blood. [10] The western blot test is used to confirm the results of ELISA because it has a greater protein specificity. Immunofluorescence Assay (IFA) identifies antigens within the nucleus of a cell. [3] Blood smear test analyzes the visual characteristics of both white and red blood cells to discover irregularities. [8] Indirect Hemagglutination Assay (IHA) causes antigens in the blood to clump together if they are present. Infect rats with the bloodstream form of Trypanosoma brucei. Wait 3 days and test to confirm infection with a blood smear. The morphology of T. brucei cells is a unique crescent containing a central nucleus with a flagellum extending from the front and a posterior kinetoplast. These characteristics will help make the confirmation through the blood smear. Run western blot, ELISA, Superoxide Dismutase, SOD, assay on blood. Draw blood every 2 to 4 days and run all tests (western blot, ELISA, SOD assay, and blood smear). To confirm the progression of the infection there should be an increased number of T. brucei cells and also some cells that have developed into a short stumpy shape. This new shape is the representation of a T. brucei cell during its quiescent stage within the human host. Once rats begin to show symptoms of stage 2 they will be euthanized.

Modified Protocol for Goal #1

The protocol detailed in this section will aid in achieving our primary goal as stated above. SOD is an extremely important protein in the elimination of ROS and it is T. brucei's only defense against oxidative stress. Being that it is so important, it is highly expressed in T. brucei. T. brucei SOD, tbSOD, is extremely different from the two SODs found in humans. In addition to the structural difference, tbSOD also calls for an iron cofactor which is different from cofactors associated with human SOD's. This makes tbSOD a great biomarker. In previous studies, SOD was used to identify T. cruzi in human blood. So we use a modified version of the protocol that undergirded the T. cruzi study to see if it would work in T. brucei. To justify using the previous T. cruzi study, a genetic comparison of both the protein and nucleotide sequences of each organism using the Clustal Omega program was performed. The exact sequences are given in table 2. The test resulted in a 99.519% similarity between them. We can infer that the SOD protein is among the similar proteins in the sequence. T. brucei and T. cruzi are also morphologically comparable

Table 2: **Genetic Comparison of T. brucei and T. cruzi**

	Protein Sequence	Nucleotide Sequence
>tbg	MAFSIPPLPWGYDGLAAKGISKEQVTFHYDKHHMGYV TKLNAAAKSNPALAAKSVEEIIR 60 TEKGPIFNLAAQIFNHNFYWESMSPNGGGEPSGKLAE AIRASFGSFAKFKEEFTNAAVGH 120 FGSGWAWLVQDTTTKKLKVFQTHDAGCPLTEADLKPI LTCDVWEHAYYIDYKNDRPAYVQ 180 TFWNVVNWDHAENQFTRKRNPGAPHSDL 208	TTTTTTTTTTTTTTTTCCCCCCCCCCCCCCCCAAAAA AAAAAAAAAAAGGGGGGGGGGGG 60 GGGG 64 TTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGGTTT TCCCCAAAAGGGGTTTTCCCCAAAA 60 GGGG 64 TCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTC AGTCAGTCAGTCAGTCAGTCAGTCAG 60 TCAG 64
>tbb1	MAFSIPPLPWGYDGLAAKGISKEQVTFHYDKHHMGYV TKLNAAAKSNPALAAKSVEEIIR 60 TEKGPIFNLAAQIFNHNFYWESMSPNGGGEPSGKLAE AIRASFGSFAKFKEEFTNAAVGH 120 FGSGWAWLVQDTTTKKLKVFQTHDAGCPLTEADLKPI LACDVWEHAYYIDYKNDRPAYVQ 180 TFWNVVNWDHAENQFTRKRNPGAPHSDL 208	TTTTTTTTTTTTTTTTCCCCCCCCCCCCCCCCAAAAA AAAAAAAAAAAGGGGGGGGGGGG 60 GGGG 64 TTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGGTTT TCCCCAAAAGGGGTTTTCCCCAAAA 60 GGGG 64 TCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTC AGTCAGTCAGTCAGTCAGTCAGTCAG 60 TCAG 64
>tb	MAFSIPPLPWGYDGLAAKGISKEQVTFHYDKHHMGYV TKLNAAAKSNPALAAKSVEEIIR 60 TEKGPIFNLAAQIFNHNFYWESMSPNGGGEPSGKLAE AIRASFGSFAKFKEEFTNAAVGH 120 FGSGWAWLVQDTTTKKLKVFQTHDAGCPLTEADLKPI LACDVWEHAYYIDYKNDRPAYVQ 180 TFWNVVNWDHAENQFTRKRNPGAPHSDL 208	TTTTTTTTTTTTTTTTCCCCCCCCCCCCCCCCAAAAA AAAAAAAAAAAGGGGGGGGGGGG 60 GGGG 64 TTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGGTTT TCCCCAAAAGGGGTTTTCCCCAAAA 60 GGGG 64 TCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTC AGTCAGTCAGTCAGTCAGTCAGTCAG 60 TCAG 64

kinetoplast. This also justifies the use of the previous T. cruzi study. However, instead of using human sera the blood of infected rats can be used.

Parasites and culture.

T. brucei should be obtained from trusted labs in the United States or abroad. Epimastigotes will be grown in axenic Grace's medium (Gibco-BRL, Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum at 28°C. Epimastigote cultures (in the exponential growth phase) will be concentrated by

centrifugation at 600 × g for 10 minutes, and cells will be washed twice and resuspended in ice-cold STE buffer (0.25 M sucrose, 25 mM Tris-HCl, 1 mM EDTA, pH 7.8)

Parasites and culture.

T. brucei should be obtained from trusted labs in the United States or abroad. Epimastigotes will be grown in axenic Grace's medium (Gibco-BRL,

Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum at 28°C. Epimastigote cultures (in the exponential growth phase) will be concentrated by centrifugation at 600 × g for 10 minutes, and cells will be washed twice and resuspended in ice-cold STE buffer (0.25 M sucrose, 25 mM Tris-HCl, 1 mM EDTA, pH 7.8)

Animal protocol

The rodents will need to be kept in a pathogen-free environment and under BLS-2 guidelines. Trypanosoma brucei is a blood pathogen and is only transferred by the tsetse fly. The rodents infected with T. brucei brucei will not infect other rodents. The rodent will be kept under NIH animal welfare guidelines. The rodents will have blood drawn before being infected. The rodents will be infected by injecting the blood form of T. brucei brucei in the pericardium. The rodents will have blood drawn every two to four days and tests, ELISA, enzyme assay, western blot, slide identification, will be run on them. Once the rodents begin to display signs of stage 2 of the disease they will be humanely euthanized.

Microscope identification

Blood will be drawn from the rodents' tails and the fluid will be examined under a microscope for visual confirmation of live parasites. We will count them to see how far along with the disease in progression.

Enzyme assay

Use SOD enzyme assays from suitable Labs. SOD Standard Wells - add 200 µl of the diluted Radical Detector and 10 µl of Standard (tubes A-G) per well in the designated wells on the plate (Sample Wells - add 200 µl of the diluted Radical Detector and 10 µl of sample to the wells. NOTE: If using an inhibitor, add 190 µl of the diluted Radical Detector, 10 µl of inhibitor, and 10 µl of sample to the wells. The amount of sample added to the well should always be 10 µl. Samples should be diluted with Sample Buffer (dilute) or concentrated with an Amicon centrifuge concentrator with a molecular weight cut-off of 10,000 to bring the enzymatic activity to fall within the standard curve range. Initiate the reactions by adding 20 µl of diluted Xanthine Oxidase to all the wells you are using. Make sure to note the precise time you started and add the Xanthine Oxidase as quickly as possible. NOTE: If assaying sample backgrounds, add 20 µl of Sample Buffer instead of Xanthine Oxidase. Carefully shake the 96-well plate for a few seconds to mix. Cover with the plate cover. Incubate the plate on a shaker for 30 minutes at room temperature. Read the absorbance at 440-460 nm using a plate reader. [12]

Polyclonal serum

Obtain specific antibodies against tbSODb1, send a sample of tbSODb to a trusted third party lab and have them make the custom antibodies.

RESULTS AND DISCUSSION

The protocol that our experiment is based on is a 15-year-old study there may be new technology that we could use in our research. The rats may not react well to T. brucei the way we expect them to. The amount of tbSOD may not correlate with how advanced the disease is then we will have to find another way to test its progression specifically in the human sera. Currently, if T. brucei is left untreated the mortality rate is approximately 100%. [14] There are treatment options for both the first and second stages of the disease, but the success rate is greater if treated as early as possible. The number of diagnosed individuals was below 10,000 for the first time in 50 in 2009. This number is continually decreasing,but there are still upwards of 65 million at risk of infection. [1] This great amount of at risk people from countries such as Guinea, Democratic Republic of the Congo proves the need to study T. brucei.

CONCLUSION

Trypanosomiasis brucei, a vector- borne disease, disproportionately affects Sub Saharan Africa. We proposed a modified protocol of a previous study of the Superoxide Dismutase protein present in T. cruzi. This experiment should work based on the 99.519% similarity between T. cruzi and T. brucei. Our modification, using rats as the host, may be an issue because the disease might not progress at the same rate and amount as it would in humans. In the future, this proposed experiment should be performed to test our hypothesis. If our assumption is true blood should be drawn from humans infected with T. brucei and the tests ELISA, western blot, IFA, blood smear, and IHA should be conducted. The results of these tests should be compared to those in our protocol to further prove T. brucei SOD is an adequate biomarker in diagnosing this disease.

REFERENCES

Brun R., Blum J., Chappuis F. & Burri C. (2010). Human African trypanosomiasis. Lancet. 375(9709):148–159.

Blood Smear - Health Encyclopedia - University of Rochester Medical Center. (2019). Retrieved from https://www.urmc.rochester.edu/encyclopedia/content.aspx?ContentTypeID=167&ContentID=blood_smear

ELISA: Purpose, Procedure, and Results. (2019). Retrieved from https://www.healthline.com/health/elisa

Eisai ATM Navigator. (2019). Retrieved from https://atm.eisai.co.jp/english/ntd/africa.html

Fèvre, E. M., Coleman, P. G., Welburn, S. C., Maudlin, I. (2004). Reanalyzing the 1900–1920 sleeping sickness epidemic in Uganda. Emerg Infect Dis, 10(4), 567–573.

Hide, G., History of sleeping sickness in East Africa. (1999). Clin Microbiol Rev., 12(1), 112–125.

Picozzi, K., Fèvre, E. M., Odiit, M. et al. Sleeping sickness in Uganda: a thin line between two fatal diseases. (2005), BMJ. 331(7527):1238–1241.

Jamonneau, V., Ilboudo, H., Kaboré, J., et al. (2012). Untreated human infections by Trypanosoma brucei gambiense are not 100% fatal. PLoS Negl Trop Dis. 6(6):e1691.

Indirect Immunofluorescence Assay (IFA) - LabCE.com, Laboratory Continuing Education. (2018). Retrieved from https://www.labce.com/spg913463_indirect_immunofluorescence_assay_ifa.aspx

Koerner, T., De Raadt, P., Maudlin I., (1995). The 1901 Uganda sleeping sickness epidemic revisited: a case of mistaken identity? Parasitol Today, 11(8), 303–306.

Simarro, P. P., Franco, J. R., Cecchi, G. et al. (2012). Human African trypanosomiasis in non-endemic countries (2000–2010). J Travel Med, 19(1), 44–53.

Superoxide Dismutase Assay Kit. (2018) (p. 14). Ann Arbor.

Trypanosomiasis, human African (sleeping sickness). (2019). Retrieved from https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness)

World Health Organization. Control and Surveillance of Human African Trypanosomiasis. Report of a WHO Expert Committee. WHO Technical Report Series 984. Geneva, Switzerland: World Health Organization; 2013