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SHORT COMMUNICATION

Brain Sialic Acid Profile of Mice Infected with *Trypanosoma brucei*

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Abstract: Sialic acids represent a family of important carboxylated sugar molecules that play vital roles in the pathophysiology of some parasitic diseases. The study investigated the changes in sialic acid concentrations (total, free and bound) in the brains of mice infected with *Trypanosoma brucei*. The infection significantly ($p<0.05$) decreased the packed cell volume and body weight of the mice but increased the bound sialic acid. The concentration of total sialic acid remained unaltered. The free sialic acid concentrations were found to decrease significantly ($p<0.05$) in the infected mice when compared with the uninfected mice. These findings point out the significance of the carboxylated sugar molecules to the sialochemistry of the brain and could provide explanations for some of the central nervous system abnormalities observed during *Trypanosoma brucei* infection.

KEYWORDS: Sialic acid, Trypanosomiasis, *Trypanosoma brucei*

1.0 Introduction

Human African trypanosomiasis is a disease endemic in sub-Saharan Africa caused by infection with the *gambiense* and *rhodesiense* subspecies of the extracellular parasite (Lejon, *et al.*, 2013). *Trypanosoma brucei* is transmitted to humans through the bite of infected tsetse flies and could lead to fatal central nervous system (CNS) impairment if not treated. This feature of the disease is due to the ability of the causative agent (*T. brucei)* to traverse the blood-brain barrier (Lejon, *et al.*, 2013). Some of the CNS symptoms attributed to the disease occur especially in the first or second stage of the infection and include behavioral changes such as mania or psychosis with speech disorders and seizures, mood swings, depression, stupor and coma (Truc *et al.*, 2012).

Over the period of a decade, increased public health efforts in the major endemic countries in sub-Saharan Africa have resulted in significant reduction of infection in cities, however, new cases still occur annually especially in the hinterlands. In some of the countries within the region, no fewer than 200 cases are reported each year (WHO, 2014). The World Health Organization (2014) reported that infection covers a total of 36 countries in sub-Saharan Africa.

Sialic acid is an important component of brain ganglioside molecules of brain cell membrane. Its presence is very important in brain development, increase learning ability and memory formation (Tram *et al.*, 1997; Wainscot, 2004). The adult human brain contains concentration of sialic acid that is 2-to 4 fold higher than those of other mammals, including chimpanzees (Wang *et al.*, 1998). Sialic acid is also thought to play structural and functional roles in the establishment of synaptic pathways (Schauer, 1982).

Clear scientific evidence has demonstrated that during the cycle of trypanosome infection between the tsetse and the host, acquisition of sialic acids is required for successful survival of trypanosomes in the midgut of tsetse flies, which could only be obtained from the host glycoconjugates through the involvement of trans-sialidase (Nagamune *et al.*, 2004). As such, for the trypanosome to find its way through to the brain which contains sialic acid-rich glycoconjugates like gangliosides, quick acquisition of the sialic acid for its survival will

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not be any difficult. Such action by the parasite would deplete the brain ganglioside structures of a very important biomolecule whose manifestation would not only be physiological but pathological, thereby likely potentiating some of the aforementioned CNS symptoms (Lundkvist, *et al.*, 2004).

In the light of the forgoing description, there is no doubt that there might be changes in the sialic acid concentrations in the brain during trypanosomiasis infection that requires investigation therefore making this study necessary. Also, considering the fact that the molecular basis of trypanosome infection is not fully understood, there is the possibility that the action of the parasite's neuraminidase might also be partly responsible for the damages caused during the CNS stage of the infection.

This study was aimed at analyzing the brain sialic acid profile of infected animals in comparison to uninfected animals.

2.0 Materials and Methods

2.1 Materials

2.1.1. Experimental Animals

Twenty, healthy mice weighing between 18-20 g were obtained from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The animals were kept in well-ventilated cages and maintained on growers mash and water *ad libitum* for two weeks to acclimatize.

2.2 Methods

2.2.1 Infection with T. brucei brucei

After acclimatization, the animals were divided into two groups of ten animals each. The animals were weighed after which one group was infected with *T. brucei brucei* while the other group was not infected. Exactly 20 microliter of virulent *T. brucei* infected blood containing about 10^6 parasites was added to 5µl of phosphate buffer saline (PBS) and was intraperitoneally injected into the first group of ten mice. Parasitemia level was assessed by monitoring the levels of parasites every 24 hours post-infection by wet mount. Blood that was obtained was dropped on a slide by pinching the tip of the pre-sterilized tail with a sterile needle. The number of parasites per ml of blood was determined by microscopic observation at × 400 magnification using the 'Rapid Matching' method as described by Herbert and Lumsden (1976). Briefly the method involved microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS pH 7.2). Logarithm values of these counts obtained by matching with the Table of Herbert and Lumsden (1976) were converted to antilogarithm, from where the absolute number of trypanosomes per ml of blood was obtained.

2.2.2. Preparation of Samples

After four days post-infection, the weights of the animals were recorded and then sacrificed. Whole blood was collected in capillary tubes for PCV determination. The brain was then removed afterwhich 5 g was cut and homogenized in 10 mM acetate buffer (pH 5.5). This was then centrifuged at 1000 x g for 10 minutes and the resulting supernatant was then used for the sialic acid assay. Weight of the animals was also determined four days post-infection.

2.2.3. Determination of Packed Cell Volume

The packed cell volume of the animals was determined using the micro hematocrit method. The capillary tubes with blood were centrifuged at 2000 x *g* for 3 minutes using the micro hematocrit centrifuge after which the PCV level was read using the micro hematocrit reader (NCCLS, 1993).

2.2.3 Determination of Total, Free and Bound Sialic Acid Concentration

The total sialic acid was determined using the thiobarbituric acid as described by Aminoff (1961). Briefly, into a test tube, 0.25 ml of the brain homogenate and 0.25 ml of 0.1N H_2SO_4 was dispensed, after which the mixture was boiled at 80°C in a water bath for one hour. After boiling, 0.125ml of periodate was added,

then the mixture was heated in a water bath at 37°C for 30 minutes. Then 0.1ml of sodium arsenite was added to the mixture, after which 1ml of thiobarbituric acid was added and the mixture was covered and heated in a boiling water bath for 7 minutes 30 seconds. The mixture was then rapidly cooled in a water bath and 2.5ml of acid-butanol (butan-l-ol containing 5% (v/v) of 12N HCl) was shaken with the mixture. The mixture was then centrifuged at 1000 *x g* for 8 minutes after which the absorbance of the butanol layer was read at 549 nm.

The free sialic acid was also determined using the thiobarbituric acid as described by Aminoff (1961). Into a test tube, 0.25ml of the brain homogenate and 0.125ml of periodate was added and the mixture was heated in a water bath at 37°C for 30 minutes. Thereafter, 0.1ml of sodium arsenite was added to the mixture, after which 1ml of thiobarbituric acid was added and the mixture was covered and heated in a boiling water bath for 7 minutes 30 seconds. The mixture was then rapidly cooled in a water bath and 2.5ml of acid- butanol (butan-l-ol containing 5% (v/v) of 12NHCl) was shaken with the mixture. The mixture was then centrifuged at 1000 *x g* for 8 minutes after which the absorbance of the butanol layer was read at 549 nm.

The bound sialic acid was determined as the difference between the total and free sialic acid concentration.

2.2.4 Data Analysis

Results are presented as Mean ± SD and Student's t-test was used in comparing the values at 95% confidence limit.

3.0 Results

The result of changes in body weight and PCV among the experimental animals is presented in Table 1. From the Table, the infection caused significant ($p<0.05$) reduction in the body weight and PCV of the experimental animals.

Figure 1 presents the sialic acid concentrations (total, free and bound) in the *T. brucei* infected and uninfected mice. There was no significant ($p>0.05$) change in the total sialic acid concentration when both groups were compared but the free sialic acids changed significantly with the infected mice having lower concentration of free sialic acids when compared with the uninfected animals. The bound sialic acids were higher in the infected mice.

Table 1: Body Weight and PCV *T. brucei* Infected Mice

Treatment Groups	Body weight (g)	PCV (%)
Uninfected mice	15.20 ± 1.40^a	48.80 ± 1.50^a
Infected mice	12.80 ± 1.02^b	44.40 ± 1.40^b

Values are means ± SD

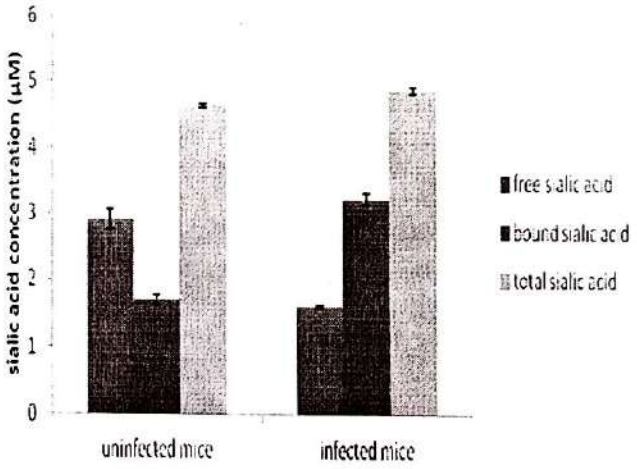

Figure 1: Free, bound and total sialic acid concentration in the brain of uninfected and *T. brucei* infected mice

4.0 Discussion

This work has demonstrated that infection with trypanosomes could cause some chemical changes in the brain of animals during infection and this may directly have association with the level of CNS lesion associated with the brain of *T. brucei* infected animals. The involvement of the CNS in *T. brucei* infection is usually associated with chronicity (WHO, 1998) and may manifest grossly as oedema of the brain and meninges. Experimental evidence by Morrison

et al. (1981) has demonstrated the ability of trypanosome to cross the blood-brain barrier. One of the enzymes involved in trypanosome pathogenesis is neuraminidase (sialidase) and this enzyme has been implicated in anaemia caused by trypanosome infection. This enzyme cleaves off sialic acids on the surface of erythrocytes and disabled them (Verma and Gautam, 1978; Igbokwe, 1994; Adamu *et al.*, 2009). It is expected that there should be an increase in free sialic acid concentration in the infected group of animals but the contrary was observed. The explanation for this could be that the presence of trans-sialidase activity in the parasite earlier reported by Engstler *et al* (1993) and Montagna *et al.* (2002). The enzyme transfers the hydrolysed sialic acid of sialic acid-rich ganglioside to the parasite's membrane surface glycosylphophatidyl inositol (GPI) anchor. Presence of surface sialic acids on the parasite membrane structure appears to serve as a form of protection to the parasite so that it evades possible host trypanocides. This singular action could account for the lower concentration of brain free sialic acids.

Furthermore, the increase in bound sialic acids in the brain further substantiates the aforementioned claim. Since the bound sialic acid concentration was higher due to infection, it implies that much of sialic acids have been transferred to the glycol-conjugates. Similarly, one wouldn't discount the activity of sialyl transferases, whose activity has as well been demonstrated in trypanosome infections (Adamu *et al.*, 2009).

Sialyltransferases can adequately transfer free sialic acids unto glycoconjugates thereby reducing the free sialic acid concentration and at the same time increasing the bound sialic acid concentration. The implication is that this neurochemical change could metamorphose into a pathogenic phenomenon that could be responsible for the CNS anomaly experienced in *T. brucei* infection. The reduction in PCV and body weight of *T. brucei* infected mice compared to the uninfected animals was due to the infection as this has been reported as characteristic clinical sign of the trypanosome infection (Adamu *et al.*, 2008; Mbaya *et al.*, 2009).

This study shows that *T. brucei* infection reduced the free sialic acid content of the brain of mice and increased its bound sialic acid content.

References

Adamu, S., Ibrahim, N. D. G., Nok, A. J. and Esievo, K. A. N. (2008). Sialyl transferase activity probably counteracts that of sialidaseas; one possible mechanisms of natural recovery or stabilization of erythrocyte mass in trypanosome-infected animals-a perspective. African Journal of Biotechnology 7:4992-5001.

Adamu, S., Maashin, U. N., Ibrahim, D. N., Nok, A. J. and Esievo, K. A. N. (2009). Erythrocyte surface sialic acid depletion as predisposing factor to erythrocyte destruction in sheep experimental model of African trypanosomosis: A preliminary Report. Slovenian Veterinary Research 46(1): 19-28.

Aminoff, D. (1961). Methods for the quantitative estimation of N-acetylneuraminic acid and their application to hydrolysates of sialomucoids. Biochemical Journal 81: 384-392.

Engstler, M., Reuter, G. and Schauer, R. (1993). The developmentally regulated trans-sialidase from *Trypanosoma brucei* sialylates the procyclic acidic repetitive protein. Molecular Biochemistry and Parasitology 61(1):1-13.

Herbert, W. J. and Lumsden, W. H. R. (1976). *Trypanosoma brucei*: A Rapid "matching" method for estimating the host's parasitemia. Experimental Parasitology 40: 427-431.

Igbokwe, I. O. (1994). Mechanisms of cellular injury in African trypanosomiasis, Veterinary Bulletin 64(7): 611-620.

Lejon V., Bentivoglio M. and Franco J. R. (2013). Human African Trypanosomiasis, *Handbook of Clinical Neurology*. 114:169-81.doi:10.1016/B978-0-444-53490-3.00011-x

Lundkvist, G. B., Kritensson, K. and Bentivovlio, M. (2004). Why trypanosomes cause sleeping sickness. Physiology (Bethesda) 19:198-206.

Mbaya, A. W., Aliyu, M. M., Nwosu, C. O., Taiwo, V. O. and Ibrahim, U. I. (2009). Effects of melarsamine hydrochloride (Cymelarsan®) and diaminazene aceturate (Berenil®) on the pathology of experimental *Trypanosoma brucei* infection in red fronted gazelles (*Gazella rufifrons*). Veterinary Parasitology 163(1-2):140-143.

Montagna, G., Cremona, M. L., Paris, G., Amaya, M. F., Buschiazzo, A., Alzari, P. M. and Frasch, A. C. (2002). The transialidase from the African

trypanosome, *Trypanosoma brucei*. European Journal of Biochemistry 269(12): 2941-2950.

induced *T. brucei* infection in the dog. I. Tissue and organ damage. American Journal of Pathology 102(2):168 - 181.

Nagamune, K., Acosta-Serano, A., Uemura, H., Brun, R., Kunz-Renggli, C., Maeda, Y., Ferguson, M. A. and Kinoshita, T. (2004): Surface sialic acids taken from the host allow trypanosome survival in tsetse fly vectors. Journal of Experimental Medicine 17:199(10): 1445-1450.

National Committee for Clinical Laboratory Satandards (1993): Procedure for determining packed cell volume by the microhematocrit method, 2nd ed.H7-A2. Villanova, Pa. NCCLS

Schauer, R. (1982). Sialic acid Chemistry, Metabolism and Function Wien, N.Y: Springer-Verlag.

Tram, T. H., Brand Miller, J. C., McNeil, Y. and McVeagh, P. (1997). Sialic acid content of infant saliva: Comparison of breast fed with formula fed infants. Archives of Disease in Childhood 77: 315-318.

Truc, P., Penchenier, L., Vatunga, G. and Josenando, T. (2012). Human African Trypanosomiasis in Angola: Clinical observations, treatment and use of PCR for stage determination of early stage of the

Morrison, W. I., Max, M., Sayer, P. D. and Preston, J. M. (1981). The pathogenesis of experimentally disease. Transactions of the Royal Society of Tropical Medicine and Hygiene 106(1):10-14

Verma, B. B. and Gautam, O. P. (1978). Studies on experimental surra (*T. evansi* infection) in Buffalo and cow calves. Indian Veterinary Journal 55: 648-653.

Wainscot, G. (2004). The role and function of sialic acid in infant neuro-development in Enfa A + Innovation. PT. Mead Johnson Indonesia working with Indonesian Pediatric Association. Jakarta.

Wang, B., Brand Miller, J., McNeil, Y. and Mc Veagh, P. (1998). Sialic acid concentration of brain gangliosides : variation among eight mammalian species. Comparative Biochemistry, Physiology and Anatomy. Molecular Integrated Physiology. 119: 435-9.

WHO (1998). Control and surveillance of African trypanosomiasis. World Health Organization Technical Report Series No 881. https://apps.who.int/iris/handle/10665/42087

WHO (2014). African Trypanosomiasis Factsheet. https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness)