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Full Length Research Paper

Role of sialic acids in the midguts of *Trypanosoma congolense* infected *Culex pipense pipiense* mosquitoes

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Free and total sialic acid concentrations were determined in the midgut extract of *Culex pipiense pipiense* mosquitoes infected with *Trypanosoma congolense*. The mean total sialic acid concentrations were found to be 1.5 to 2 fold higher than the mean free sialic acid concentrations in the midgut extracts of all the groups of the *T. congolense* infected *C.p. pipiense*. Infusion of 10 mg/ml galactose and 10 mg/ml lactose did not change the pattern of this difference but resulted to 1.3 to 1.4 fold decrease in the total sialic acid concentration. The relevance of these findings to the role of sialic acids in the midgut of *T. congolense* infected *C.p. pipiense* mosquitoes is discussed in this paper.

Key words: *Trypanosoma congolense*, *Culex pipiense pipiense*, sialic acid, midgut.

INTRODUCTION

The *Culex pipiense pipiense* mosquito is a member of the *Culex pipiense* complex. Apart from serving as a vector for the agent that transmits lymphatic filariasis, this mosquito also transmits several viral, bacterial and protozoal infections. (Vinodogradova, 2000). As a biting and blood sucking insect, one would expect the possibility of transmitting a disease like trypanosomiasis, as vectors that transmit this disease are biting and blood sucking (tsetse flies) as well. To date, there has been no report on the transmission of trypanosomiasis by the *C.p. pipiense* or any other member of the *C.p. pipiense* complex. Sialic acids comprise a family of 9 carbon carboxylated sugars that are components of soluble or cell bound glyconjugates (Engstler and Schauer, 1993), and are involved in a variety of biological events. These molecules have been reported (Pontes de Carvallio *et al.,* 1993) to lie on the surface coat of the insect form of the trypanosome parasite (procyclic) anchored by the glycosyl-phosphatidylinositol (GPI) and is also found to be necessary for the survival of the procyclics in the midgut of the tsetse fly. Reports on cultured procyclics *Trypanasoma brucei* reveal that it acquires the sialic acid unto its surface coat from the environment around it and that such action is being aided by a glycohydrolase (sialidase) that has a unique trans-sialidase activity (Engstler et al., 1993; Schenkman et al., 1992).

The aim of this experiment therefore, is to examine the presence of sialic acid in the midgut of *C.p. pipiense* to establish whether it plays any role in the midgut of *T. congolense* infected *C.p. Pipiense* mosquitoes with the view to harnessing possible significance of sialic acid to the infection biology of *C.p. Pipiense* mosquitoes.

MATERIALS AND METHODS

All reagents and chemicals used for this experiment were purchased from Sigma Chemical Company, St. Louis, USA.

Trypanosomes

T. congolense (Kauru strain) was obtained from the National Institute of Trypanosomiasis Research NITR, Vom Plateau State, Nigeria.

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Table 1. Mean sialic acid concentrations obtained from midgut extracts of *trypanosoma congolense* infected *C.p. pipiense* mosquitoes.

Total Sialic Acid concentration (mM)	Groups	Free Sialic Acid concentration (mM)
3.29 ± 0.00	*C. p. pipiense* mosquitoes infected with *Trypanosoma congolense* from mice given no sugar solution	1.51 ± 0.36
2.30 ± 1.17	*C. p. pipiense* mosquitoes infected with *Trypanosoma congolense* from mice infused galactose solution	1.58 ± 0.10
2.53 ± 0.00	*C.p. pipiense* mosquitoes infected with *Trypanosoma congolense* from mice infused lactose solution.	1.71 ± 0.01

Results above are mean ± S.D for two independent Experiments (η = 2).

Table 2. Mean total sialic acid concentrations in obtained from midgut extracts of *T. congolense* infected *C.p. pipiense* mosquitoes.

Groups	Total Sialic Acid Concentration (mM)
C. p.pipiense mosquitoes infected with *T. congolense* from mice given no sugar solution	3.29 ± 0.00
C. p. pipiense mosquitoes infected with *T. congolense* from mice infused galactose solution	2.30 ± 1.17
C. p. pipiense mosquitoes infected with *T. congolense* from mice infused lactose solution	2.53 ± 0.00

Results Above Are Mean ± S.D for Two Independent Experiments (η = 2).

Mosquitoes

The puparium of *C.p. pipiense* mosquitoes were identified and scooped unto a container from a sewage outlet at Samaru village, Nigeria. They were then transferred inside the container into a cage with supplements of yeast until they hatched. On hatching, the mosquitoes were given 5% sucrose solution soaked cotton swabs as source of food. Seventy (70) mosquitoes were used for the experiment and were starved for 12 h prior to the commencement of the experiment.

Sialic acid assay

Free sialic acid concentration was determined in the midgut extract by the thiobarbituric acid method of sialic acid assay described by Warren, (1959). Total sialic acid concentration were carried out using the same procedure after hydrolyzing the sample (midgut extract) by heating at 80^0C for 1 h in 0.1 NHCl.

Midgut extract preparation

Midgut extract was prepared by dissecting five mosquitoes and removing their midgut contents into the well of a titre plate containing 50 µl of 66 mM phosphate buffer pH 7.2. This was homogenized and used immediately for assays. The dissected midgut was also observed at x400 magnification for the presence of trypanosomes.

Sialidase assay

This was carried out by incubating midgut extract from the starved *Cp. pipiense* mosquitoes that have been prefed 0.1% cotrimoxazole with freshly prepared fetuin (25 mg/ml). The free sialic acid released was then assayed. One unit of the enzyme activity was defined as the amount of enzyme that hydrolises 1µM fetuin per hour.

Treatment of Mosquitoes

Three Balb/C mice were experimentally infected with *T. congolense*. At peak parasiteamia, the mice were restrained into three separate cages. Cage I, contained mouse not given any sugar infusion, whereas cages II and III contained mice given intraperitoneal infusion of 5 ml (10 mg/ml) galactose and lactose solutions, respectively, at 30min interval for 1 h. After the second infusion, 10 starved mosquitoes were introduced each to the three different cages for infection (feeding) serving as group I, II and III mosquitoes, respectively.

The remaining 40 starved mosquitoes were further divided into 4 groups of 10 mosquitoes each. Group IV mosquitoes were dissected and their midgut extracts analysed for free sialic acid, (no sugar was administered to this group). Group V mosquitoes comprise of those that were allowed to feed on 10 mg/ml galactose solution soaked cotton swabs after the 12 h starving and thereafter their midgut extract prepared for free sialic acid analysis. Group VI are *C.p. pipiense* mosquitoes fed on 10 mg/ml lactose solution soaked cotton swabs after the 12 h starvation and thereafter the mosquitoes were dissected and their midgut extract prepared for

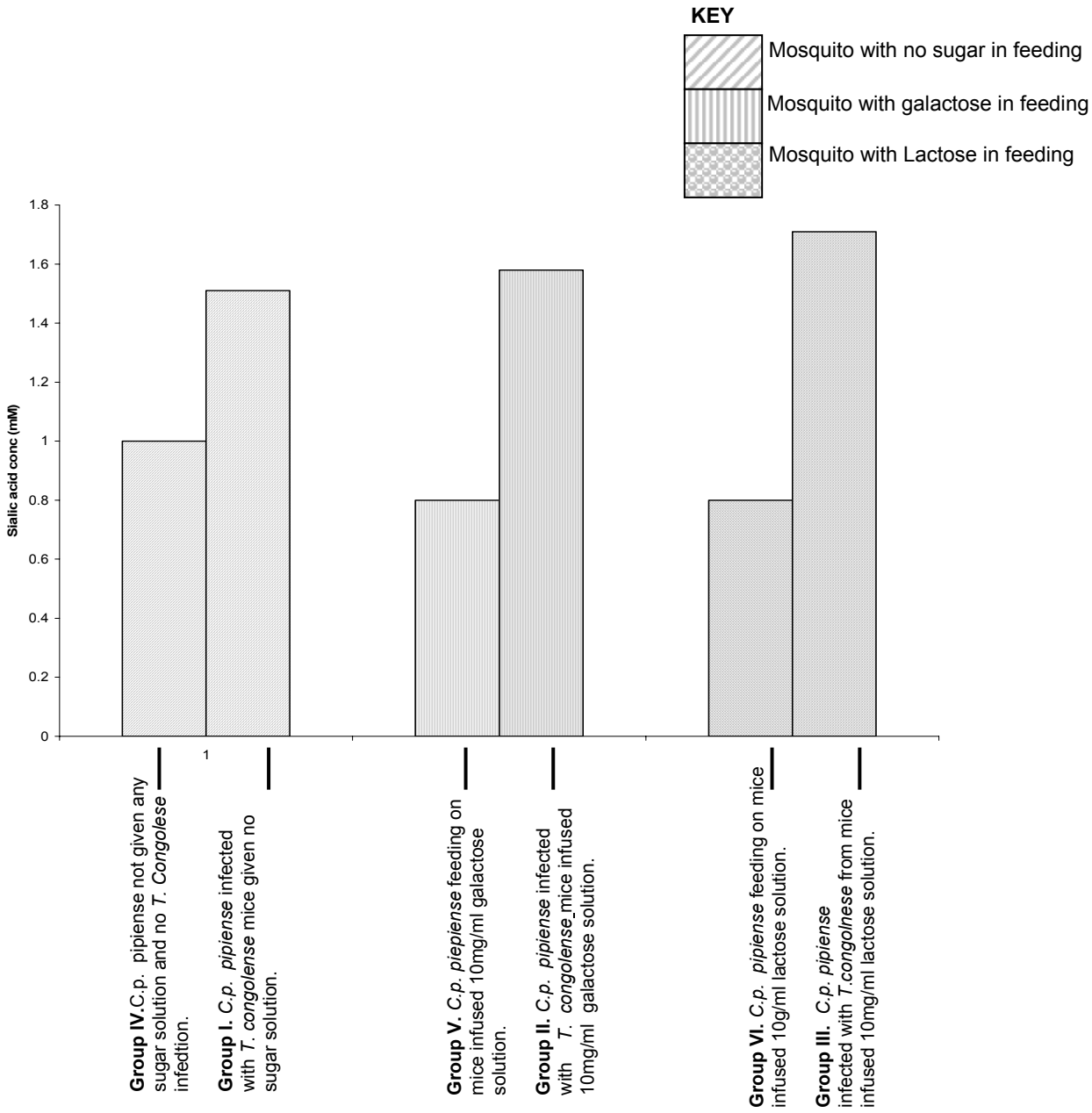

Figure 1. A bar diagram comparing free sialic acid concentrations in the midgut extracts of *C.p. pipiense* mosquitoes not infected *T. congolense* and midgut extracts of *C.p. pipiense* mosquitoes infected with *T. congolense*.

free sialic acid analysis. The group VII mosquitoes were those from which midgut extract was obtained for sialidase assay.

RESULTS

The mean total sialic acid concentrations are 1.5 to 2 fold higher than the mean free sialic acid concentrations in the midgut extracts of all the groups (Table 1). However, the pattern of the difference in the sialic acid concentrations remained the same even with the infusion of galactose and lactose solutions, though there was a 1.3 and 1.4 fold decrease in total sialic acid concentration in group II and III mosquitoes. Figure 1 illustrates the increase in free sialic acids in all the groups of *C.p. pipiense* infected with *T. congolense* when compared with the groups of *C.p. pipiense* uninfected with the parasite. When all the *C.p. pipiense* mosquitoes infected with *T. congolense* were dissected for midgut preparation and observed under the microscope (x400), not a single trypanosome was observed in the midgut. Analysis of the midgut extracts of group VI and group VII mosquitoes for sialic acids and sialidase gave readings of 1.01 mM free

sialic acid and sialidase (neuraminidase) activity of 0.2 µM/h respectively.

DISCUSSION

The consistency in the pattern of this 1.5 to 2.0 fold higher mean total sialic acid concentration (Table 1) implies that galactose and lactose infusions did not inhibit the availability of sialic acids in the *T. congolense* infected *C.p. pipiense* midgut. Though, there was a notable 1.3 and 1.4 fold decrease respectively in mean total sialic acids in group II and III which could be explained (in case of group III) to be as a result of the inhibitory effect of lactose (a potential trans-sialidase acceptor) on sialidase (Nok et al., 2002) in the midgut of *C.p*. *pipiense*. This supports the possibility of *C.p. pipiense* harbouring a tras-sialidase activity whose inhibition will result in the diminished transfer of sialic acids to acceptors and hence decreased mean total sialic acid concentration.

The main function of sialic acids in the midgut of *C.p. pipiense* is not known but since sialic acids have been implicated in variety of biological protective mechanisms (Kelm and Schauer, 1986), it is possible that the protozoas carried by the *C.p*. *pipiense* acquire the sialic acid in their midgut onto their surfaces to protect themselves against host defense hydrolytic systems The presence of sialic acids in the midgut of *C.p*. *pipiense* even with the absence of trypanosomes suggests that the sialic acid molecule may have a role to play with other agents transmitted by the *C.p*. *pipiense*. The occurrence of sialic acids in the midgut could as well buttress the fact that the *C.p. pipiense* midgut sialic acids have been correlated with transialidase enzyme activity (Engstler et al., 1993)

Although the function of the sialidase found in the midgut of *C.p*. *pipiense* is not known, the explanations given above could suggest that this enzyme has an effect on the sialic acids presence in the midgut of the mosquito. Viral and bacterial sialidase have been found to play key role in viral and bacterial infections, respectively (Muller, 1974). *C.p. pipiense* midgut sialidase could therefore be speculated to also play a significant role in the disease transmission of the mosquito.

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