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EFFECTS OF MIDGUT BACTERIA AND TWO PROTEASE INHIBITORS ON THE TRANSMISSION OF *WUCHERERIA BANCROFTI* BY THE MOSQUITO VECTOR, *CULEX PIPIENS*

By

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Abstract

Laboratory investigations were carried out to study the effect of two protease inhibitors on the transmission of *W. bancrofti* filarial by *Culex pipiens* and to study the susceptibility interaction between filaria and protease inhibitors. The results obtained revealed that, infection rates were variable among untreated and treated symbiotic and aposymbiotic *Cx. pipiens* females resulted from third instar larvae treated with E-64 and EDTA. The survival rate was variable among untreated and treated symbiotic and aposymbiotic females resulted from third instar larvae treated with E-64 and EDTA. Protease inhibitor (E-64) caused inhibition of the parasite development and transmission by means of ceasing catalytic activity- responsible for parasite migration-caused by parasitic larval stages inside the mosquito vector.

Key words: *Culex pipiens, W. bancrofti,* interaction, protease inhibitors.

Introduction

Lymphatic filariasis tops the nematode parasites of which man is the definitive host. *Wuchereria bancrofti* is spread by several species of mosquitoes causing filariasis or elephantiasis.

The parasite is distributed throughout tropical regions (Central and South America, Africa, Asia, Pacific areas) and also in subtropical countries in the Middle East (Service 1996). The current estimates (Abdel-Hamid *et al*, 2011) suggested that approximately 120 million people are infected with all types of lymphatic filarial, particularly bancroftian filariasis. The high fecundity of *Culex pipiens*, together with the increase in number of breeding places due to uncontro-lled urbanization, may have contributed to the recent increase in prevalence rates of *W. bancrofti* from 9.7% (Azevedo and Dobbin 1952) up to 14.9% (Maciel *et al*, 1996).

Mosquitoes play a serious role as vectors of many vertebrate blood pathogens. *Cx. pipiens* is a very common mosquito species in Egypt it is the predominant vector of *W. bancrofti* that causes filariasis or elephantiasis in humans (Khalil *et al*, 1930; Abdel-Hamid *et al*, 2012), Rift Valley fever virus (Meagan *et al*, 1980; El Bahnasawy *et al*, 2013a) and West Nile virus (El Bahnasawy *et al*, 2013b).

The present work aimed to study the effect of two protease inhibitors on the

development of the vector host and consequently on the transmission of the *W. bancrofti* filaria, also, to study the susceptibility interaction between filaria and protease inhibitors in mosquito midgut.

Materials and Methods

Cx. pipiens was collected from Abu Rawash, Giza Governorate, and safely transmitted to the laboratory for rearing. The 3rd larval instars from the second generation were placed in plastic cups (diameter 12 cm x height 7 cm) containing 250 ml of the proteases inhibitors solution. Twenty five cross matched larvae were placed in 250 ml dechlorinated tap water as control. The larvae were daily provided with fish food as a diet, which proved to be the most preferable food for their development and a well female fecundity (Kasap and Demirhan, 1992).

The emerging adult females were fed 10% sugar solution for 24 hr. and then they were exposed simultaneously to the volunteer the hand back or forearm for 20-30 min between 10 pm and midnight. This feeding time coincided with the peak of microfilaraemia activity (Rocha *et al*, 1991).

Infection rate was calculated as follows:

$$\frac{\text{No. infected x 100}}{\text{No. dissected}}$$

And survival rate was as follows:

$$\frac{\text{No. survived for 14 days after blood meal x 100}}{\text{No. maintained mosquitoes}}$$

Transmission Electron Microscope (TEM): female *Cx. pipiens* were dissected 3, 6, 9, and 12 days post blood meal. Grids were examined by JEOL JEM 1010 using image analysis document at Al-Azhar University Regional Center for Mycology and Biotechnology.

Results

Effects induced by filarial infection: The infection rate (tab. 1) was variable among untreated and treated symbiotic females resulted from third instar larvae treated with E-64 and EDTA, where the infection rate was 70 % & 80%; respectively, compared to 90% for the control females. Also, the rate was variable among untreated and treated aposymbiotic females resulted from larvae treated with E-64 and EDTA, where the infection rate was 80 % and 90%; respectively, compared to 90% for the control females (aposymbiotic).

Table 1: Symbiotic and aposymbiotic *Cx. pipiens* females affected by 3rd larval instars treatment with different protease inhibitors post blood feeding on *W. bancrofti* microfilaraemic volunteer

Treatments	No. dissected	No. infected	Infection %
Symbiotic	10	8	80
E-64	10	7	70
EDTA	10	8	80
Aposymbiotic	10	9	90
E-64	10	8	80
EDTA	10	9	90

The survival rate (tab. 2) was variable among untreated and treated symbiotic females emerged from third instar larvae treated with E-64 and EDTA, 8.7 % and 10.5%; respectively, compared to 14.3% for controls. Also, the survival rate was variable among untreated and treated aposymbiotic females emerged from larvae treated with E-64 and EDTA, 5% and 14.3 %; respectively, compared to 4.3% for controls (aposymbiotic).

Table 2: Symbiotic and aposymbiotic *Cx. pipiens* females affected by 3rd larval instars treatment with different protease inhibitors post blood feeding on *W. bancrofti* microfilaraemic volunteer.

Treatments	No. tested	No. dead	No. survived	survived 7 days post blood meal	survived 14 days post blood meal	14 days post blood meal Survival %
Symbiotic	25	4	21	8	3	14.3
E-64	25	2	23	5	2	8.7
EDTA	25	6	19	6	2	10.5
Aposymbiotic	25	2	23	5	1	4.3
E-64	25	5	20	6	1	5
EDTA	25	4	21	4	3	14.3

Effect of protease inhibitor (E-64) on parasite development and transmission: The midgut structure and the thoracic muscles of *Cx. pipiens* females infected with *W. bancrofti* emerged from 3rd larval instars treated with E-64 and controls were examined by TEM (fig. 1).

DISCUSSION

In Egypt, *Cx. pipiens* tops all mosquitoes in prevalence and distribution (Micheal *et al*, 2009). In the present study, a high infection rate percentage was observed in treated and untreated infected symbiotic *Cx. pipiens* females affected by the treatment of 3rd larval instars with two protease inhibitors post blood feeding on *W. bancrofti* microfilaraemic volunteer. Infection rates were 70 & 80 % for females emerged from third larval instars treated with E-64 and EDTA; respectively. This result agreed with Anosike *et al*. (1992) and Sabatinelli *et al*. (1994) working on *Cx. quinquefasciatus*.

The present study has shown also that infection rate of aposymbiotic *Cx. pipiens* females affected by treatment of 3rd larval instars with the two protease inhibitors post blood feeding on the *W. bancrofti* microfilaraemic volunteer was higher than those of symbiotic ones. Infection rate showed high percentages in treated and untreated infected aposymbiotic females. Infection rates were 80 and 90 % for females resulted from larvae treated with E-64 and EDTA; respectively. These results may indicate that symbiotic bacteria play a very important role in the transmission of the parasite by mosquitoes.

These results are similar to those demonstrated by Curtis *et al.* (1983), Pumpuni *et al.* (1993) and Pais *et al.* (2008) against *Culex quinquefasciatus*. On contrary, to those of Ganushkina (1992), Straif *et al.* (1998), Mourya *et al.* (2002a, b) and Gonzalez-Ceron *et al*. (2003) where infection rate of apo-

symbiotic insects was lower than those of symbiotic ones. In the present study, the **s**urvival rate of *Cx. pipiens* females affected by treatment of 3rd larval instars with the two protease inhibitors post blood feeding on *W. bancrofti* microfilaraemic volunteer showed very low percentage in treated and untreated symbiotic and aposymbiotic females. Survival rates of symbiotic females were 8.7 and 10.5 % for females resulted from larvae treated with E-64 & EDTA; respectively. While survival rate of aposymbiotic females was 5 & 14.3 % for females resulted from larvae treated with E-64 and EDTA, respectively. This agreed with Crans (1973) working on *Cx. pipiens* infected with *W. bancrofti* and Seitz *et al.* (1987) against *Anopheles stephensi* infected with *Plasmodium berghei*. Calheiros *et al.* (1998) showed that survival rates of insects living for 21 days after blood meal on microfilaraemic patients were not significantly differed from controls.

In the present study, of particular interest was the inhibition of parasite development and transmission by protease inhibitor (E-64) ceasing the catalytic activity–responsible for parasite migration- caused by parasite larval stages inside the mosquitoes. The mortality suffered by *Cx. pipiens* females post blood feeding on *W. bancrofti* microfilaraemic volunteer showed high sensitivity to infection based on survival rates. By 6 days (PBM), the second larval stage (L2) of *W. bancrofti* was found in treated and untreated females' midgut. In treated females, (L2) stage was found penetrating the midgut epithelial cells with no traces in the surrounding haemoceal. While in untreated ones, the (L2) stage was found in the haemoceal and in the midgut epithelial cells suggesting the absence of peritrophic membrane during penetration. These results are similar to those demonstrated by Perrone and Spielman (1986) against *Aedes aegypti*. They found that the mosquito's midgut was disrupted by *Brugia malayi* passage.

The present study showed disruption of the parasitized muscle fiber in the thoracic muscles of *Cx. pipiens* control females infected with *W. bancrofti* 12 days (PBM). The results are similar to that of Kan and Ho (1973) against *Ae. togoi* infected with *Brugia pahangi*. They found disarray or disruption of parasitized muscle fiber, reduction and disorganization of the inter-febrile mitochondria, reduction of muscle glycogen and, finally, complete dissolution of the muscle fiber.

Conclusion

The infection rate varied among untreated and treated symbiotic and aposymbiotic females resulted from larvae treated with E-64 and EDTA. The survival rate was also variable among untreated and treated symbiotic and aposymbiotic females resulted from larvae treated with E-64 and EDTA.

The protease inhibitor (E-64) caused inhibition of development and transmission of the *W. bancrofti* microfilariae by means of ceasing the catalytic activity-responsible for parasite migration-caused by larval stages of the parasite inside the *Culex pipiens* vector.

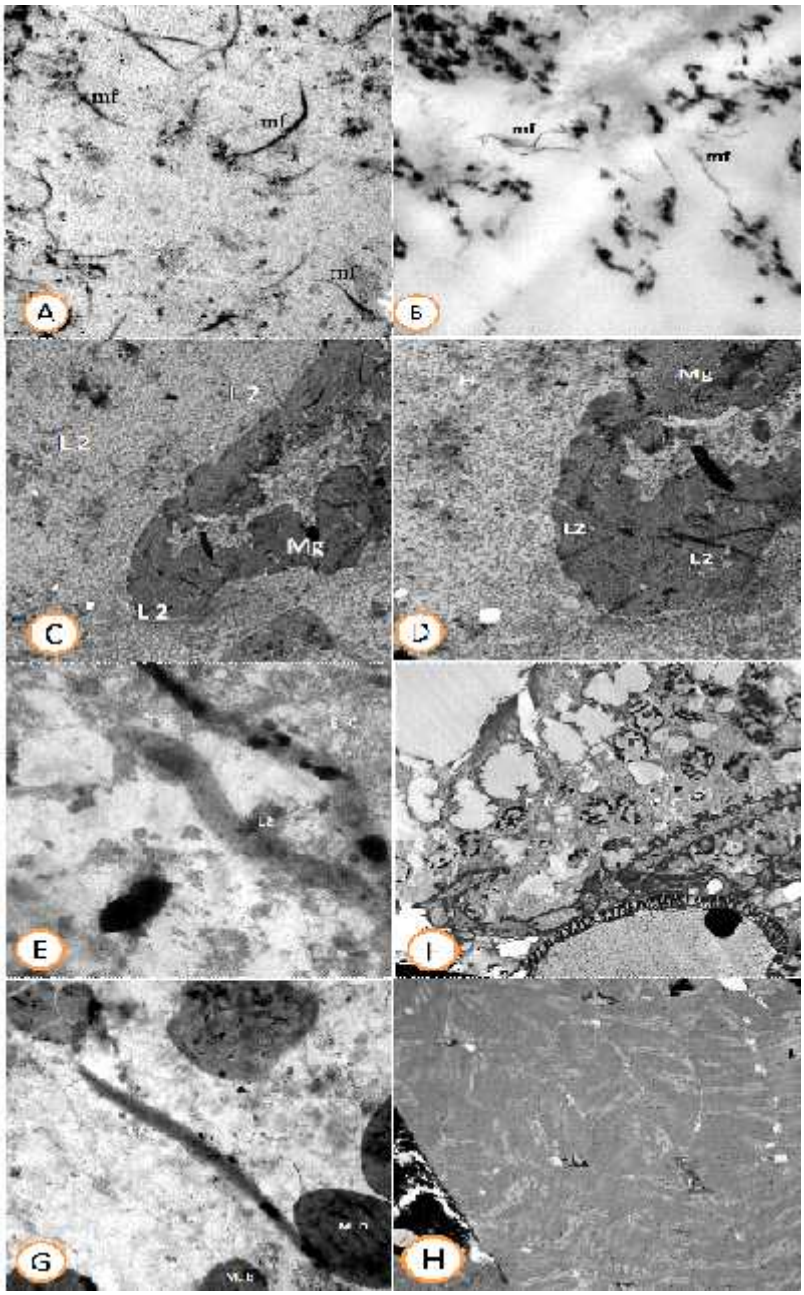

Fig. 1: Electron-micrograph of *Cx. pipiens* females infected with *W. bancrofti*.
A- Control, 3 days (PBM). X =10000, B- Treated, 3 days (PBM). X =30000, C- Control, 6 days (PBM). X =4000, D- Treated, 6days (PBM). X =10000, E- Control, 9 days (PBM). X =20000, F- Treated, 9 days (PBM). X =2000, G- Control, 12 days (PBM). X =15000, H- Treated, 12 days (PBM). X =3000.

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