Tetracycline, a tool for transmission blocking of *Brugia malayi* in *Mastomys coucha*

In the absence of an ideal macrofilaricidal agent to kill adult filarial parasites, interference with the transmission of infection appears to be a logical approach. Efforts made towards elimination and control of filariasis have been vector control, general improvement in hygiene, reducing microfilaria (mf) density in human patients by treating them with available filaricides, namely diethylcarbamazine and Ivermectin which are principally microfilaricidal with limited action against adult filariids[1–4]. However, none of these approaches was found to be promising because of recurrence of microfilaraemia after treatment. Studies with rodent filariid revealed that tetracycline, when administered during the moulting of L_3 to L_4 or L_4 to L_5, affects their further development as evidenced by reduced worm burden in vertebrate host[5–7]. We wanted to investigate whether tetracycline treatment, if carried out in animals harbouring a patent infection, interferes with the subsequent development of microfilaria to L_3 stage in the vector mosquito fed on the blood of these treated animals. The present study was therefore designed with this aim using a sub-periodic strain of human lymphatic filariid, *Brugia malayi*, maintained in rodent host *Mastomys coucha*.

B. malayi infection was experimentally maintained in *M. coucha* through the vector, *Aedes aegypti*. In brief, laboratory-bred mosquitoes were fed on microfilaraemic mastomys, and L_3 were recovered by gentle crushing of infected mosquitoes after 10 ± 1 days of feeding. Hundred L_3 were inoculated subcutaneously in each of the six-weeks-old male mastomys into the neck region.

Two different approaches were made to observe the effect of tetracycline on the development of mf to L_3 stage. (i) Treatment was given to vertebrate host showing progressive rise in peripheral microfilaraemia and the effect was observed on the recovery of L_3 from mosquitoes fed on these treated animals. (ii) Mosquitoes were fed on the blood of untreated infected animals and were served with glucose solution to which tetracycline was added, to observe the effect of tetracycline on the development of normal ingested mf to L_3.

Twenty-eight mastomys harbouring 150-day-old infection and exhibiting high mf counts (> 100 mf/10 µl) were divided into three groups. Groups I and II consisting of 6 and 10 animals respectively, were treated with tetracycline at 200 mg/kg, orally, whereas 12 animals constituting group III were kept as untreated control. Animals of group I received treatment for one week (5 days), whereas animals of group II received treatment for six consecutive weeks (5 days/week).

Different batches of 3–4-day-old mosquitoes were allowed to feed on animals of groups I–III at different time points, i.e. on day 15, 30, 45, 65 and 120 of treatment. Only one animal (whether treated or untreated) was used per cage of mosquito for blood feeding.

Six cages of mosquitoes (each containing at least 100 females) were fed on mf-positive mastomys and maintained thereafter on glucose solution for ten days. Four out of six cages were provided with glucose solution supplemented with tetracycline at 100 µg/ml concentration.

Ten µl of tail blood of microfilaraemic mastomys was drawn at 12.00 noon and spread as thick smear. Smears were air-dried, de-haemoglobinized, stained with Leishman's stain and examined under a compound microscope. Assessment of mf density was made just prior to commencement of treatment, before mosquito feeding, i.e. on day 15, 30, 45, 65 and 120 and thereafter at monthly intervals till 180 days post-treatment (p.t.).

Eight to ten engorged female mosquitoes were taken out of the cage each time, just after blood feeding. They were teased individually in a drop of insect saline (0.6%) on clean glass slide, air-dried and stained as above to examine the number of mf ingested/mosquito.

On day 10 ± 1 post blood-feeding, at least 20 mosquitoes were dissected individually from each cage and the number of L_3 recovered was assessed.

It was observed that five-day-treatment of host did not exert any effect either on the intensity of mf in the peripheral blood of mastomys or on the development of mf to L_3 in mosquitoes. In contrast, the six-week-treatment led to later decrease in the density of microfilaraemia in the blood of mastomys and also showed adverse effect on the larval development in mosquitoes. Decline in microfilaraemia started after 30 days of treatment and continued up to day 120 p.t., resulting into persistence of significantly low mf counts. Untreated animals on the other hand, showed continuous increase in mf counts. Average mf ingestion and L_3 recovery per mosquito in the six-week-treatment group are shown in Figures 1 and 2. It was interesting to note that in spite of almost identical mf ingestion by mosquitoes fed on treated and untreated mastomys on day 45 and day 65 p.t. (Figure 1), recovery of infective larvae from mosquitoes fed on treated mastomys was affected (Figure 2). Tetracycline supplementation in glucose solution did not have any adverse effect on the development of mf to L_3 stage in the vector (data not shown).

Intracellular bacteria in filarial nematodes were first described more than 20 years ago[8]. Various studies done so far, exhibited either the intimate association between bacteria with their filarial hosts[9–11]

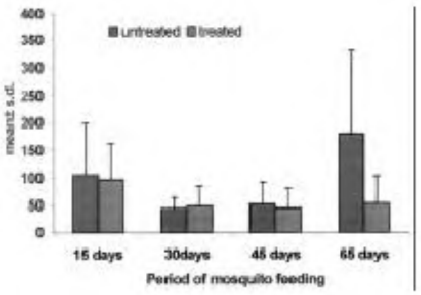

Figure 1. Number of mf ingested/mosquito after feeding on tetracycline (200 mg/kg for six weeks) treated and untreated donor mastomys.

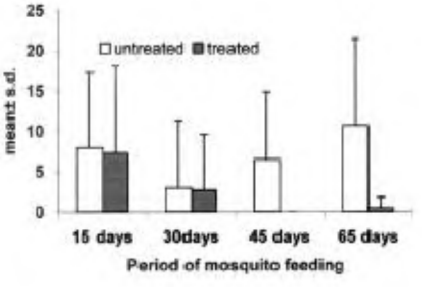

Figure 2. Number of L_3/mosquito obtained after feeding on tetracycline (200 mg/kg for six weeks) treated and untreated mastomys.

or the effect of tetracycline treatment on the establishment of filarial infection in the mammalian hosts[5,6]. The current study was carried out to see whether tetracycline treatment, if carried out in animals harbouring a patent infection, interferes with the subsequent development of mf to L_3 stage in the vector mosquito fed on the blood of these treated animals as well as on the existing microfilaraemia in the peripheral blood of the treated mastomys. Tetracycline, being an antibiotic, was initially tried for five days, but this regimen did not show any effect either on existing microfilaraemia in vertebrate host or on recovery of L_3 from mosquitoes. On the other hand, a longer treatment for six weeks showed miraculous effect on recovery of L_3 from mosquitoes. This treatment also demonstrated lowering of microfilaraemia in vertebrate host. The findings thus reveal that a longer treatment of host with tetracycline is required to obtain antifilarial efficacy. Almost similar findings have been reported by Hoerauf *et al.*[6] who exhibited reduced survival, size and fertility of adult worms of *L. sigmodontis* obtained from jirds treated with tetracycline for 28 days. These worms contained neither bacterium as detected by microscopy nor amplifiable Wolbachia DNA. Their findings thus indicate that the arrested development of filarial parasite after treatment with tetracycline in our study could be due to clearance of Wolbachia. The bacteriostatic activity of tetracycline is known to be related to its accumulation by both active[12,13] and passive[13] transport mechanisms in susceptible species of bacteria and inhibit protein synthesis in prokaryotes by interacting with 16S ribosomal RNA[14]. The present study also revealed that prolonged treatment of filarial host with tetracycline adversely affected the development of ingested microfilariae to infective stage in mosquitoes, without affecting their mf ingestion. Smith and Rajan[7], working with *B. malayi*, *B. pahangi* and *Dirofilaria immitis* in a serum-free *in vitro* system, observed that tetracycline was capable of inhibiting molting of L_3 to L_4 within a dosage range similar to that reported for susceptible rickettsial organisms. Bosshardt *et al.*[5], working with *B. pahangi*, observed low microfilaraemia in jirds treated with tetracycline between 27 and 54 days of infection. It is difficult to explain the exact mechanism for arrested development of mf to L_3 stage in vector mosquitoes. Findings conclude that prolonged treatment with tetracycline provides a good tool to check transmission of filariasis from one host to another, by restricting the larval development in vector mosquitoes as well as by lowering the existing mf in the peripheral blood of the vertebrate host. Nevertheless, reported adverse effects of tetracycline[15,16] associated with possible development of drug resistance limit its frequent use in filaria endemic areas. Therefore, synthesis of a molecule having similar potential with no toxicity would be of use in controlling filarial infection.

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Normapolles group of pollen grains in the Indian Palaeogene palynoassemblage from Ganga Basin, India

Normapolles group of pollen grains with specialized morphocharacteristics of evolutionary significance is a well-recognized group for its restricted stratigraphic occurrence during Upper Cretaceous to Early Tertiary periods[1–3] and also restricted phytogeographical distribution in west Siberia across Europe to eastern North America.

The restricted distribution of the group during late Cretaceous to Early Tertiary was considered to recognize a specific palaeophytoprovince, viz. Euramerian 'Normapolles province' by Zaklinskaya[4]. Three more palaeophytoprovinces have been recognized on the basis of palynological records at this geological period in restricted geographical areas on the globe (Figure 1). The characteristic palynomorphs and the corresponding late Cretaceous–Early Tertiary phytogeographic