EFFECT OF IVERMECTIN ON TWO FILARIA-VECTOR PAIRS

Brugia malayi-Aedes aegypti; Litomosoides sigmodontis-Bdellonyssus bacoti

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SUMMARY

The effect of ivermectin was studied on two filaria-vector pairs, Brugia malayi-Aedes aegypti and Litomosoides sigmodontis-Bdellonyssus bacoti. The rodent hosts, respectively Mastomys coucha and Meriones unguiculatus, were treated with ivermectin doses of 0.05 mg/kg, 0.2 mg/kg or 2 mg/kg. Batches of vectors were fed on rodents, infected or not, treated or not, from H7 to D43 post-ivermectin. Vector survival was observed and dissections were performed to study the filarial development.

It appears that ivermectin has no systemic effect on vectors, or very little. The drug acts on transmission because it affects the microfilariae. Transmission of L. sigmodontis is blocked because microfilariae are eliminated from the blood. Transmission of B. malayi is blocked although microfilaremia remains present at a low level. Two particular features are observed: microfilariae are hyper-ingested, but they do not cross the stomach wall (in contrast, they cross at a high rate in the control batch of Aedes, due to the « stomach wall limitation »). These events might be explained by a muscular passivity of the microfilariae treated with ivermectin. Transmission of the two filarial species is restored normally about D25-40 post ivermectin because a new population of microfilariae has appeared.

INTRODUCTION

The transmission of a filarial worm from an infected vertebrate to an uninfected one requires a combination of phenomena: ingestion of microfilariae by the vector, migration through the vector stomach wall, larval development, vector survival and filarial inoculation.

The goal of this study is to evaluate the effects of ivermectin on two filaria-vector pairs Brugia malayi-Aedes aegypti and Litomosoides sigmodontis-Bdellonyssus bacoti, and to try to define what are the steps of the cycle which are affected by the treatment. This study is particularly concerned with the weak points of the cycle during the development of the filaria in the vectors which are: (1) ingestion of microfilariae (Petit, 1978, 1985; Dickerson et al., 1989) and (2) passage of microfilariae through the stomach wall.
stomach wall of the vector (Bain, 1971; Brengues et Bain, 1972; Chabaud et al., 1986).

**GENERAL METHODS**

Ivermectin (Ivomec bv® for veterinary use, Merck, Sharp and Dohme) was administrated by subcutaneous injection after dilution in propylen glycol. The control rodents received the solvant alone.

Vectors took blood meals at 14-15 hours. Prior to each experiment the microfilaraemia of rodents was evaluated from 10 mm³ of blood taken from the retro-orbital sinus. Microfilariae were counted in Giemsa stained thick blood smears.

Dissection of vectors was done in RPMI/20 % of new born-calf serum.

**I —PAIR BRUGIA MALAYI-AEDES AEGYPTI**

**Material and methods**

a — Biological material

The subperiodic strain of *B. malayi* was maintained in *Mastomys coucha*. The experimental vector was *A. aegypti*, Liverpool strain (isolated by McDonald, 1963). In order to obtain homogeneous breeding, eggs were hatched in hay water and the density was maintained at 200 larvae per liter; adults were obtained after 7/8 days and were used at the age of 5/6 days for the various experiments.

Mosquitoes were maintained 11 days subsequent to the infective feed in order to count the filarial larvae (L3). Some mosquitoes were dissected 17 hours after the blood meal. For each *Aedes*, microfilariae were counted separately in the stomach and in the rest of the abdomen and the thorax; this established the total number of ingested microfilariae (Mfi) and the number of microfilariae which had passed through the stomach wall (MFP).

b — Ivermectin (Iv) doses

A preliminary experiment was done to define the effect of a high dose (5 mg/kg) on microfilaraemia and vectors. The microfilaraemia reached 25 % of initial value at 8 days (D8). It remained very low from day 21 to day 43. About twenty *Aedes* were fed 7 hours after treatment; most were unable to fly 17 hours after the blood meal. This dose was considered too high to allow a proper analysis of *B. malayi* development in the vector. The doses of 0.2 mg/kg and 2 mg/kg was then chosen.

c — Experimental protocol

Four groups of rodents (*M. coucha*) were used:

1 — infected and treated rodents: three at 0.2 mg/kg Iv (Nos. 5, 6, 7), one at 2 mg/kg Iv (No. 8),
2 — infected and untreated rodents: four (Nos. 1, 2, 3, 4),
3 — uninfected and treated rodents: one at 0.2 mg/kg Iv and one at 2 mg/kg Iv,
4 — uninfected and untreated rodent: one.

For each group and each dose, one rodent was chosen for *Aedes* blood meals (Nos. 1, 5, 8 and the 3 uninfected). Following a previous study by Chandre (1990) the choice of the infected untreated rodent was done taking into account the fact that the proportion of microfilariae escaping from the *A. aegypti* stomach increases when the number of ingested microfilariae decreases (« phenomena of stomach wall limitation », Bain, 1971). Thus, it was ensured that the microfilariae of the untreated rodent was low, at a value similar to the value of the microfilaraemia in the rodents after treatment. The blood meals were done at H7, H55, D8, D15, D21, D28, D35 and D43 post-ivermectin. For a given time, batches of *Aedes* were taken from the same larval breeding pool and the blood meals were done simultaneously for the different doses and the control. Forty eight batches of about 73 females each were used for the complete study.

**RESULTS**

a — Mortality of *A. aegypti* 11 days after treatment (Table I)

The dead females were counted and discarded daily. In most cases, mortality was the same in the batches feeding on treated and untreated rodents (< 9 %). A significant mortality was observed only with an ivermectin dose of 2 mg/kg: at H7, for rodents infected or not (respectively 33 % and 24 %) and at H55 only for infected rodents (14 %).

**Table I. — Mortality of *A. aegypti*: comparison of the cumulative percentages 11 days after the blood meal on *M. coucha* (infected or not, treated or not) according to the time post-ivermectin.**

The values in bold type indicate the mortalities significantly higher than those of the controls; H7 to D43: time after injection of ivermectin in hours (H) or days (D); Iv: dose of ivermectin in mg/kg.

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<th>Rodent</th>
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b — Time course of microfilaraemia in *Mastomys coucha*

The average time course of microfilariae is shown on Figure 1; the individual values are presented in Table II.

**Fig. 1. — *B. malayi-M. coucha*: time course of microfilaraemia expressed as percentage of initial microfilaraemia (HO).**

(!): Control; (□): 0.2 mg/kg; (♦): 2 mg/kg; (○): 5 mg/kg.
The microfilaremias remained constant, or increased, for the four infected control rodents (Nos. 1 to 4). The microfilaremias decreased from H7 and reached 25-30% of the initial value at D8 with the five infected treated rodents (Nos. 5 to 9), whatever the dose of ivermectin. The microfilaremias remained at low level until D42; but, between D15 and D42, the reduction of microfilaremias was slightly more pronounced with ivermectin doses of 2 and 5 mg/kg (93-99% of reduction, rodents Nos. 8 and 9) than with the dose of 0.2 mg/kg (87-92% of reduction, rodents Nos. 5, 6, 7).

The microfilariae fell to zero in one case only: rodent No. 6 with ivermectin dose of 0.2 mg/kg, between D21 and D35.

c — Ingestion of microfilariae (Table III)

This study was made with the rodent No. 5 treated at 0.2 mg/kg and the control rodent No. 1.

Results differed between the early phase (D1-D21) and the late phase (D21-after). During early phase, a much higher ingestion of microfilariae was observed with the treated rodent compared to the untreated one, although both had the same microfilaria. The « ingestion efficiency », i.e., the ratio of microfilariae ingested/microfilaria was about three times higher for Aedes batches fed on the control. During late phase, the « ingestion efficiency » was identical in both cases.

d — Passage through the stomach wall (Table III)

Due to the phenomenon of stomach wall limitation the effect of the treatment could be under-estimated if the number of ingested microfilariae is too low. However, the dose 0.2 mg/kg was clearly effective at H7 until D28. The passage through the stomach wall was restored almost to normal at D43.

The number of ingested microfilariae is too low. However, the effect of the treatment could be underestimated if the ratio ingested microfilariae to the microfilaria was identical in both cases.

The mean number of larvae fluctuated between 2.6 and 5.2 per Aedes fed on control rodent No. 1.

— No larvae were found in Aedes fed on rodent No. 5 treated at 0.2 mg/kg, from H7 to D15. In fact, the few microfilariae which had passed through the midgut wall did not develop during this period. The first larvae appeared at D21. A normal amount of larvae were found in Aedes fed at D35 or later, indicating that the transmission had returned to normal.

— No larvae were found in Aedes fed on rodent No. 8 treated at 2 mg/kg, from H7 to D28. The first larvae appeared at D35. At D43, the transmission was normal, for the level of microfilaria.

e — Number of filarial larva recovered (Table IV)

Almost all larvae found at D11 were L3 larvae. The mean number of larvae fluctuated between 2.6 and 5.2 per Aedes fed on control rodent No. 1. No larvae were found in Aedes fed on rodent No. 5 treated at 0.2 mg/kg from H7 to D15. In fact, the few microfilariae which had passed through the midgut wall did not develop during this period. The first larvae appeared at D21. A normal amount of larvae were found in Aedes fed at D35 or later, indicating that the transmission had returned to normal.

— No larvae were found in Aedes fed on rodent No. 8 treated at 2 mg/kg, from H7 to D28. The first larvae appeared at D35. At D43, the transmission was normal, for the level of microfilaria.
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To Summarize

Ivermectin did not affect the survival of *Aedes* at the dose of 0.2 mg/kg Iv but some mortality occurred at the dose of 2 mg/kg Iv; however, in this latter case, the lethal effect was not observed after H55. Ivermectin decreased microfilariae but was unable to completely eradicate the microfilariae. It blocked the transmission from H7 although an hyper-ingestion occurred when microfilariae were taken up from the treated rodents. This interruption was due to the inhability of microfilariae to pass through the midgut wall of mosquitoes.

II — PAIR LITOMOSOIDES SIGMODOntIS-BDEloNYSsuS Bacoti

Material and Methods

a — Biological material

The experimental mammal host of *Litomosoides sigmodontis* (misnamed *L. carinii*, cf. Bain *et al.*, 1989) was the jird *Meriones unguiculatus*. The experimental vector is the mite *Bdellonyssus bacoti*. Breeding methods of this mite have been described by Diagne *et al.* (1990). Female mites only were used for the experiments. For each experiment, a batch of 75 females was poured on a given rodent. Fed females were collected on a water basin, placed under the rodent cage. They were kept in small tubes containing 20 mites each.

b — Experimental protocol

Four groups of jirds were used:

1. infected treated rodents: one at 0.05 mg/kg (No. 1), six at 0.2 mg/kg (Nos. 2 to 7) and three at 2 mg/kg (Nos. 8 to 10),
2. infected untreated rodents: three,
3. uninfected treated rodents: one at 0.05 mg/kg, one at 0.2 mg/kg and one at 2 mg/kg,
4. uninfected untreated rodent: one.

In each group and for each dose, one rodent was chosen for the mite blood meals (8 rodents in total). The blood meals were done at H7, H31, H55, D4, D8, D16 and D29 post-ivermectin. Fifty-six batches of 75 mites were used for the complete study.

Results

a — Feeding capacity of *B. bacoti* and mortality during the blood meal

An average of 42.4 mites per batch were engorged on rodents, treated or not. A low percentage of mites died during the blood meal, not related to the ivermectin doses.

b — Mortality of *B. bacoti* 11 days after bloodmeal

The dead mites were counted daily, and not removed from the tubes. The percentage of dead mites was similar in treated and untreated batches, with an average of 18% overall (extremes: 3% - 58%).

c — Fecundity of *B. bacoti*

The protonymphs were counted at D11. Their mean numbers per female mite did not vary in the different batches; these means were 6.7, 7.2, 6.7 and 6.2 respectively for the batches fed on the controls, on the rodents treated at 0.05 mg/kg Iv, or 0.2 mg/kg, or 2 mg/kg.

d — Time course of microfilaraemia in *Meriones unguiculatus* (Fig. 2)

The mean microfilaraemia of the three infected but untreated controls remained stable from DO to D42 and was about 2,400 Mf/10 mm³.

The mean pretreatment microfilaraemias in the 10 treated rodents were 1,860 Mf/10 mm³ for the rodent No. 1, 5,250 Mf/10 mm³ for Nos. 2 to 7, and 6,910 for Nos. 8 to 10. The mean microfilaraemias were reduced to more than 99% from H7, irrespective of the ivermectin doses. In all cases, microfilaraemias fell to zero, from H55 generally. Positive microfilaraemias reappeared in all rodents, and more or less rapidly according to the ivermectin dose: on D8 for No. 1 treated at 0.05 mg/kg, D16 for No. 2 to 7 treated at 0.2 mg/kg, D29 for Nos. 8 to 10 treated at 2 mg/kg. Microfilaraemias did not reach the pretreatment values at D42: 870 Mf/10 mm³ for No. 1, 950 Mf/10 mm³ for Nos. 2 to 7, and 15 Mf/10³ for Nos. 8 to 10.

e — Number of filarial larvae recovered

The mean numbers of L3 larvae per mite fluctuated from 1.0 to 3.9 L3 in the batches fed on the untreated rodent, and these figures followed the fluctuations of its microfilaraemia (1,850 to 5,580 Mf/10 mm³). These mean L3 values were in accord with those from Diagne *et al.* (1900).

No L3 larvae were recovered from H7 to J16 post-ivermectin in the mite batches fed on treated infected rodents, whatever the ivermectin doses. Transmission was...
restored at D29 for 0.05 and 0.2 mg/kg Iv, with respective means of 1.2 and 0.6 L3 per mite after feeding on microfilaremias of 790 et 1,050 Mf/10 mm³; these L3 values were in accord with dose from Diagne et al. (1990). Transmission was not restored at D29 with the rodent treated at 2 mg/kg Iv and with a very low microfilaremia (12 Mf/10 mm³).

To summarize

Ivermectin had no effect on the feeding, survival and fecundity of the female B. bacoti.

The mite vectorial capacity depended strictly upon the microfilarial density in the jird: transmission, which was temporarily blocked because microfilariae were eliminated from the blood, was restored to normal as soon as the microfilarial density was sufficient.

Discussion and conclusion

In the two pairs of filaria-vectors studied, ivermectin has little or no effect on the vector. The drug acts on the filarial transmission because it affects the microfilariae.

With L. sigmodontis, the microfilaremia drops down during the first hours following the treatment; this fits with the results of Soeffner and Wenk (1985), who show that it is caused by the accumulation of blood microfilariae in the deep organs, such as the spleen and the liver, in which they are destroyed over the next two days. The release of the uterine microfilariae is also interrupted temporarily by the paralyzing action of the drug on the female filariae (Zahner et al., 1987; Rao et al., 1990a). Later, the positive microfilaremias correspond to the recovery of microfilarial release by the female worms. These new microfilariae are not altered, as demonstrated by the transmission rate, which is normal (Fig. 3A).

With B. malayi, the ivermectin effect is more complex: transmission is blocked for several days although the microfilariae, which decrease, generally remain present (Fig. 3B).

Rao et al. (1990b) have shown that Aedes aegypti ingest microfilariae, after the ivermectin treatment, but that no larval development occurs. Our experiments demonstrate 1) an hyper-ingestion of the microfilariae by the mosquitoes fed on the treated rodents, 2) the inability of these ingested microfilariae to pass through the stomach wall of the vector. These two phenomena may be explained by an ivermectin induced inhibition of the neuro-muscular coordination in the microfilariae: these passive microfilariae might be drawn up more easily by the vector, but they cannot cross the barrier of the stomach epithelium. The hyper-ingestion in B. malayi-A. aegypti may be compared to the hypo-ingestion observed after treatment of Onchocerca volvulus in black flies (Prod’hon et al., 1987); in that case, the surviving microfilariae are found deeper in the skin (Jurgens and Schulz-key, 1990) because they are unable to maintain themselves in the peripheral lymphatic vessels, and so are passively drained with the lymph into the deep lymphatic vessels (Vuong et al., 1992).

The B. malayi transmission, with an ivermectin dose of 0.2 mg/kg, is recovered at low level at D21-28, then normally at D35, although the microfilaremia is not increased. This suggests that the release of microfilariae from the female filariae is recovered after D21, but microfilaremia remains constant because an equilibrium is created between the progressive destruction of the previous microfilariae and the release of the new microfilariae. About one month after treatment, most of the microfilariae are new, the transmission is recovered, with an excellent rate of development due to the phenomenon of stomach wall limitation. A similar event occurs in blackflies transmitting O. volvulus in the African Savanna; this explains why ivermectin treatment has not been able to block the transmission in that region (Remme et al., 1990).

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REFERENCES


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