

Cumulative mortality rates in *Aedes polynesiensis* after feeding on polynesian *Wuchereria bancrofti* carriers treated with single doses of ivermectin, diethylcarbamazine and placebo

J.-L. Cartel, Y. Sechan, A. Spiegel, L. Nguyen, Ph. Barbazan, P. M. V. Martin, J.-F. Roux
Institut Territorial de Recherches Medicales Louis Malarde, Papeete, Tahiti, Polynesie Francaise

Abstract

During a therapeutic trial, batches of 672 to 1979 laboratory-bred *Aedes polynesiensis*, the mosquito vector of lymphatic filariasis in French Polynesia, were fed on *Wuchereria bancrofti* carriers one, three and six months after they had been treated with either single doses of ivermectin at 100 mcg/kg, diethylcarbamazine (DEC) at 3 and 6 mg/kg or placebo. High mortality rates were observed during the 15-day period following the blood-meal in mosquitoes fed on carriers treated with microfilaricidal drugs and were significantly higher in mosquitoes fed on carriers treated with ivermectin than in those fed on carriers treated with DEC. Though its intensity decreased with the passage of time, the phenomenon was observed in mosquitoes fed on carriers up to six months after treatment, especially in those fed on carriers treated with ivermectin. By decreasing the number of mosquitoes able to transmit the infection, this lethal effect on *Ae. polynesiensis* might represent an additional advantage of ivermectin in lymphatic filariasis control programmes.

Introduction

The results of a single-blind, dose-ranging study conducted in French Polynesia between 1986 and 1988 have indicated that a single dose of 100 mcg/kg ivermectin resulted in the best efficacy and treatment tolerance for *Wuchereria bancrofti* carriers (Roux et al., 1989). Also, a single 200 mcg/kg dose induced a reduction in transmission by the vector *Aedes polynesiensis* (Cartel et al., 1990). Therefore, in 1989, a double-blind, placebo-controlled trial was implemented, the objectives of which were (i) to compare the efficacy and safety of single doses of ivermectin at 100 mcg/kg, diethylcarbamazine (DEC) at 3 mg/kg (the current standard regimen for mass treatment of Bancroftian filariasis in French Polynesia) and DEC at 6 mg/kg and (ii) to determine the effects of these 3 regimens on transmission by mosquitoes fed on carriers treated with effective drugs as compared to those fed on placebo treated carriers.

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Materials and methods

Of 68 microfilariae (mf) carriers who were included in the clinical trial and allocated randomly to treatment with a single dose of ivermectin 100 mcg/kg, DEC 3 or DEC 6 mg/kg, or placebo, 38 participated in the entomological study. For the present study of mortality rates, batches of laboratory-bred *Ae. polynesiensis* were blood-fed on 26 mf carriers (8, 7, 3 and 8 respectively were treated with ivermectin 100 mcg/kg, DEC 3 mg/kg, DEC 6 mg/kg and placebo). All mosquitoes were 3 days old when blood fed. At 1, 3 and 6 months after treatment, one arm of each carrier was enclosed for 10-15 minutes in a cage of mosquitoes (range 672 to 1979 mosquitoes per cage, median 1314). For the following 15 days the cages were stored at 27-29°C in a constant-temperature room. The 10% sucrose solution used for mosquito feeding was removed 18 h before the blood-meal. Every morning, from the 2nd to the 15th day after the blood-meal, both the numbers of "naturally-dead" mosquitoes and mosquitoes killed for dissection were recorded. On the 15th day, the surviving mosquitoes were killed and numbered.

The study was kept blind until 7 months after treatment, neither carriers, nor clinicians or entomologists were aware of the nature of the drug administered. Cumulative survival probabilities were calculated by the life-table method (Laplanche et al., 1981). The overall differences between the life-table survival rates were estimated using the log-rank summary chi-square statistic (Peto et al., 1977).

Results

The main results of both the clinical and entomological studies have been reported elsewhere (Barbazan et al., 1990; Cartel et al., 1991). In the current study, the percentages of fed and unfed mosquitoes (less than 2% were unfed until the 3rd day after the blood-meal, all had taken a blood-meal by the 4th day) as well as the mean numbers of *W. bancrofti* larvae at different stages were similar in "naturally-dead" and in killed mosquitoes. The overall cumulative survival probabilities were significantly lower ($p < 0.001$) both in mosquitoes fed on carriers treated with single doses of microfilaricidal drug (either ivermectin or DEC) compared to those fed on placebo treated carriers. Survival was also lower in mosquitoes fed on ivermectin treated carriers compared to those fed on DEC treated carriers at 1, 3 and 6 months after treatment (fig. 1, 2 and 3). Nevertheless, because the DEC 6 mg/kg and ivermectin 100 mcg/kg survival curves cross at 3 months after treatment (fig. 2), caution has to be taken in interpretation. The cumulative mortality rates were roughly similar (about 90%) by the 15th day after blood-meal, in mosquitoes fed on carriers 1 month after they had been treated with either single dose of ivermectin or DEC (6 and 3 mg/kg); by comparison, they were less than 20% by the same day after blood-

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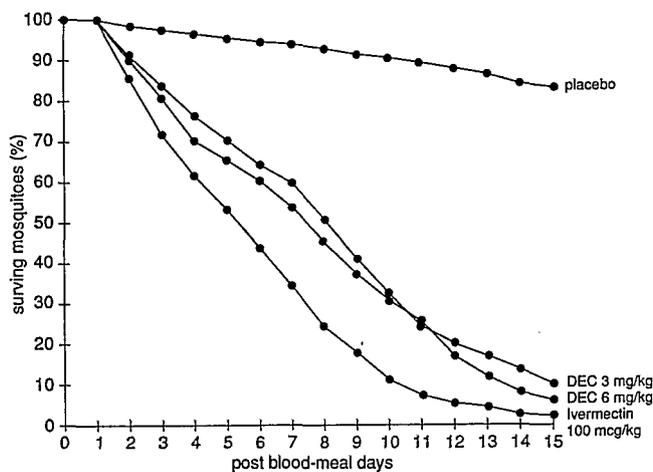


Fig. 1 Cumulative survival probabilities in *Ae. polynesiensis* fed on *Wuchereria bancrofti* carriers by one month after treatment with placebo, DEC and ivermectin single doses

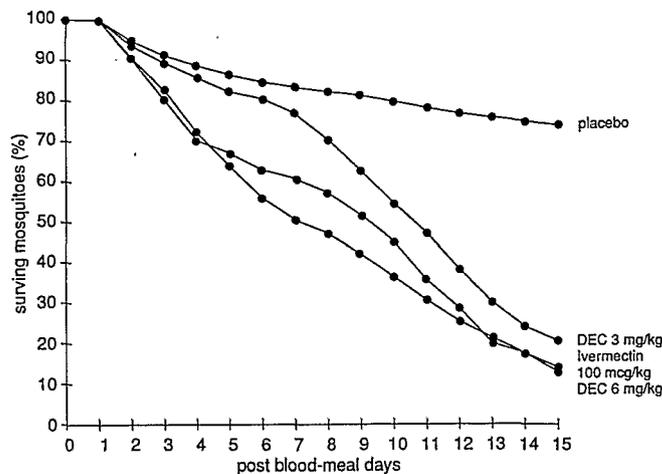


Fig. 2 Cumulative survival probabilities in *Ae. polynesiensis* fed on *W. bancrofti* carriers by three months after treatment with placebo, DEC and ivermectin single doses.

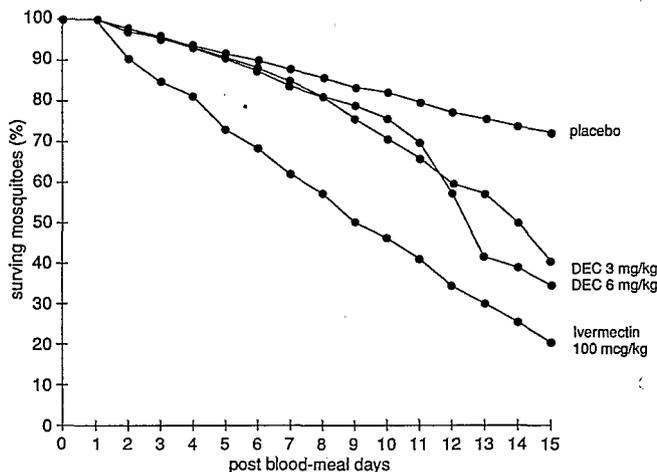


Fig. 3 Cumulative survival probabilities in *Ae. polynesiensis* fed on *W. bancrofti* carriers by six months after treatment with placebo, DEC and ivermectin single doses

meal in mosquitoes fed on carriers treated with placebo (table 1). In mosquitoes fed on carriers 3 months after they had been treated with microfilaricidal drugs, cumulative mortality rates were still of 80% or more by the 15th day after blood-meal when they were of 26% in mosquitoes fed on carriers treated by placebo. They were of 80% in mosquitoes fed on carriers 6 months after they had been treated with ivermectin, significantly higher ($p < 0.01$) than in mosquitoes fed on carriers who had been treated with DEC (either at 6 or 3 mg/kg).

The summarized results of our study may be expressed as follows: (1) mortality rates were much higher in mosquitoes fed on carriers treated with microfilaricidal drugs than in those fed on placebo treated carriers and significantly higher in mosquitoes fed on ivermectin than on DEC treated carriers, (2) they were roughly similar each day during the 15-day period following the blood-meal and (3) the phenomenon was observed up to 6 months after treatment though its intensity decreased with the passage of time.

Discussion

The efficacy of ivermectin in killing ectoparasites in animals has been well established (Benz et al., 1989; Soll, 1989). In a study conducted in Australia, ivermectin administered subcutaneously to cattle resulted in immediate high mortality of *Culicoides brevitarsis* (Standfast et al., 1984). More recently, acute toxicity and 100% mortality have been observed in *Ae. aegypti*, *Ae. albopictus* and *Culex quinquefasciatus* fed a blood-ivermectin mixture through a chick skin membrane (Tesh and Guzman, 1990). Nevertheless, to our knowledge, mortality has not been reported in insects fed either on animals or on humans such a long time after treatment as 1, 3 or 6 months. In the above studies, the death of insects was nearly 100% in the few days (1–2) following the blood-meal although in our study the daily mortality rate remained stable (about 8%) over the 15-day period following the blood-meal. This is possibly due to the fact that, in the current study, mosquitoes were not fed on carriers immediately after treatment. Regarding DEC, its efficacy in reducing the transmission of filariasis by the vector mosquitoes was first reported by Laigret et al. (1965) on the basis of epidemiological analysis, and also from entomological studies (Chen and Fan, 1977) but the reduction was attributed to the effect of DEC on the development of *W. bancrofti* larvae and not to the death of vectors.

The reasons why a single dose of 100 mcg/kg ivermectin induced important mortality in mosquitoes fed on carriers several months after they had been treated remain difficult to explain. It is known that, in humans treated with a 6 mg dose, concentration of ivermectin peaks at 4 hours after the intake and that peak plasma concentration metabolites occur somewhat later (Fink and Porras, 1989). Concerning elimination of ivermectin from the body fluids and tissues, it has been shown in rats that radioactive residues have practically disappeared from blood at 4 days after treatment (Chiu and Lu, 1989). Presently, to our knowledge, there is no evidence that residues of ivermectin or its metabolites could persist for months in humans and be the cause of death of biting insects. Same remarks apply to DEC which has been demonstrated, at dosage of 10 mg/kg, to provoke a peak blood concentration in

Table 1 Cumulative mortality rates at 8th and 15th days after blood-meal in *Ae. polynesiensis* fed on *W. bancrofti* carriers by 1, 3 and 6 months after treatment with ivermectin, DEC, and placebo

| Post-treatment months | Post blood-meal days | Cumulative mortality in mosquitoes fed on carriers treated with: | | | |
|-----------------------|----------------------|--|-------------|-------------|---------|
| | | Ivermectin 100 mcg/kg | DEC 6 mg/kg | DEC 3 mg/kg | Placebo |
| 1 | 8 | 76% | 55% | 49% | 7% |
| | 15 | 98% | 94% | 90% | 17% |
| 3 | 8 | 53% | 43% | 29% | 18% |
| | 15 | 86% | 87% | 79% | 26% |
| 6 | 8 | 43% | 19% | 19% | 14% |
| | 15 | 80% | 65% | 60% | 28% |

3 hours which decreases to zero within 48 hours (Mackenzie and Kron, 1985). Nevertheless, when considering the phenomenon we have reported and the decrease of its intensity with the passage of time, it might be speculated that drug residues persist in tissues and affect mosquitoes when biting treated humans. Whatever the explanation, the death of mosquitoes feeding on treated populations, more especially on populations treated with ivermectin, may represent an additional advantage in filariasis control strategy. This finding should be taken into account when efficacy of mass treatments in filariasis control programmes is assessed not only on the evolution of microfilaremia in carriers but also on the transmission by the vector mosquitoes. Further studies are planned in French Polynesia to assess whether similar results are observed in *Ae. polynesiensis* and in *Ae. aegypti* fed on *W. bancrofti* carriers as well as on non microfilaremic volunteers treated with single doses of either ivermectin or DEC.

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Jean-Louis Cartel

Institut Territorial de Recherches Médicales Louis Malardé
B. P. 30, Papeete
Tahiti, Polynésie Française