[Jpn. J. Parasitol., Vol. 40, No. 2, 142–146, April, 1991]

Suppression of Fecundity of *Brugia malayi* in the Jird, *Meriones unguiculatus*, by Repeated Administration of Diethylcarbamazine

RYUICHIRO MAEDA[1], YOSHIHIRO HAYASHI[2], EMI MAKITA[1], TOSHIRO SHIBUYA[1] AND HIROSHI TANAKA[3]

(Accepted for publication; March 12, 1991)

Abstract

The purpose of this study was to describe more clearly the efficacy of diethylcarbamazine (DEC) on microfilaremia in jirds. Previously it was shown that microfilaria (Mf) density in peripheral blood decreased within one hour after a single administration of DEC, but returned to pretreatment level after 24 hours at dose up to 200 mg/kg. In this study, when DEC was administered intraperitoneally to jirds infected with *Brugia malayi* at 50 mg/kg for 5 consecutive days, the average Mf density decreased to the level lower than 10% of the initial counts on the 5th day after the onset of treatment and was suppressed at a low level for more than 4 weeks. The recovery rates of adult worms ranged from 11.3% to 45.4% in treated animals and 13.8% to 17.0% in control animals. There was no significant difference in recovery rates between the two groups. The proportion of female worms found to be producing Mf ranged from 13.3% to 37.5% in the treated group and 33.3% to 73.3% in the control group. There was a significant difference in fecundity between these two groups ($p<0.05$). It is clear from this experiment that the repeated administration of DEC has microfilaricidal effect and suppresses Mf productivity of female worms in the *B. malayi*-jird model.

Key words: *Brugia malayi*, jird, diethylcarbamazine, fecundity, filariasis, chemotherapy

Introduction

In a previous paper (Maeda *et al.*, 1988a), the time course of microfilaremia after infection and the periodicity of microfilaremia were described for jirds infected with *B. malayi*. The paper also reported the development of an efficient method for generating and selecting infected jirds for quantitative studies of microfilaremia.

Using this *B. malayi*-jird model, the microfilaricidal effect of DEC was studied and a new method of macrofilaricide assessment, by monitoring microfilaremia after test drug and DEC administration, was established (Maeda *et al.*, 1988b). Microfilaria (Mf) density in peripheral blood was found to decrease within one hour after 50 mg/kg of DEC was given intraperitoneally. After 24 hours, Mf levels recovered to pretreatment levels. Even though the dose of the drug was increased to 200 mg/kg, the average Mf density went back to initial level after 24 hours. Prolonged suppression of Mf density at low levels, as seen in human cases, was not observed.

The efficacy of DEC on Mf in jirds is not clear from previous works (Denham *et al.*, 1977, Hawking, 1973, Matsuda *et al.*, 1976, Tanaka *et al.*, 1981, Yamashita *et al.*, 1983) except for one report using the *Litomosoides carinii*-jird model (Sturm *et al.*, 1974). However, preliminarily, we have found that when DEC was administered repeatedly to the *B. malayi* infected jirds. Mf density decreased and was maintained at low levels for 4 weeks after treatment (Maeda *et al.*, 1988b).

In this paper, the effect of repeated ad-

[1] *Department of Parasitology, Teikyo University School of Medicine, Itabashi-ku, Tokyo 173, Japan*
[2] *Department of Veterinary Anatomy, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*
[3] *The Public Health Laboratory of Chiba Prefecture, Chiba-shi, Chiba 280, Japan*
前田龍一郎　牧田絵美　渋谷敏朗（帝京大学医学部寄生虫学教室）
林　良博（東京大学農学部獣医学科解剖学教室）
田中　亮（千葉県衛生研究所）

ministration of DEC on adult and Mf of *B. malayi* in jird is described in detail.

Materials and Methods

Infected jirds: The method of inoculation of larvae into jirds was described elsewhere (Maeda *et al.*, 1988a). Briefly, jirds were inoculated subcutaneously with 100 to 200 infective stage larvae of *B. malayi*. After 6 to 9 months, Mf density was examined and the animals harbouring more than 10 Mf/μl of blood were selected for the experiment.

DEC administration: DEC citrate, donated by TANABE SEIYAKU CO. LTD., was given intraperitoneally at a dose of 50 mg/kg for 5 consecutive days. Saline was given to control animals on the same schedule.

Blood examination: To examine Mf density, 4 films were prepared with 5 μl of blood taken from the tail vein of each animal as described in Fig. 1 and Fig. 2.

Recovery of adult worms: The jirds were anesthesized and necropsied after the last examination of Mf density. Adult worms were recovered according to the method described previously (Maeda *et al.*, 1988b). Adult worms were placed in the wells of a cell culture plate and kept at 37°C for 4 hours in Tyrode's solution to be examined under the microscope for sex and viability.

Examination of fecundity of female worms: After the determination of the sex of worms, the fecundity of female worms was examined by existence of active Mf produced in the solution.

Results

Microfilaria density in peripheral blood decreased within one hour after a single administration of DEC, but it returned to pretreatment level after 24 hours even when 200 mg/kg DEC was given. This temporary decrease and recovery of Mf density was observed not only in newly infected jirds but also in old ones.

However, when diethylcarbamazine was administered intraperitoneally into jirds infected with *B. malayi* at 50 mg/kg for 5 consecutive days, the average Mf density decreased to the level less than 10% of the initial count on the 5th day after the onset of treatment.

Fig. 1 shows a typical change in Mf density in peripheral blood during and after the repeated administration of DEC. Each point and vertical bar in the figure indicate the mean and the standard deviation of Mf counts in 4 blood films.

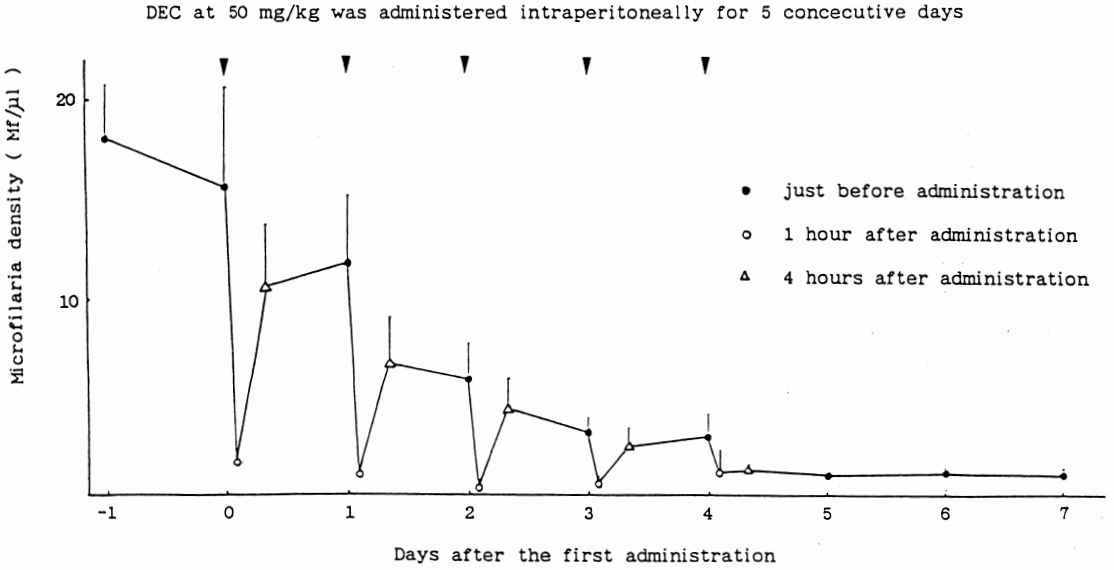
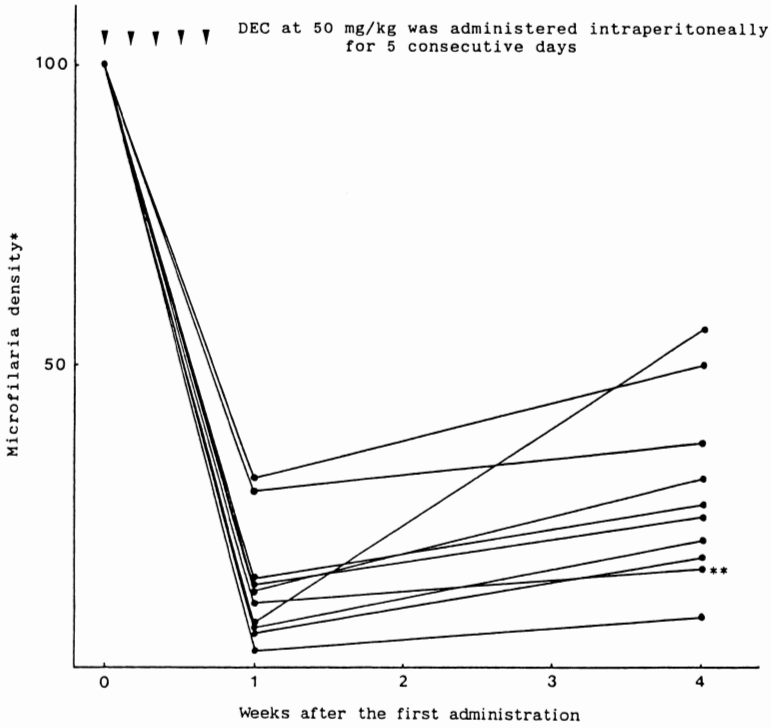


Fig. 1. Effect of repeated administration of diethylcarbamazine on microfilaria density of *Brugia malayi* in jird.



* microfilaria density before treatment was set to be 100.
** The difference between microfilaria density at 1 and 4 weeks was not significant in this animal.

Fig. 2. Effect of repeated administration of diethylcarbamazine on microfilariae of *Brugia malayi* in jird.

Prolonged filaricidal effect of repeated administration of DEC was observed for 4 weeks using 10 jirds (Fig. 2). The Mf density was clearly decreased in all jirds 1 week after the first administration of the drug. In 8 of 10 animals, no Mf were observed 4 hours after the 4th administration of drug. All jirds showed no Mf at 4 hours after the 5th administration.

In nine of 10 jirds used in this experiment, Mf density increased significantly from 1 to 4 weeks after the treatment. This increase was not observed in five jirds which had been patent for more than 12 months before treatment.

After the observation of Mf density for 4 weeks, the jirds were anesthesized and necropsied and the adult worms were recovered. The recovered worms were individually incubated at 37°C for 4 hours in Tyrode's solution to examine their viability and productivity of Mf.

The recovery rate of adult worms ranged from 11.3% to 45.4% in treated animals and 13.8% to 17.0% in control animals. There was no significant difference of recovery rate between the two groups.

The proportion of female worms which produced Mf ranged from 13.3% to 37.5% in the treated group and 33.3% to 73.3% in the control group (Table 1). There was a significant difference between the proportions of two groups ($p<0.05$).

Female worms were also recovered from four untreated animals with chronic infections. None of these produced Mf after 4 hour cultivation.

Table 1 The number of adult worms recovered and the productivity of microfilaria in females

	Months after infection	No. of infective larvae incoulated	No. of adult worms recovered				Microfilaria productivity in females	
			Male	Female	Total	Recovery (%)	No. of females with Mf	Mf positive rate (%)
DEC treated	7	151	15	24	39	25.8	9	37.5
	8	194	13	25	38	19.6	8	32.0
	8	149	9	18	27	18.1	5	27.8
	8	195	13	26	39	20.0	4	15.4
	6	196	42	47	89	45.4	14	29.8
	8	144	11	15	26	18.1	2	13.3
	8	194	10	16	26	13.4	3	18.8
	8	186	10	11	21	11.3	2	18.2
	8	194	28	45	73	37.6	16	35.6
	9	150	5	14	19	12.7	4	28.6
Control	8	195	10	18	28	14.4	10	55.5
	8	200	11	23	34	17.0	15	65.2
	7	176	10	15	25	14.2	11	73.3
	8	196	12	15	27	13.8	5	33.3
	15	182	17	22	39	21.4	0	0.0
	16	171	17	16	33	19.3	0	0.0
	16	192	18	19	37	19.3	0	0.0
	15	196	15	27	42	21.4	0	0.0

Discussion

Diethylcarbamazine citrate has been widely used as a filaricide for the last 40 years and has resulted in the reduction of microfilarial rate and densities in many endemic areas of lymphatic filariasis, yet its mode of action remains unknown. Jirds are highly susceptible to *Brugia* infection and they would seem to be an animal model of choice for studying DEC action. But the efficacy of DEC on Mf in jirds is not clear from most previous work (Denham *et al.*, 1977, Hawking, 1973, Matsuda *et al.*, 1976, Tanaka *et al.*, 1981, Yamashita *et al.*, 1983), except for one report using the *L. carinii*-jird model (Sturm *et al.*, 1974). In a previous paper (Maeda *et al.*, 1988b), we reported that when 50 mg/kg DEC was administered for 5 consecutive days to *B. malayi* infected jirds, Mf density decreased and was suppressed at a significantly low level for 4 weeks.

In the chemotherapy of microfilaraemic patients, it is generally accepted that it is unimportant to the final outcome whether the drug is given daily, weekly or monthly as long as sustained treatment is given to a final total dose of 36 mg/kg for *B. malayi* and 72 mg/kg for *Wuchereria bancrofti* infections (Sasa, 1976). However, the regimen of drug administration has an influence on microfilaremia in the *B. malayi*-jird model. The repeated administration continuously suppressed Mf density to low level, but the single administration did not suppress Mf density even when the same amount of DEC in total was used.

According to Kimura *et al.* (1984), the concentration of DEC in the blood of jirds increased just after 100 mg/kg was given by intraperitoneal injection and then decreased immediately. Four hours after the administration, DEC could not be detected by gas-liquid chromatography. This rapid metabolism or excretion of DEC may explain the lack of change in the Mf density and ineffectuality of single dose administration in the jird. The quick recovery of Mf density in blood 4 hours after drug administration may be the

result of Mf temporarily sequestered when the concentration of DEC was transiently high in the blood. This recovery, following the single dose, was much faster than the gradual increase in Mf density that occurred after five doses. The slow increase in Mf density might be caused by new Mf production by female worms, suggesting that after the 4th or the 5th administration sequestered Mf may be unable to repopulate circulating blood. On the other hand the decreases of the fecundity in female worm caused by repeated DEC may also contribute to the differences observed between single dose and multiple dose Mf density recovery.

The Mf density was suppressed after the repeated administration of DEC, but low microfilaraemia was still observed in all jirds. It has been observed in human cases that some patients remained microfilaraemic at low level for a few months after DEC treatment. Mak and Zaman (1980) reported that 36% and 9% of *B. malayi* patients given total DEC doses of 36 mg/kg and 42 mg/kg respectively, continued to have low density microfilaraemia. It was shown in our experiment that the repeated administration of DEC significantly suppressed Mf productivity in female worms. But this suppression was not complete and there remained some worms which continued to produce Mf after treatment in each jird.

However, the prolonged low density microfilaraemia following the 5th administration could not be explained by the number of females that lost the ability of Mf production altogether. If all the Mf produced by female worms from one week until four weeks after the treatment remained alive, the Mf density at 4 weeks should have been much higher than the observed level. The possibility of a higher level of Mf attrition from one to four weeks post treatment may be due to DEC effects on the female worm or the host immune/inflammatory mechanism.

It is clear from this experiment that the repeated administration of DEC has a microfilaricidal effect and suppresses Mf productivity of female worms in the *B. malayi*-jird model.

Acknowledgment

The authors wish to thank Professor Bruce Hammerberg, Department of Parasitology, North Carolina State University, for his reviewing the manuscript.

References

1) Denham, D. A. and McGreevy, P. B. (1977): Brugian filariasis. In Advances in Parasitology, Dawes, B., ed., Academic Press, London, 243–309.
2) Hawking, F. (1973): Chemotherapy of tissue nematodes. In Chemotherapy of Helminthiasis, Cavier, R. and Hawking, F., eds., Pergamon Press, Oxford, 437–500.
3) Kimura, E., Aoki, Y., Nakajima, Y. and Niwa, M. (1984): Plasma level of diethylcarbamazine in jirds and hamsters. Southeast Asian J. Trop. Med. Pub. Hlth., 15, 74–79.
4) Maeda, R., Hayashi, H. and Shibuya, T. (1988a): Basic studies on the laboratory assessment of macrofilaricides using *Brugia malayi* in the jird, *Meriones unguiculatus*. 1. Longevity and periodicity of microfilaremia. Jpn. J. Exp. Med., 58, 39–44.
5) Maeda, R., Hayashi, H. and Shibuya, T. (1988b): Basic studies on the laboratory assessment of macrofilaricides using *Brugia malayi* in the jird, *Meriones unguiculatus*. 2. Establishment and evaluation of a new method of macrofilaricide assessment. Jpn. J. Exp. Med., 58, 45–49.
6) Mak, J. W. and Zaman, V. (1980): Drug trials with levamisole hydrochloride and diethylcarbamazine citrate in Bancroftian and Malayan Filariasis. Trans. Roy. Soc. Trop. Med. Hyg., 74, 285–291.
7) Matsuda, H., Takaoka, M. and Tanaka, H. (1976): Effect of diethylcarbamazine on microfilariae of *Litomosoides carinii* in jird, *Meriones unguiculatus*. Jpn. J. Parasitol., 26, 101–112.
8) Sasa, M. (1976): Properties of main chemicals to be used for the control of filariasis. In Human filariasis, University of Tokyo Press, 643–647.
9) Sturm, P. A., Henry, D. W., Thompson, P. E., Zeigler, J. B. and McCall, J. W. (1974): Antifilarial agents. Diazabicyclooctanes and diazabicycloheptanes as bridged analogs of diethylcarbamazine. J. Med. Chem., 17, 481–487.
10) Tanaka, H., Takaoka, T., Matsuda, H. and Hayashi, Y. (1981): Effect of diethylcarbamazine on microfilariae of *Brugia malayi* in the jird, *Meriones unguiculatus*. Jpn. J. Exp. Med., 51, 195–196.
11) Yamashita, S., Shigeno, S., Kimura, E. and Aoki, Y. (1983): The effect of diethylcarbamazine on microfilariae of *Brugia pahangi* in Mongolian jirds (*Meriones unguiculatus*). Trop. Med., 25, 113.