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Delayed macrofilaricidal activity of diethylcarbamazine against *Brugia pahangi* in Mongolian jirds

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Abstract

The macrofilaricidal activity of diethylcarbamazine (DEC) was confirmed in jirds infected with *Brugia pahangi*. Seventy jirds were inoculated subcutaneously with 100 infective larvae. At 20 weeks post-infection, the microfilaraemic jirds were divided into two groups, untreated and treated. For the treated group, 200 mg kg^{-1} of DEC was injected intraperitoneally for 5 consecutive days. One, 4, 8, 12, 16 and 27 weeks after the final treatment, 4–7 jirds in each group were sacrificed to measure adult worm burdens. The number of adult worms recovered from treated jirds was comparable to controls at earlier necropsy (1 and 4 weeks post-treatment). However, at late necropsy (8 weeks and later) the recovery rate of adult worms in treated jirds was significantly lower than that in untreated controls, indicating an adultcidal effect of DEC. The present study demonstrates that DEC requires 8 weeks to kill *B. pahangi* adult worms in jirds and that the Mongolian jird is a useful model for screening antifilarial activity.

Introduction

Diethylcarbamazine (DEC) is still the most important drug for the treatment of lymphatic filariasis since it was discovered by Hewitt *et al.* (1947) to be effective against filarial infections. The drug is known to have marked microfilaricidal activity but it has no appreciable action against adult worms. Thus the development of effective macrofilaricides and inhibitors of microfilarial production has been identified by the World Health Organization (WHO) as goals for research on the chemotherapy of filarial infections (TDR, 1997). To develop new drugs, animal experiments are required before human trials. For primary *in vivo* screening of potential compounds, adult worms of *Brugia pahangi* and *Dipetalonema viteae* transplanted into the peritoneal cavity of jirds (*Meriones unguiculatus*) have been used (Suswillo & Denham, 1977; TDR, 1997). However, the *B. pahangi*–jird model produced by subcutaneous infection with infective larvae is still important as a simulation of human lymphatic filariasis.

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In this model, several compounds were assessed for their potential activity against adult stages of various species of filarial worms (Denham *et al.*, 1976, 1990; Surin & Denham, 1990). The adultcidal activity of DEC was also evaluated but the drug was found to have no efficacy against adult worms (Denham *et al.*, 1978) when the effect was evaluated 4 weeks after treatment.

In contrast, there are several reports that macrofilaricidal effects increased with time after treatment with flubendazole (Zahner & Schares, 1993), mebendazole (Shibuya *et al.*, 1979; Zahner & Schares, 1993) and suramin (Duke, 1991). Tyagi *et al.* (1986) reported a better efficacy of DEC against adult *B. malayi* in *Mastomys coucha* when necropsies were performed 91 days after treatment.

In the present study the adultcidal property of DEC in the *B. pahangi*–jird model is described.

Materials and methods

The filarial worm, *Brugia pahangi* used in this study has been maintained in Mongolian jirds for many years in the Animal Research Center of the Institute of Tropical Medicine, Nagasaki University. Male jirds were each

inoculated subcutaneously with 100 infective larvae of *B. pahangi*. Larvae were obtained from mosquitoes (*Aedes aegypti*) which had been fed on microfilaraemic jirds 14 days previously.

Microfilariae were counted by the Knott's concentration technique with 20 μl of blood samples from the retro-orbital sinus before treatment and at various intervals after the final treatment. The average microfilarial (mf) density of each group was calculated using the geometric mean of mf counts per jird. On the 20th week post-infection, microfilaraemic jirds were randomly divided into untreated control and treated groups. The treated group was given DEC (Supatonin®, Tanabe Ltd, Japan) intraperitoneally at 200 mg kg^{-1} for 5 consecutive days. The untreated group was given normal saline solution through the same route for the same period.

At 1, 4, 8, 12, 16 and 27 weeks after treatment, 4–7 jirds in both groups were sacrificed to recover adult worms according to the method of Ash & Riley (1970). Briefly, heart, lungs, kidneys, testes, adipose tissues in axillar, inguinal, perirenal and peritestes regions, carcass and pelt were examined for adult worms.

Results

The number of adult worms recovered at various times after treatment is summarized in table 1. In the untreated control group, the number of adult worms recovered remained relatively stable (28.5–36.4% of the number of larvae inoculated) up to 16 weeks after treatment, and then decreased slightly to 24.8% at 27 weeks. In the DEC-treated group, at earlier necropsy (1 and 4 weeks after treatment) no change was observed in the number of adult worms recovered. However, a significant reduction in the number of adult worms recovered was observed from 8 weeks onwards compared with the respective control group ($P < 0.01$ at 8 and 27 weeks; $P < 0.05$ at 12 and 16 weeks).

A time-course over 27 weeks of changes in microfilaraemia after 5 days treatment is shown in fig. 1. In the untreated control group, mf densities remained relatively stable (57.2–123.6% of the pretreatment density (PTD)) except on day 98 where mf levels dropped (41.8%). On the other hand, in the treated group, mf levels dropped to 44.4% of the PTD on day 1 after the final treatment, then decreased gradually to 15.2% on day 98. Thereafter, mf levels gradually increased and had recovered to 52.2% of the PTD by day 189.

Table 1. The number of adult *Brugia pahangi* recovered from jirds up to 27 weeks after diethylcarbamazine (DEC) treatment.

Recovery time fter treatment (weeks)	Control	DEC	% reduction rate
1	36.4 ± 11.1(5)	32.0 ± 7.1(5)	12.1
4	30.8 ± 14.3(5)	28.0 ± 4.6(5)	9.1
8	32.0 ± 7.8(5)	10.6 ± 4.2(5)*	66.9
12	28.5 ± 7.5(4)	19.0 ± 5.5(6)**	33.3
16	29.0 ± 4.3(5)	17.8 ± 8.8(5)**	38.6
27	23.2 ± 2.2(5)	13.9 ± 7.9(7)*	40.1

( ) No. of jirds necropsied. *Significantly different from recovery rate of control worms (Mann-Whitney test, $P < 0.01$). **Significantly different from recovery rate of control worms (Mann-Whitney test, $P < 0.05$).

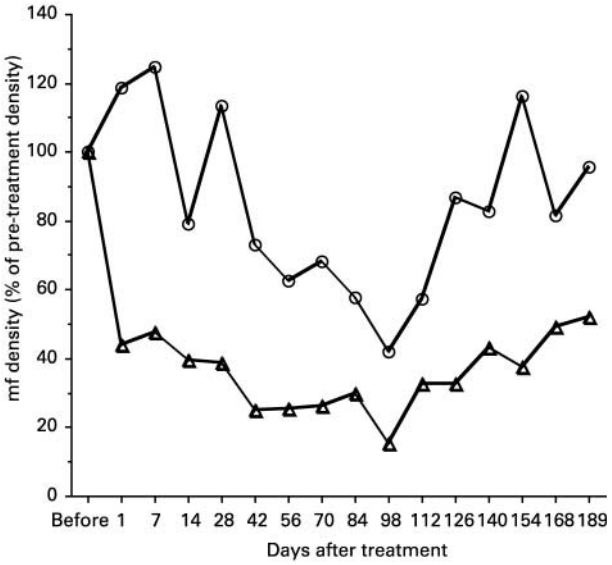

Fig. 1. Variation in the density of microfilariae of *Brugia pahangi* in jirds after diethylcarbamazine treatment expressed as a percentage of pretreatment density (Δ, treated jirds; ○, untreated controls).

Discussion

The effect of drugs against filarial worms depends on many factors such as the host (species, age, sex of animals), the species and stage of the parasite and the drug administration schedule (dose, period, and route). Thus *in vivo*-testing of antifilarial drugs should not be restricted to a particular model system.

In animal experiments using jirds, Denham *et al.* (1978) found that DEC was inactive against *B. pahangi* adult worms when jirds were killed 30 days after the end of treatment. This finding was confirmed in our present study when jirds were sacrificed at 4 weeks post-treatment although the dose of DEC (200 mg kg^{-1}) in the present study was lower than the 300 mg kg^{-1} administered in the previous study. Also at 1 week after treatment comparable numbers of adult worms were observed in both treated and untreated groups. However, at 8 weeks and later (12, 16 and 27 weeks) a significant reduction in the number of adult worms recovered was observed in the treated group. We have obtained similar results in another study where 108 adult worms were recovered from 9 DEC-treated jirds and 306 worms from 10 non-treated ones (unpublished data).

From the viewpoint of microfilarial levels, this is the first report to show that mf were observed in jirds for up to 27 weeks after DEC treatment, i.e. 47 weeks after the infection. By 112 days (16 weeks) mf levels seemed to be recovering. This suggests that surviving females resume mf release and that DEC may temporarily suppress embryogenesis of the females for 14 weeks. Further study on the effect of DEC against embryogenesis is required.

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The relationship between DEC concentration in the serum and its macrofilaricidal effect has not been studied either in humans or in animal models. The highest concentration of DEC obtained in jirds was 20–25 $\mu g\,ml^{-1}$ (Kimura *et al.*, 1984) and about 10 $\mu g\,ml^{-1}$ (Mitsui *et al.*, 1996) at 10 min after a single intraperitoneal injection of 100 $mg\,kg^{-1}$. In the present study the concentration of DEC was not measured after 5 consecutive days administration of a single dose of 100 $mg\,kg^{-1}$. However, such a repeated dosed schedule does not show a tendency for the drug to accumulate since DEC is very rapidly excreted in the urine and faeces of jirds and the plasma level fell to an undetectable level 4 h after administration (Kimura *et al.*, 1984). Moreover, the ineffectiveness of DEC against adult filarial worms in jirds described by Denham *et al.* (1978) was considered to be due to this rapid metabolism (Zahner & Schares, 1993).

The present study, however, demonstrates that DEC, probably at a low concentration in the serum, could to some extent affect adult filarial worms resulting in death or in the suppression of embryogenesis. The study also suggests that jirds should be held for a minimum of 8 weeks after treatment with DEC to assess macrofilaricidal action. Diethylcarbamazine has been reported to have efficacy against microfilariae (Yamashita *et al.*, 1983) as well as the third and fourth stage larvae of *B. pahangi* in jirds (Shigeno *et al.*, 1983). Thus, combined with the present study, DEC appears to be effective against any stage of *B. pahangi* in the jird. The *B. pahangi*–jird is therefore a good model for screening of antifilarial activity. Furthermore, the macrofilaricidal effect of DEC in the jird model in the present study has encouraged the administration of DEC in cooking salt as an alternative measure for the control of filariasis.

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