The Effect of Diethylcarbamazine on Microfilariae of *Brugia pahangi* in Mongolian Jirds (*Meriones unguiculatus*)

Seiji YAMASHITA

Nagasaki University, student

Shizugi SHIGENO, Eisaku KIMURA and Yoshiki AOKI

Department of Parasitology, Institute for Tropical Medicine, Nagasaki University

Abstract: Diethylcarbamazine (DEC) was effective in reducing microfilarial densities of *Brugia pahangi* in Mongolian jirds (*Meriones unguiculatus*) when it was administered intraperitoneally at the daily dosage of 300 mg/kg of body weight either for 5 consecutive days or every 3 days for 43 days with the total of 15 doses. The latter treatment scheme seemed to be more effective than the former, suggesting that the effect was dose-dependent.

The DEC treatment by stomach intubation seemed to be less effective than the intraperitoneal administration of the medicine.

Key words: Diethylcarbamazine, Chemotherapy, Microfilariae, *Brugia pahangi*, Mongolian jird.

INTRODUCTION

Since diethylcarbamazine (DEC) was first demonstrated to be effective against filarial worms, *Litomosoides carinii*, in naturally infected cotton rats (Hewitt *et al.*, 1947), it has been known to be a safe and most effective drug against lymphatic-dwelling human filaria, and used in many endemic areas in the world for the purpose of controlling or eradicating the disease. However, the mechanisms of DEC effect on filarial worms have been only poorly understood, and a variety of studies aimed at solving the problem is still being conducted.

With regard to microfilaricidal activities of DEC, it was effective against *L. carinii* in cotton rats (Hewitt *et al.*, 1947; Hawking, 1950) and in albino rats (Ramakrishnan *et al.*, 1963), *Brugia malayi* (Wilson, 1950) and *Wuchereria bancrofti* in man (Santiago-Stevenson *et al.*, 1947). Whereas, DEC was inactive against *Dipetalonema witei*, *Brugia pahangi* or *L. carinii* in jirds (Worms and Terry, 1961; Denham *et al.*, 1978; Matsuda

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et al., 1976) and *D. perstans* in man (Hawking, 1950).

Recently, we confirmed that DEC was effective against developing stages of larvae of *B. pahangi* in Mongolian jirds (Shigeno *et al.*, in preparation). This fact necessitated us to study the effect of DEC on *B. pahangi* microfilariae in jirds using our own strain of *B. pahangi*, in order to know to what extent DEC was ineffective or effective against the microfilariae.

In the present study, four different schemes of DEC treatment were compared for the microfilaricidal effect.

MATERIALS AND METHODS

Brugia pahangi used has been maintained for years in our laboratory in domestic cats and Mongolian jirds (*Meriones unguiculatus*). Jirds were infected subcutaneously with 100 infective larvae which were obtained from *Aedes aegypti* (Liverpool strain) fed on microfilariae positive jirds 11-12 days previously. Seven or more months after infection, 22 jirds were selected for the experiments from the stock of infected jirds. Four groups of jirds, which consisted of 5-7 animals each, were made and treated with DEC citrate (Supatonin®) by four different schemes. Group Ⅰ (5 jirds) was served as a control group. The animals were given an intraperitoneal dose of physiological saline (0.7-0.9 ml/animal) daily for 5 consecutive days. Group Ⅱ (5 jirds) was treated intraperitoneally (i.p.) at the daily dosage of 300 mg/kg of body weight (B.W.) for 5 consecutive days. Group Ⅲ (5 jirds) was treated i.p. at 300 mg/kg B.W. every three days for 43 days (15 doses). Group Ⅳ (7 jirds) was treated by stomach intubation at 300 mg/kg B.W. every 3 hours for 21 hours (8 doses). The treatment given to Group Ⅱ was the same as tested by Denham *et al.* (1978).

Microfilariae were counted by Knott's concentration technique with 20 cmm of blood samples from retro-orbital sinus twice before treatment and then at various intervals of days after the initiation of treatment. The microfilarial density (mf density) of each group was calculated as an average (geometric mean) of mf counts of 5 (Groups Ⅰ, Ⅱ and Ⅲ) or 7 animals (Group Ⅳ). The pretreatment density (PTD) was an average (geometric mean) of two determinations of mf counts in each group.

In this paper, numbering of days starts from the first day of treatment.

RESULTS

The results are shown in Fig. 1, in which the change of mf densities is expressed as percentages of the PTD.

A big fluctuation of mf counts was observed in all four groups. In the control group, a rather anomalous and sharp drop of mf density was recorded at 26 days. In

Group Ⅰ, mf densities remained relatively stable (80.2–115.9% of the PTD) in the first 22 days, and then began to show big fluctuations with a slight tendency of gradual decrease. In Group Ⅱ, the mf density dropped to 41.5% of the PTD at 5 days, 4 hours after the completion of treatment, and then increased to 52.5–78.3% of the PTD at 18–20 days. After this, densities decreased again in the similar pattern which was observed in Group Ⅰ. In Group Ⅲ, mf densities reduced rapidly after a fifth dose of treatment at 13 days, reached 26.8% of the PTD at 22 days and then remained between 12.6–26.5% of the PTD. In Group Ⅳ, the mf density dropped sharply to 13.0% of the PTD at 2 days, 3 hours after the completion of treatment. The density, however, bounced up to 44.9% at 3 days and then increased gradually, reaching 74.4% at 31 days.

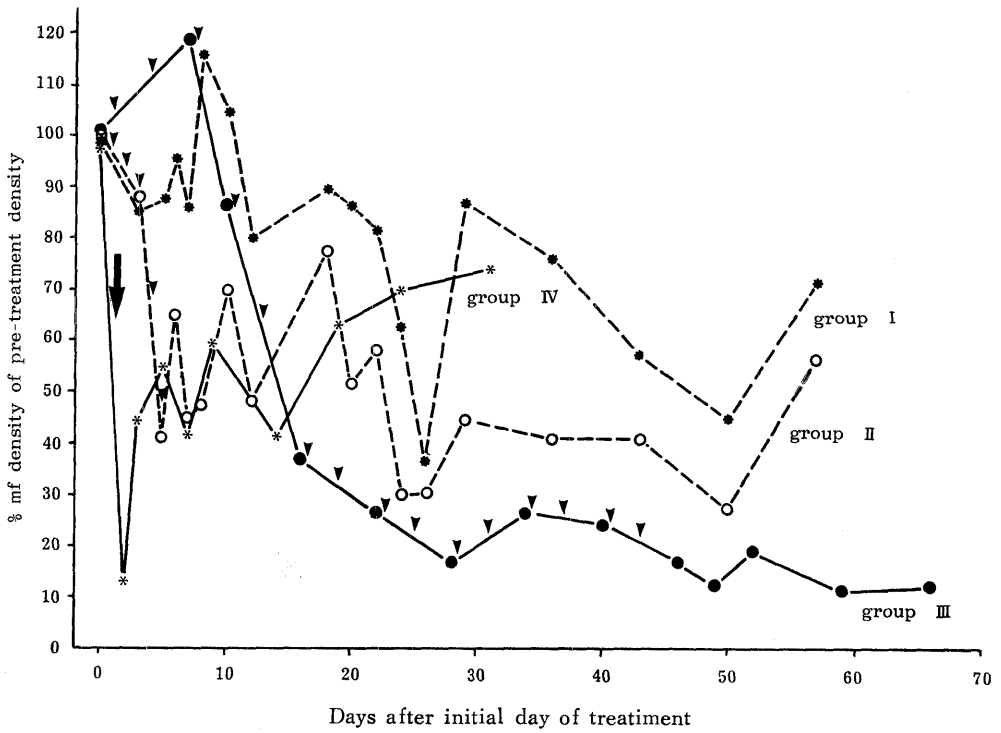

Fig. 1. The change of microfilarial densities of *Brugia pahangi* in Mongolian jirds after DEC treatment with different schemes

Group I : non-treated control group.
Group II : treated daily at 300 mg/kg for 5 consecutive days by intra-peritoneal route (i.p.).
Group III : treated i.p. every three days at 300 mg/kg for 43 days (15 doses).
Group IV : treated every three hours at 300 mg/kg for 21 hours (8 doses) by stomach intubation.

A small arrow (▼) denotes a single administration at 300 mg/kg.
A large arrow denotes 3-hourly 8 dose treatment.

DISCUSSION

The evaluation of DEC effect on microfilariae is hampered by difficulties in determining mf counts which are fluctuating greatly by nature. In addition, the degree of mf reduction and its duration, which were the most essential criteria in assessing microfilaricides, could not always be reliable due to the reproduction and release of new microfilariae by adult worms. Furthermore, Mongolian jird, as a desert-dwelling animal, is said to have a specialized renal physiology, and little is known about blood DEC levels in the animal. Thus, the influence of different DEC dosages, routes of administration and treatment schedules is not easily predictable.

In our present study, several different schemes of DEC treatment were compared, and the following facts were demonstrated. 1). the DEC treatment given i.p. at 300 mg/kg B.W. daily for 5 consecutive days was effective in reducing mf densities. The effect continued at least for a week, which could not be explained by a simple sequestration of microfilariae to other organs. 2). the DEC treatment given i.p. at 300 mg/kg B.W. every 3 days for 43 days (15 doses) was also effective against microfilariae. The reduction of densities was much greater and more continuous than the former 5 dose treatment. 3). the DEC treatment given by stomach intubation at 300 mg/kg B.W. every three hours for 21 hours (8 doses) immediately and sharply reduced mf density, but experienced a kind of rebound phenomenon.

The results of Groups II and III suggested that the effect of DEC on microfilariae was dose-dependent. However, even the 15 dose treatment failed to eliminate all the microfilariae. In the case of *L. carinii* in cotton rats, or *W. bancrofti* in man, the microfilaricidal effect of DEC is known to be much more dramatical. The relative ineffectiveness of DEC in jirds could be explained, partially, by the insufficient blood level of DEC in the animals. DEC which was administered intraperitoneally into jirds at a single dose of 300 mg/kg B.W. was excreted very quickly, in 5 hours. It is not known whether the quick excretion of DEC is the peculiar characteristic of this animal, but at least, hamsters could retain blood DEC level much longer than jirds (Kimura, in preparation). Thus, it might be that our dosage rate, 300 mg/kg B.W., which is already a toxic dosage to jirds, is still not enough to maintain required DEC concentration in blood to kill microfilariae completely. If this is the case, Mongolian jirds are not suitable for chemotherapeutic studies using DEC.

The 8 dose treatment by stomach intubation seemed to be least effective among the three treatment schemes with DEC. This could be explained, again partially, by the finding that the blood DEC level attained by stomach intubation was only 1/3−1/8 of the i.p. administration (Kimura, in preparation). This result suggests that the intraperitoneal (and probably subcutaneous) route is preferable to oral route in treating jirds with DEC. The short period of time in oral administration (*i.e.* 21 hours) may have relevance to this relative ineffectiveness.

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スナネズミに感染させた *Brugia pahangi* の仔虫に対するジエチルカルバマジンの治療効果

山下征二（長崎大学・学生）
重野鎮義，木村英作，青木克己（長崎大学熱帯医学研究所寄生虫学部門）

ジエチルカルバマジン（DEC）を，スナネズミ体重 1kg 当り300mg 宛，腹腔内に１日１回，５日間連続投与した場合，或は，同量の DEC を43日間にわたり２日おきに15回投与した場合，スナネズミ血液中の *B. pahangi* 仔虫数に明らかな減少を認めた．DEC の15回投与は，５回投与に比し，その効果はより強く持続的で，薬量依存の傾向がみられた．

胃内挿管法により DEC 300mg/kg を８回投与した場合の治療効果は，前２者に比べて劣ることが観察された．

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