Plasma Levels of Diethylcarbamazine and Their Effects on Implanted Microfilariae of *Brugia pahangi* in Rats

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ABSTRACT. Plasma level of diethylcarbamazine (DEC) was measured by using gas chromatography and was compared to the changes of microfilaremia after an intraperitoneal injection with 200 mg/kg of DEC in rats. The microfilaremia was induced artificially by an intravenous implantation with 2×10^5 *Brugia pahangi* microfilariae (mf) 1 day before DEC treatment. The rats treated with DEC showed a rapid and significant decrease in mf number in the circulation within 30 min, continued for 4 hr, and then increased rapidly. DEC seemed to cause transient but significant suppression of microfilaremia of *B. pahangi* in rats directly. — KEY WORDS: *Brugia pahangi*, diethylcarbamazine (DEC), microfilaremia.

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Diethylcarbamazine (DEC) is one of the commonest drugs in the treatment of filariasis, however its precise mode of action has not been understood fully [10]. Effect of DEC vary among host-filaria combinations, e.g., DEC is effective to cause suppressed microfilaremia of *Wuchereria bancrofti* and *Brugia malayi* in man [7] but not to *B. malayi* and *B. pahangi* in the Mongolian gerbil [1, 11, 13]. Regarding this, it has been reported that DEC is eliminated rapidly from the blood of gerbils [6] as compared to man [2, 9]. However, correlation between blood level of DEC and microfilaria (mf) changes in the circulation has not been studied. In this study, in order to clarify the effect of DEC on microfilaremia, mf counts after DEC injection were monitored over a time course and compared to the changes of plasma level of DEC in rats.

Outbred male Wistar rats (10-weeks old) were used in this study. Microfilariae were collected from the peritoneal cavity of gerbils 3 months after intraperitoneal (i.p.) inoculation with 300–400 infective third-stage larvae of *B. pahangi* [8]. Implantation was carried out as previously described [5]. Briefly, peritoneal adherent cells were removed by incubating at 37°C and mf were suspended in Hanks' balanced salt solution (HBSS) at a concentration of 4×10^5 mf/m*l*. Rats were injected intravenously (i.v.) via penis vein with 2×10^5 mf in 0.5 m*l* of HBSS 1 day before DEC injection. DEC solution (Supatonin: Tanabe, Osaka, Japan) containing DEC citrate at 200 mg/m*l* was diluted with saline and injected i.p. Rats received an i.p. injection of DEC citrate at 200 mg/kg. Control rats received equal amount of saline as same manner. Blood samples were collected at intervals during 0.5 to 24 hr for DEC measuring or 0 to 10 days for mf count from the retro-orbital sinus of rats under ether anesthesia with heparinized capillary tube.

The extraction and measurement of DEC were carried out according to the method described previously [6]. Briefly, DEC in the plasma (0.5 m*l*) was extracted with 5 m*l* of ethyl acetate twice, then the ethyl acetate was dried under nitrogen gas at room temperature. The residue was redissolved in 200 μl of hexane and immediately injected into the gas chromatograph. A gas-liquid chromatograph (Shimazu, GC-R1A) equipped with a flame ionization detector was used for measurement of DEC. The column consisted of 2% Carbowax 20 M, 5% KOH, on Chromosorb G AW DMCS (100–120 mesh). The column temperature was 160°C and the loading temperature 180°C. The carrier gas was nitrogen at a flow rate of 60 ml/min. Five microliters of sample was loaded. Under these conditions, the retention time of DEC was 9.4 min. A standard curve was made using powdered DEC citrate dissolved in normal rat plasma (0.2 μg/m*l*, 1 μg/m*l*, 10 μg/m*l* and 100 μg/m*l*). The amount of DEC in samples was calculated from the standard curve.

Microfilarial count was made in wet films prepared from 20 μl aliquots of blood collected from mf-inoculated rats as described above. Student's *t*-test was used for statistical analysis. Data were considered significantly different from each other at $P<0.05$.

The plasma level of DEC in the rats following an i.p. injection with 200 mg/kg of DEC is shown in Fig. 1. The plasma level of DEC was about 30 μg/m*l* at 30 to 60 min after the injection, and then decreased rapidly. DEC at an average concentration of 1.5 μg/m*l* was detected in the blood at 4 hr, and 0.1 μg/m*l* at 8 hr after the injection. No DEC was detected by gas chromatography at 24 hr after the injection. The kinetics of mf counts of rats after an i.p. injection with 200 mg/kg of DEC and those of controls without DEC treatment are shown in Fig. 2. Microfilarial counts of DEC-treated and untreated control rats before treatment were 18.8 ± 4.6 and 18.9 ± 10.2, respectively. After DEC treatment, mf count decreased significantly ($P<0.05$) at 30 min (0.9 ± 1.2, 95% reduction) and continued for 4 hr (1.9 ± 1.6, 90% reduction), and then rapidly increased. At 8 hr after the injection, mean mf count of DEC-treated rats was about a half of that of controls but was not statistically significant. At 3 days after injection, mf count of DEC-treated rats recovered to the same level as control.

The present results clearly show that artificially induced microfilaremia in rats decreased rapidly by an injection of DEC. When the changes of mf counts in the blood of the

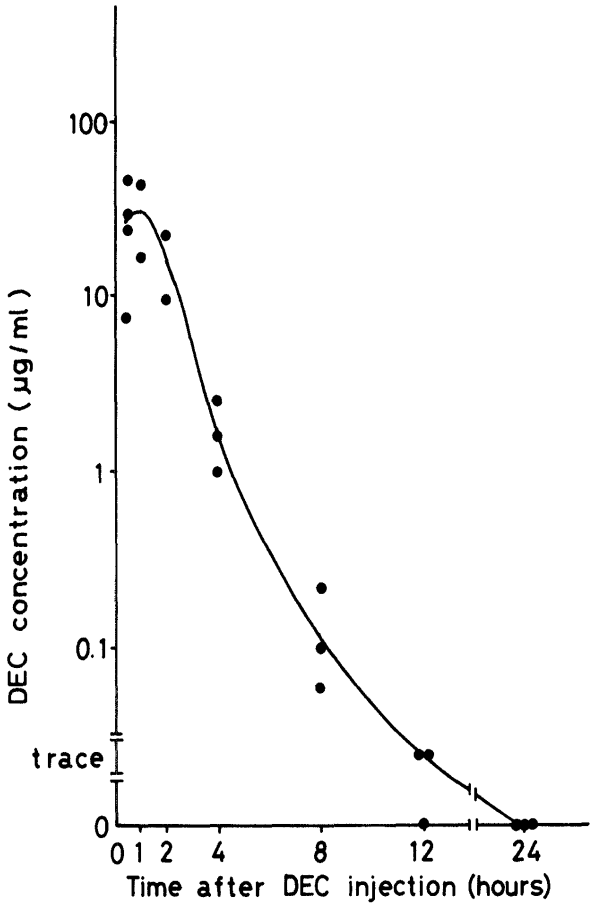

Fig. 1. Kinetics of plasma level of DEC in rats after an i.p. injection of DEC citrate at 200 mg/kg body weight. Each point indicates a DEC value from each rat.

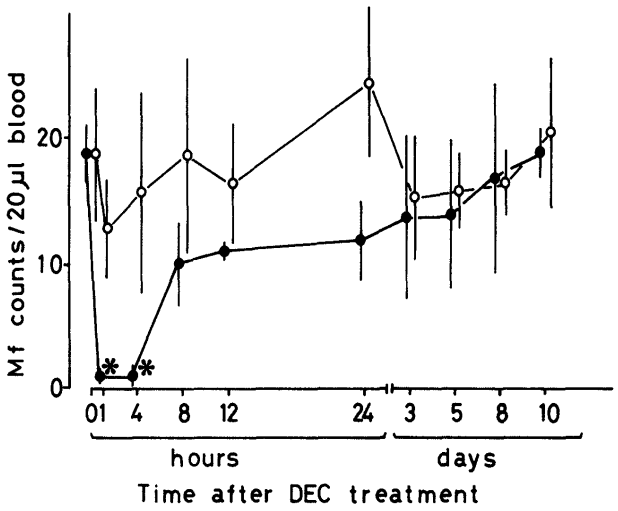

Fig. 2. Kinetics of microfilarial count in the blood of rats after an i.p. injection of DEC. Closed circle (●) indicates DEC-injected rats and open circle (○) indicates untreated controls. Rats received 2×10^5 mf 1 day before DEC treatment. Each value is the mean ± SEM of 5 rats. Asterisk indicates statistical significance at $P<0.05$.

rats were compared to the plasma level of DEC (Fig. 1), the presence of DEC in the plasma at a concentration of 1.5 μg/ml or more led to a significant reduction (90–95%) in mf count. However, these declines of microfilaremia were transient and recovered to about the same level as controls within a few hours after the treatment. A similar transient decline of mf count of *B. pahangi* in cats [1] and *Litomosoides carinii* in Mongolian gerbils [4] after DEC treatment have been reported. However, the precise mechanism underlying this is not clear due to complicated factors in the infected animals, e.g., reproduction of mf or influence of host's immunity, etc. The transient changes observed in this study may be attributed to the action of DEC, since the recruitment of mf and influence of specific immunity were almost abolished as a result of the use of artificially-induced microfilaremic animals implanted with mf just one day before DEC treatment. Several evidence on the direct action/effect of DEC on filariae at concentrations comparable to those used in the present study have been reported. DEC has been found to inhibit the motility of *Dirofilaria immitis in vitro* at concentrations of 10^{-7} to 3×10^{-4} M through a gabergic mechanism [12]. DEC also inhibits filarial 5, 10-methylenetetrahydrofolate reductase and serine hydroxymethyltransferase at concentrations of 10^{-5} M and 5×10^{-6} M, respectively [10].

The distribution of mf throughout the body of cotton rats infected with *L. carinii* has been examined before and after DEC treatment [3]. In untreated cotton rats about 63% were in the circulating blood, 30% in the capillaries of lungs, and 3% in the liver. However, soon after DEC treatment, almost 75% of the mf were found in the capillaries of the liver, whereas only 20% and 4% were found in the blood and lungs, respectively. The effect of DEC on the decline of mf count might be related to the changes in the distribution of mf after treatment. The inhibitory action of DEC on parasite motility [12], in part, may cause distributional changes of mf.

In conclusion, the effect of DEC on the transient decline of artificially induced microfilaremia in the rat was dependent on the plasma level of DEC concentration. The present study suggests that a low level of microfilaremia can be achieved by maintaining of DEC in the blood at the minimum required concentration without any specific immunity of the host.

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