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The Effect of Diethylcarbamazine Citrate on the 3rd- and 4th-stage Larvae of *Brugia pahangi* Inoculated Intratesticularly into Inbred GN Hamsters (*Mesocricetus auratus*)

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Abstract: Inbred GN hamsters were infected intratesticularly with infective larvae of *Brugia pahangi*. The new route of infection produced localized infection of filariae in the testis and its peritesticular tissues. Using this model, developing 3rd- and 4th-stage larvae in hamsters were treated with diethylcarbamazine. The results showed that the recovery rate of worms in the non-treated control group was 80%, while those of the treated groups were 14.2 and 26.9%.

Key words: *Brugia pahangi*, Diethylcarbamazine, Chemotherapy, *Mesocricetus auratus*.

The intratesticular inoculation of infective larvae of *Brugia pahangi* into inbred GN hamsters (*Mesocricetus auratus*) was reported to be a useful technique which could produce localized infection in the inoculated testis and resulted in very high recovery rate of developing larvae (Kimura *et al.*, 1984a). In the testis, the 3rd molt occurred at 7–8 days postinoculation, and the 4th molt was seen after 24 days. In the present study, this new route of infection was used in studying the effect of diethylcarbamazine citrate (DEC) on the 3rd- and 4th-stage larvae of *B. pahangi*.

Infective larvae of *B. pahangi* were obtained from *Aedes aegypti* fed on a microfilariae positive Mongolian jird (*Meriones unguiculatus*) 11 days previously. They were washed five times in sterilized Hanks' balanced salt solution (HBSS). Twenty GN hamsters of 3–5 months old were infected by inoculating 28–30 infective larvae in about 0.05 ml of HBSS into the left testis of each animal using a 22-gauge needle. The hamsters were then divided into the following four groups of five hamsters each. Group I was treated intraperitoneally with DEC (Supatonin®, Tanabe Seiyaku Co., Ltd.) at 300 mg/

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kg/day at days 1, 2, 3, 4 and 5 postinoculation, and necropsied one animal per day at days 6, 7, 8, 9 and 10. Group II was treated at the same dosage at days 11, 12, 13, 14 and 15, and necropsied one animal per day at days 16, 17, 18, 19 and 20. Group III and Group IV were control groups matching respectively with Group I and Group II. They were treated intraperitoneally with 0.7 ml of normal saline, with which DEC solution was diluted to the required concentration. The autopsy was carried out following Kimura et al. (1984b) and the number of larvae were recorded by site of recovery.

In control groups, the average recovery rate of larvae was 79.3%. Most of the recovered larvae (88.9% in Group III, 54.7% in Group IV) were obtained from the inoculated testis, which was followed by the peritesticular tissues of the inoculated side (5.1% in Group III, 33.3% in Group IV). The "peritesticular tissues" include epididymis, ductus deferens and adipose tissue attaching to the testis. In DEC -treated groups, the average recovery rates for Group I and Group II were 14.2% and 26.9% respectively. Again the majority of recovery (81.0% in Group I, 74.4% in Group II) was obtained from the inoculated testis. The peritesticular tissues of the inoculated side accounted for 14.3% and 20.5% respectively in Group I and Group II (Table 1).

These results indicate that DEC is effective against 3rd- and 4th-stage larvae of *B. pahangi* in GN hamsters. There was no significant difference of recovery between the

Table 1. Distribution and recovery rate of *B. pahangi* inoculated intratesticularly into GN hamsters and treated with DEC against 3rd- and 4th-stage larvae

	3rd-stage larvae		4th-stage larvae	
	treated (Group I)	control (Group III)	treated (Group II)	control (Group IV)
Total No. larvae inoculated	148	148	145	147
Site of recovery				
Testis (inoculated)	17 (81.0)	104 (88.9)	29 (74.4)	64 (54.7)
Peritest. tissues (inoculated side)	3 (14.3)	6 (5.1)	8 (20.5)	39 (33.3)
Testis and peritest. tissues (non-inoculated side)	0 (0.0)	1 (0.9)	0 (0.0)	2 (1.7)
Heart and lungs	0 (0.0)	0 (0.0)	0 (0.0)	6 (5.1)
Kidney and perirenal fat tissue	1 (4.8)	5 (4.3)	1 (2.6)	2 (1.7)
Carcass	0 (0.0)	1 (0.9)	1 (2.6)	2 (1.7)
Others	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.7)
Total No. larvae recovered	21 (100)	117 (100)	39 (100)	117 (100)
% Recovery	14.2	79.1	26.9	79.6

( ) : Percentage to the total number of larvae recovered.

two stages of development statistically. The reason why DEC could not eliminate all the larvae was not known, but the low plasma concentration of DEC in rodents (Kimura *et al.*, 1984c) may have relevance to this.

When we use subcutaneously-infected rodent models for experimental chemotherapy of filariasis, the recovery rates of worms from control animals are not satisfactorily high. For example, Shigeno *et al.* (1983) reported that, when developing larvae of *B. pahangi* in subcutaneously-infected Mongolian jirds were treated with DEC at 300mg/kg/day for 5 consecutive days, the recovery rates for 3rd- and 4th- stage larvae were 9.1% and 4.9% respectively, compared with 43.1% in the non-treated control. The results have raised a question on what would happen to more than 50% of the inoculated larvae which could not be recovered in the control group. Our new model showed high recovery rate (about 80%) in control groups, and thus provided more confirmative evidence of the effect of DEC against 3rd- and 4th- stage larvae. In addition, by using the present animal model, 88-95% of the recovered worms were obtained from the inoculated testis and the peritesticular tissues of the inoculated side. Thus the examination of only these two sites, which can be easily excised en bloc, will probably be sufficient in future chemotherapeutic studies.

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近交系ハムスターの睾丸内接種モデルを用いた *Brugia pahangi* 3期・4期幼虫に対するジエチルカルバマジンの治療効果

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近交系ハムスターの睾丸内に, *Brugia pahangi* 感染幼虫を接種すると, 睾丸とその周囲に限局するフィラリア感染を起すことができる. このモデルを用い *B. pahangi* 3期・4期幼虫に対

するジエチルカルバマジンの治療実験を行なった．その結果，非治療対照群では接種数の約80％の虫体が回収されたのに対し，治療群では14－27％であった．回収虫体のほとんどは接種睾丸とその周囲組織より得られるので，今後の治療実験ではこの部分の検査のみで十分である．この新しいモデルにより，治療実験における虫体回収率は従来の約2倍となり，かつ回収作業が著しく容易になった．

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