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Studies on *Brugia pahangi* in Inbred Hamsters 3. The susceptibility of CBN hamsters and treatment experiment with diethylcarbamazine

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Abstract: The susceptibility of inbred male CBN hamsters to *Brugia pahangi* infection was studied. The animals infected subcutaneously with 100 infective larvae showed 100% microfilaremia with the average prepatent period of 74.5 days. The average microfilarial density (geometric mean) at 20 weeks postinfection was 53.5/40 cmm of blood. The rate of adult worm recovery ranged from 5 to 54% with the average of 25.4%. They were recovered from the testis, peritesticular tissues, and the heart and lungs.

The effect of diethylcarbamazine citrate (DEC) against microfilariae, 3rd and 4th stages of larvae, and adult worms was studied. For the treatment of developing stages of larvae, intratesticularly-infected CBN hamsters were used. DEC was confirmed to be effective against microfilariae and 3rd- and 4th-stage larvae, but the effect was less marked against adult worms. The advantages of intratesticular route of infection for chemotherapeutic studies were discussed.

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

INTRODUCTION

Hamsters were tested for the susceptibility to *Brugia* infection by several workers (Ash and Riley, 1970a,b; Sucharit *et al.*, 1972; Harbut, 1973; Malone *et al.*, 1975), but with limited success. Malone *et al.* (1979) compared inbred and outbred hamsters and reported that PD-4 inbred hamsters were more susceptible than outbred hamsters.

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Crandall *et al.* (1982) compared five inbred strains and reported that MHA and PD-4 hamsters were slightly more susceptible than the others. Recently, Shigeno *et al.* (1983) showed that inbred GN hamsters were very susceptible to *Brugia pahangi* infection, and APG hamsters were less susceptible.

It will be beneficial if there are several inbred strains of hamsters available which have different susceptibility to filarial infection, or produce different results in the chemotherapy of parasites. With a hope of finding such a hamster strain, inbred CBN hamsters were studied in connection with the susceptibility to *B. pahangi* infection and the effect of diethylcarbamazine treatment against microfilariae, developing stages of larvae and adult worms.

MATERIALS AND METHODS

A total of seven male CBN hamsters were infected subcutaneously in the inguinal region with 100 infective larvae of *Brugia pahangi*. The CBN hamster (*Mesocricetus auratus*) is a inbred strain developed at Nippon Institute for Biological Science. Starting from the seventh week after infection, 40 cmm of blood samples obtained from the retro-orbital sinus were examined for microfilariae. At 26 weeks, five hamsters were autopsied and adult worms were recovered following the method by Ash and Riley (1970a).

The effect of diethylcarbamazine citrate (DEC) against developing stages of larvae in CBN hamsters was studied by using an animal model infected intratesticularly (Kimura et al., 1984). A total of 23 hamsters were inoculated each with 30 infective larvae into the left testis. A group of six animals (Group I) was treated intraperitoneally with DEC at the dosage of 300 mg/kg/day for five consecutive days between 1 and 5 days postinoculation, when larvae were expected to be in the third stage. As a control group (Group II), five animals were treated with normal saline, which was used to dilute DEC solution (Supatonin® Tanabe Seiyaku Co. Ltd). Another six animals (Group III) were treated as in Group I between 10 and 14 days, when larvae were expected to be in the fourth stage. Six control animals (Group IV) were treated as in Group II. In order to recover worms, Groups I and II were sacrificed at 6-9 days, and Groups III and IV at 15-18 days. As almost all of the intratesticularly-inoculated parasites are found from the inoculated testis and its peritesticular tissues (epididymis, ductus deferens and adipose tissue attaching to the testis), and the heart and lungs (Kimura et al., 1985), only these three parts of animal body were examined for parasites.

In another experiment, the effect of DEC against microfilariae and adult worms was studied using 11 CBN hamsters, which had been infected subcutaneously with 100 infective larvae 18 weeks previously. Animals were divided into a DEC-treated group (6 animals) and a control group (5 animals). The method and dosage schedule of DEC treatment were the same as in Group I or III. Microfilarial (mf) counts were checked

before and after treatment (up to 16 days) using 40 cmm of blood from the retro-orbital sinus. The 11 hamsters were then sacrificed and adult worms were recovered.

RESULTS

Microfilaraemia was seen in all the hamsters with the average prepatent period of 74.5 days. The mf density (geometric mean) at 20 weeks averaged 53.5/40 cmm (range 8-211) (Table 1). The recovery rate of adult worms ranged from 5 to 54% with the average of 25.4%. They were recovered from the testis, peritesticular tissues and the heart and lungs, but none from the other parts of animal body (Table 2).

The results on treatment experiments against developing stages of larvae are shown in Table 3. In control groups (Groups II and IV), 67.2-76.7% of the inoculated larvae were recovered, while, the DEC treatment reduced recovery rate to 2.8-4.4%. There was

Table 1. Microfilarial counts in CBN hamsters infected subcutaneously each with 100 infective larvae of *Brugia pahangi*

Animal No.	Weeks after inoculation									
	7	8	9	10	11	12	13	15	17	20
1.	0	0	0	0	1	14	5	108	326	211
2.	0	0	0	1	0	6	2	7	18	8
3.	0	0	0	0	0	0	1	35	64	98
4.	0	0	0	0	1	0	0	2	8	9
5.	0	0	0	0	0	0	3	14	34	43
6.	0	0	0	4	6	6	9	32	77	150
7.	0	0	0	2	3	9	18	93	137	131

Table 2. Distribution and recovery rate of *Brugia pahangi* adults in CBN hamsters infected subcutaneously each with 100 infective larvae

Animal No.	Site of recovery						Total	% Recovery
	Testis	Peritesticular tissues	Heart and lung	Carcass and pelt	Intestinal organs	Others		
1.	1	5	11	0	0	0	17	17.0
2.	5	1	14	0	0	0	20	20.0
3.	3	0	2	0	0	0	5	5.0
4.	8	0	46	0	0	0	54	54.0
5.	14	1	16	0	0	0	31	31.0
Total	31 (24.4)	7 (5.5)	89 (70.1)	0 (0.0)	0 (0.0)	0 (0.0)	127 (100)	25.4

( ) : Percentage to the total recovered.

no difference of effect of DEC between the third and fourth stages.

Figure 1 shows the effect of DEC treatment on microfilariae. The mf density is expressed as percentage to the pre-treatment level. The mf density of control group fluctuated between 66.7 and 114.4%, whereas, in the treated group, it reduced to about 10% of the pre-treatment level and maintained the level until 12 days after the completion of treatment.

Table 3. Distribution and recovery rate of *Brugia pahangi* larvae in CBN hamsters infected intratesticularly and treated with DEC at 300 mg/kg/day for 5 days

	3rd-stage larvae		4th-stage larvae	
	Treated (Group I)	Control (Group II)	Treated (Group III)	Control (group IV)
No. animals used	6	5	6	6
Total No. larvae inoculated	180	150	180	180
Site of recovery				
Testis (inoculated)	3 (37.5)	109 (94.8)	3 (60.0)	57 (47.1)
Peritesticular tissues (inoculated side)	0 (00.0)	6 (5.2)	2 (40.0)	59 (48.8)
Heart and lungs	5 (62.5)	0 (00.0)	0 (00.0)	5 (4.1)
Total No. larvae recovered	8 (100)	115 (100)	5 (100)	121 (100)
% Recovery	4.4	76.7	2.8	67.2

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

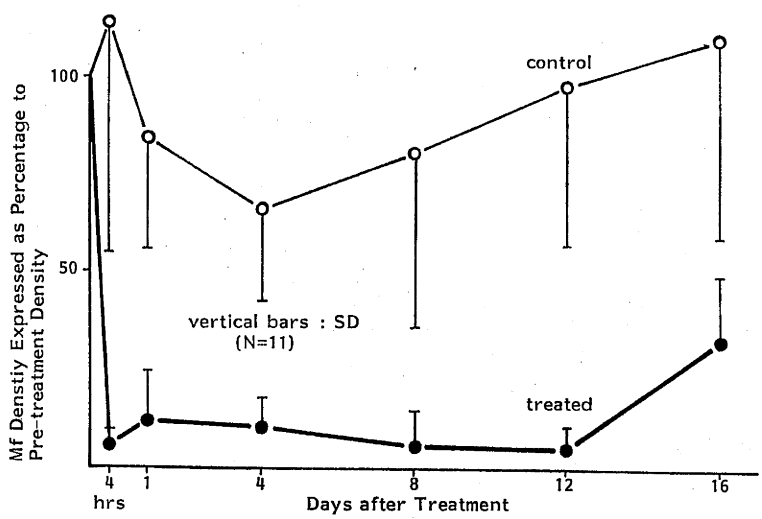

Fig. 1 Effect of Diethylcarbamazine against Microfilaremia

The DEC treatment against adult worms showed that the average recovery rate of the treated group was 5.5% in comparison with 16.0% of the control group (Table 4). There was no difference between two groups at a statistically significant level. In this experiment, a few adults were found in the carcass, but most were from the testis, peritesticular tissues and the heart and lungs.

Table 4. Distribution and recovery of *Brugia pahangi* adults in CBN hamsters infected subcutaneously each with 100 larvae and treated with DEC at 300 mg/kg/day for 5 days

Animal No.	Site of recovery					Total	% Recovery
	Testis	Peritesticular tissues	Heart and lungs	Carcass	Others		
Treated group							
1.	7	0	0	0	0	7	7.0
2.	2	0	3	0	0	5	5.0
3.	2	0	0	0	0	2	2.0
4.	0	0	0	0	0	0	0.0
5.	1	0	8	0	0	9	9.0
6.	5	0	5	0	0	10	10.0
Total	17 (51.5)	0 (0.0)	16 (48.5)	0 (0.0)	0 (0.0)	33 (100)	5.5
Control group							
1.	3	12	3	4	0	22	22.0
2.	9	2	13	0	0	24	24.0
3.	1	0	1	2	0	4	4.0
4.	2	1	8	0	0	11	11.0
5.	8	0	11	0	0	19	19.0
Total	23 (28.8)	15 (18.8)	36 (45.0)	6 (7.5)	0 (0.0)	80 (100)	16.0

( ) : Percentage to the total recovered.

DISCUSSION

Infected CBN hamsters showed 100% patency with 74.5-day prepatent period. The average mf density at 20 weeks and the adult recovery rate at 26 weeks were respectively 53.5/40 cmm and 25.4% When the susceptibility to filarial infection is measured by adult recovery rate, CBN hamster could be placed next to GN hamsters, whose adult recovery rate was reported to be 36.0% (Shigeno *et al.*, 1983). All the adult worms were recovered from the testis, peritesticular tissues and the heart and lungs. This agrees with the results obtained by Shigeno *et al.* (1983) using GN and APG hamsters. Thus, for the recovery of adult worms in these hamsters infected subcutaneously, it will be enough to examine only these three parts of the body in future studies.

In experimental chemotherapy using rodents, it is required that a host rodent should

be sufficiently susceptible to filarial infection, that the effect of chemotherapy is clearly recognizable and reproducible and that the high worm recovery rate is obtainable with minimum efforts. Concerning treatments of developing third- and fourth-stage larvae in hamsters, the intratesticular inoculation method can meet all these requirements. Using that method, almost all of the developing larvae can be obtained by examining only the inoculated testis and its peritesticular tissues, regardless of whether animals are treated with DEC or not (Kimura *et al.*, 1985). It would be more safe to include the number of larvae recovered from the heart and lungs as did in the present study.

The effectiveness of DEC against third- and fourth-stage larvae of *B. pahangi* in CBN hamsters was confirmed. The total 94.3-95.8% reduction of worm burden from the control groups was much higher than the similar treatment with GN hamsters, which showed 66.2-82.0% reduction (Kimura *et al.*, 1985).

The treatment against microfilariae also showed clear effect of DEC. The rate of reduction of mf density was roughly 90% from the pre-treatment level. In Mongolian jirds (*Meriones unguiculatus*) which were infected and treated by the same methods as in the present study, the DEC treatment was reported to have reduced mf density by about 50% (Yamashita *et al.*, 1984).

The DEC treatment against adult worms showed that the recovery rates of the control and treated groups were 16.0% and 5.5% respectively. The difference was not statistically significant. This is due to an exceptionally low rate of recovery (4%) included in the control group. If this is excluded, the difference becomes significant ($p<0.01$). It is most probable that, even if a fixed number of infective larvae was inoculated, the number of adult worms in rodents showed wide individual variation by the time the treatment started, and thus produced such an exceptional result. To solve this kind of problem and to simplify procedures of recovering worms, Suswillo and Denham (1977) reported a new system of testing for macrofilaricidal activity using intraperitoneally-transplanted adult *Brugia* in the Mongolian jird. In hamsters, the transplantation was not successful (Kimura, unpublished data).

The present study revealed that CBN hamsters were sufficiently susceptive animal host to *B. pahangi* infection and that they can be used for chemotherapeutical studies against microfilariae, developing stages of larvae and, with some disadvantages, adult worms. A CBN hamster seems to be less susceptible to *B. pahangi* infection than a GN hamster, but produce better effect of DEC treatment against developing stages of larvae. Comparative studies between these strains would provide means to clarify mechanisms of drug action and, more generally, the host-parasite relationship.

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近交系ハムスターにおける B. pahangi の研究3．CBN ハムスターの感受性と diethylcarbamazine を用いた治療実験

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近交系 CBN ハムスター雄の B. pahangi に対する感受性を調べた．感染幼虫100隻を皮下接種すると，すべての動物の末梢血中に仔虫が出現した．prepatent period は平均74.5日であった．感染後20週時の血中仔虫密度は40cmm 中53.3隻（幾何平均）であった．成虫の回収率は5～54％（平均25.4％）で，虫体は睾丸およびその周囲組織，心臓，肺臓より回収された．以上のことから，CBN は先に報告した GN ハムスターについで B. pahangi に対する感受性が高いことが明らかとなった．

CBN ハムスターを用いて，diethylcarbamazine の B. pahangi 仔虫，3期・4期幼虫，および成虫に対する効果を調べた．仔虫，成虫に対する効果は，鼠径部皮下感染動物を用い，3期・4期幼虫に対する効果は睾丸内接種動物を用いて判定した．仔虫，幼虫に対しては著明な効果（血中仔虫密度の減少と幼虫回収率の低下）が認められたが，成虫治療群と対照群の虫体回収率には有意の差が認められなかった．糸状虫の治療実験モデルとしてハムスターへの睾丸内接種法の有用性を述べた．

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