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Mobilization of *Dirofilaria immitis* Microfilariae in Mice during the Treatment with Diethylcarbamazine

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Abstract: The provocative activity of diethylcarbamazine (DEC) on the skin-dwelling microfilariae was examinend by using a mouse-*Dirofilaria immitis* microfilariae model. The mice were inoculated subcutaneously with 30,000 *D. immitis* microfilariae and given DEC at dosage of 300 mg/kg body weight on 3,4 and 5 days after inoculation. Ten animals treated with DEC and ten control animals were necropsied on the 6 th day after infection in order to recover microfiariae alive. DEC treatment induced the reduction in the total number of microfilariae recovered from a mouse. The remarkable reduction was recognized in the number of microfilariae from pelt ($p<0.001$). On the contrary, the number of microfilariae from viscera was likely to increase, though the difference was not statistically significant. These facts suggested that DEC might cause the mobilization of microfilariae into viscera, especially to liver and kidneys. The possible invasion of large number of *D. immitis* microfilariae into deep organs during the treatment was discussed.

Key words: *Dirofilaria immitis* microfilariae, Alternative host, Onchocerciasis, Mice, Diethlcarbamazine, Mobilization

INTRODUCTION

Since diethylcarbamazine (DEC) was widely utilized as treatment on the patients with filariasis, it has been revealed that DEC had given effects on the distribution of

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microfilariae in host. When DEC was given to the patients infected with onchocerciasis, microfilariae were commonly found in the blood, cerebrospinal fluid, sputum, urine, epidermis, cornea and lymphatic system (Buck *et al.*, 1969; Fuglsang and Anderson, 1973). This mobilization of onchocercal microfilariae is considerable interest in research, because it may be closely correlated with microfilarial destruction following the administration of DEC.

Recently, we reported that the subcutaneous inoculation of *Dirofilaria immitis* microfilariae into mice was useful as a tool for the studies of the skin-dwelling microfilariae (Sakamoto *et al.*, 1984). The present study describes the effects of DEC on the mobilization of *D. immitis* microfilariae which were inoculated into mice.

MATERIALS AND METHODS

The procedures used for the inoculation of microfilariae of *D. immitis* and necropsy in this study were described in detail earlier (Sakamoto *et al.*, 1985). Briefly, *D. immitis* microfilariae obtained from the infected dog were washed with Hanks' solution (PH 7.2) several times. An aliquot of solution containing 30,000 microfilariae was inocutaled into each of 20 ICR mice subcutaneously in the inguinal region. Ten mice were given DEC intraperitoneally at the daily dosage of 300 mg/kg body weight on 3, 4, and 5 days after inoculation of microfilariae. Ten control mice were treated with saline solution by the same regimen of experimental group. On the 6th day after infection, the animals were sacrificed with ether. The ears and tail were removed. The mice were then skinned, and the eyes, heart, liver, spleen, kidneys, mesentery and genital organs were isolated. To recover microtilariae, all tissues and organs, except a head, were minced in Hanks' solution in petri dishes. After soaking for 2 hours at room temperature, the tissues and organs were removed and the remaining fluid was centrifuged at 300g force for 10 mim. The supernatant was discarded and the sediment was examined for microfilariae. Knott's concentration technique (1939) was applied to 60 cmm of the blood taken from the orbital sinus by a fine capillary tube in order to detect microfilariae. Statistical analysis was performed where appropriate using the t-test.

RESULTS

Table 1 represents the results of the postmortem examinations of control mice. Active microfilariae were recovered from all mice necropsied. The recovery rate ranged from 21.2 to 44.3% of the original inoculum with the mean of 35.4%. The majority of microfilariae were recovered from carcass, pelt and viscera though they were recognized in various parts of the body. The results of postmortem examinations of mice given DEC

were shown in table 2. The DEC treatment led the significant reduction of recovery rate ($p<0.05$). The microfilarial recovery rate was 21.1% of the original inoculum on the average, though it varied from 8.5 to 41.8%. The reduction in the number of microfilariae recovered from the pelt was statistically significant ($p<0.001$). The number of microfilariae recovered from the viscera of mice treated with DEC, however, increased.

Table 1. Distribution of *D. immitis* microfilariae in mice on 6th day after subcutaneous inoculation.

Mouse No.	% Recovery	No. microfilariae recoverd								
		Eyes	Ears	Pelt	Carcass	Viscera	Tail	Orbital blood	Others*	Total
1	33.8	2	21	3590	2317	858	2	7	3325	10122
2	38.9	1	10	3447	3600	796	27	7	3774	11662
3	24.5	2	16	3048	1548	484	2	4	2232	7336
4	52.8	2	6	3334	4568	1092	16	15	6795	15828
5	21.2	1	4	2632	2253	317	3	4	1125	6339
6	25.3	1	5	2942	2842	360	3	2	1468	7623
7	28.0	2	11	3486	2324	530	2	4	2051	8410
8	43.7	3	7	4267	5133	1046	4	9	2540	13009
9	44.3	2	2	5299	4062	1097	2	4	2835	13303
10	41.9	3	3	5315	3865	789	7	3	2380	12557
Total	35.4	19	85	37360 (35.2)	32512 (30.6)	7369 (6.9)	68	59	28525 (26.9)	106189

*Recovered from Hanks' solution rinsed tissues, organs, pleural and peritoneal cavities.

Table 2. Distribution of *D. immitis* microfilariae in mice treated with DEC on 6th day after inoculation.

Mouse No.	% Recovery	No. microfiariae recoverd								
		Eyes	Ears	Pelt	Carcass	Viscera	Tail	Orbital blood	Others*	Total
1	8.6	2	4	1880	326	74	0	4	287	2577
2	20.8	3	6	1172	1124	2073	32	9	1817	6236
3	17.7	1	15	1162	1768	359	7	2	1978	5292
4	29.3	8	5	1422	2856	1777	18	30	2682	8798
5	11.5	1	5	774	1193	297	0	2	1170	3442
6	12.0	0	3	1126	1262	383	3	1	819	3597
7	15.1	1	1	1141	2340	420	12	1	621	4537
8	41.8	4	19	3706	5376	1480	12	9	1942	12548
9	18.4	1	4	1027	2554	634	8	2	1290	5520
10	35.9	4	5	2366	5001	1943	19	2	1419	10759
Total	21.1	25	67	15776 (24.9)	23800 (37.6)	9440 (14.9)	111	62	14025 (22.2)	63306

*Vide note (*) in Table 1.

Table 3 shows the distribution pattern of microfilariae in viscera of control mice. When the frequency of distribution was expressed as the percentage of microfilariae recovered from an organ to total number of microfilariae recovered from the viscera examined, the rates were 6.0% in the liver, 21.3% in the kidneys, 0.7% in the spleen, 28.3% in the heart, 32.0% in the lungs, 6.4% in the mesentery and 5.3% in the genital organs. Table 4 represents the microfilarial distribution in viscera after DEC treatment. The number of microfilariae which were recovered from the liver, kidneys and spleen tended to increase following DEC administration, but the difference was not statistically significant.

Table 3. Distribution of *D. immitis* microfilariae in viscera in control mice.

Mouse No.	Total	No. microfilariae recoverd						
		Liver	Kidneys	Spleen	Heart	Lungs	Mesentery	Genital organs
1	858	74	84	3	41	608	5	43
2	796	10	97	1	397	214	26	51
3	484	44	50	6	210	86	66	22
4	1092	19	419	12	207	212	152	71
5	317	24	30	6	191	54	4	8
6	360	24	31	8	196	72	18	11
7	530	46	38	5	196	202	18	25
8	1046	76	271	3	350	223	51	72
9	1097	74	291	4	168	456	34	70
10	789	48	255	6	129	232	101	18
Total	7369	439 (6.0)	1566 (21.3)	54 (0.7)	2085 (28.3)	2359 (32.0)	475 (6.4)	391 (5.3)

Table 4. Distribution of *D. immitis* microfilariae in viscera in mice treated with DEC.

Mouse No.	Total	No. microfilariae recoverd						
		Liver	Kidneys	Spleen	Heart	Lungs	Mesentery	Genital organs
1	74	10	29	3	3	12	7	10
2	2073	73	655	179	267	623	179	97
3	359	39	88	13	76	88	32	23
4	1777	441	358	47	278	515	105	33
5	297	27	72	12	84	83	16	3
6	383	30	101	19	54	154	8	17
7	420	16	153	9	78	156	4	4
8	1480	93	607	36	390	246	105	3
9	634	58	255	6	129	168	6	12
10	1943	222	660	63	111	628	184	75
Total	9440	1009 (10.7)	2978 (31.5)	387 (4.2)	1470 (15.6)	2673 (28.3)	646 (6.8)	277 (2.9)

DISCUSSION

The mobilization of microfilariae caused by DEC was first described by Katamine *et al.* (1952). They reported that when DEC was given the patients infected with *Wuchereria bancrofti* during the daytime, at which microfilariae count was absolutely low, microfilariae count was much increased with a peak after 5 min. and returned to zero by 4 − 5 hours. A somewhat similar effect of DEC has been reported to occur in onchocerciasis. *O. volvulus* microfilariae were occasionally present in the blood and the urine of untreated patients (Buck *et al.*, 1969). In the patients treated with DEC, however, microfilariae were found more often in the blood, urine, sputum, cerebrospinal fluid, epidermis, cornea and lymphatic system (Buck *et al.*, 1969; Fuglsang and Anderson, 1973; Taylor *et al.*, 1980). When DEC has been administered, the number of microfilariae in the blood and the urine rose rapidly on the second third days of treatment and might remain high for 1 − 2 weeks (Hawking, 1979).

In the present study, the provocative action of DEC was tested 24 hours after the final administration of DEC at dosage of 300mg/kg body weight for 3 consecutive days by using a mouse-*D. immitis* microfilaria model. The distribution pattern of microfilariae in the mouse did not differ much between DEC treated mouse and control one. The majority of microfilariae recovered were from the pelt and carcass. However, DEC treatment induced the reduction in microfilarial recovery rate ($p<0.05$). The remarkable reduction was recognized in the number of microfilariae recovered from the pelt after DEC treatment ($p<0.001$). The number of microfilariae recovered from the carcass also decresed, but the difference was not statistically significant. The number of microfilariae in the blood remained unchanged. On the contrary, the number of microfilariae from viscera was likely to increase though statistical significance could not be obtained. These facts suggested that DEC caused the mobilization of microfilariae into the viscera, especially to liver and kidneys. Based on our study, it was anticipated an unexpected large number of *O. volvulus* microfilariae might invade into deep organs during DEC treatment. Onchocerca microfilariae which migrated to the epidermis were reported to encapsulation in micro-abscess 24 hours after DEC administration (Mimori, 1985). For the better understanding of Mazzotti reaction, the unpleasant reaction caused by DEC treatment, it is absolutely necessary to elucidate the histopathological changes in visceral organs which may be trigered by the mobilization of *O. volvulus* microfilariae.

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Diethylcarbamazine 治療前後に於ける *Dirofilaria immitis* ミクロフィラリアのマウス体内での分布の差異について

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Dirofilaria immitis ミクロフィラリア 30,000隻を ICR マウスの鼠径部皮下に接種し，Diethylcarbamazine (DEC) 300mg/kg 治療によるミクロフィラリアの マウス体内での 分布の変動を観察した．DEC 投与前の平均仔虫回収率は35.4%で，投与後3日目には21.1% に減少した．仔虫の体内での分布は DEC 投与により Pelt より Carcass，Viscera に移動する傾向がみられ，特に内臓では肝臓，腎臓，脾臓，肺臓，腸間膜等に仔虫が多くみられ，心臓では仔虫の減少が観察された．

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