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Low Responsiveness of *Dipetalonema viteae* in the Jird to Filaricides

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Introduction

There are only a few drugs practically used for the treatment of human filariasis. Development of new and safe filaricidal drugs, especially on adult filariae, is necessary and important in the world. For this reason, experimental models for drug testing which simulates human filariasis have been sought by many workers (Denham, 1979; Shibuya *et al.*, 1978). *Dipetalonema viteae* is considered to be useful for its characteristics as a subcutaneous filaria like *Onchocerca volvulus* and also regarded as an alternative experimental filariasis of *Brugia malayi*, *B. pahangi* or *Litomosoides carinii* (Hawking, 1973; WHO, 1974). In the present study, the value of *Dipetalonema viteae* in the jird as an experimental filariasis for the test of macrofilaricides is studied using the existing drugs such as suramin and Mel-W as macrofilaricides, and diethylcarbamazine and metrifonate as microfilaricides. In some expriments, *L. carinii* in the jird was also used for comparison with *D. viteae*.

Materials and Methods

1. Animal

The Mongolian jird, *Meriones unguiculatus*, was obtained from the colony of Jms: MON bred in this laboratory.

2. Filaria

Dipetalonema viteae was maintained in the jird and in a soft tick, *Ornithodoros moubata*, as the intermediate host (Worms *et al.*, 1961). Jirds were infected by subcutaneous inoculation in the inguinal region with 30 infective larvae of *D. viteae* which had been collected from the infected *O. moubata* using the Baermann apparatus.

For the infection of animals with *Litomosoides carinii*, they were infected by intrathoracic inoculation with 8 day-old larvae collected from the pleural cavity of the infected cotton rat, *Sigmodon hispidus*, 8 days after exposure to infective mites, *Ornithonyssus bacoti* (Petranyi and Mieth, 1972).

3. Examination

Microfilaria (mf) density in the peripheral blood of animals was counted by microscopical observation of thick films of blood collected quantitatively from the tail vein with a micropipette and stained with Azur II. At the end of experiment, animals were sacrificed by cardiopunctural bleeding and adult worms of *D. viteae* were collected from subcutaneous tissues and intermuscular fasciae of jird by dissection, for observing the effect of test compound on adult worms. Adult worms of *L. carinii* in the pleural cavity of the jird were also observed by autopsy.

4. Test drugs

The following drugs were used in the present study;

1) suramin (Germanin)

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2) Mel-W (Trimelarsan)

3) diethylcarbamazine citrate (Supatonin, abbreviated as DEC)

4) metrifonate

Suramin, Mel-W and metrifonate were dissolved in physiological saline and DEC was with phosphate buffered saline at pH 7.4. Concentrations were adjusted so as to inject 0.2 ml per 100 g of animal body weight.

Results

The effect of suramin was examined by injection at doses of 50 and 25 mg/kg weekly for 5 times in the jirds 14 weeks after infection with *D. viteae*. Animals were sacrificed 6 weeks after the first injection, and the effect of suramin on adult worms was observed by dissection. Mf counts were observed once a week during the course of experiment. The mf density observed and adult worms recovered are shown in Table 1. No effect of suramin on mf density or adult worms was observed, comparing with the control group. And live adult worms with active movement were recovered even at a large dosage of 50 mg/kg.

Mel-W was examined in jirds 16 weeks after infection by intramuscular injection at 100 mg/kg and by intraperitoneal injection at 20 mg/kg, both daily for 5 consecutive days. By intramuscular injection, two of five treated jirds died within 5 weeks, no dead worm was found at autopsy in the three tolerated jirds 6 weeks after treatment (Table 2). Transient reduction of mf density was observed by intraperitoneal injection.

Effect of DEC on mf of *D. viteae* was examined in jirds 14 weeks after infection by intraperitoneal injection at a daily dose of 50 mg/kg for 5 consecutive days. Changes of mf density are illustrated in Figures 1 and 2 by the percentage to the initial mf density. Anti-microfilarial effect of DEC was not observed in two of five treated jirds. DEC was also tested in jirds 14 weeks after infection with *D. viteae* together with *L. carinii*. Jirds were treated with DEC at a dose of 50 mg/kg for 5 consecutive days by intraperitoneal injection. Anti-microfilarial effect of DEC was found against *L. carinii* in all treated jirds (Fig. 2), but no effect was observed on *D. viteae* in two of five jirds.

Effect of metrifonate on mf of *D. viteae* was examined in jirds 10 to 14 weeks after infection, by intraperitoneal injection at doses of 25 mg/kg and 100 mg/kg daily, and subcutaneous in-

Table 1 Effect of suramin (Germanin) on *Dipetalonema viteae* in jirds by intraperitoneal injection weekly for 5 times

Weekly dosage (mg/kg)	Sex of host	No. of mf in blood (Mf/10 μl)				No. of worms recovered			
		Weeks after the first injection				active		in-active	dead or degenerated
		0	1	3	5	♂	♀		
50	♂	79.4	25.9	59.9	122.9	4	2	0	0
	♂	326.4	83.1	321.6	251.9	4	8	1(♂)*	0
	♀	13.4	2.0	2.0	4.8	0	1	0	0
	♀	53.4	23.0	74.2	160.0	2	4	0	0
	♀	31.6	21.6	50.0	224.0	2	6	0	0
25	♂	68.1	28.8	63.5	58.0	3	2	1(♂)*	0
	♂	9.8	4.0	11.8	2.0	3	6	0	0
	♀	8.5	2.0	10.6	41.8	3	3	0	1
	♀	9.8	5.7	2.0	4.9	1	2	1(♂)*	0
	♀	15.5	6.9	14.1	16.7	4	3	1(♂)*	2
0	♂	29.4	25.5	138.6	67.1	9	5	0	2
	♀	3.4	5.7	10.4	15.5	3	1	0	0

* Sex of the adult worm.

Table 2 Effect of Mel-W (Trimelarsan) on *Dipetalonema viteae* in jirds by injection for 5 consecutive days

Daily dosage (mg/kg)	Animal no.	No. of microfilariae in blood (Mf/2.5 μl) Weeks after the first injection						Adult worms recovered
		0	1	2	4	6	10	
100(im)	E-1*	7.3	13.3	12.0	47.3	—	—	alive
	-2	0.7	0.7	1.6	3.3	6.7	—	alive
	-3	3.7	3.3	5.3	10.0	13.7	—	alive
	-4*	13.0	8.3	—	—	—	—	alive
	-5	1.0	0.7	9.0	9.3	5.0	—	alive
20(ip)	F-1*	46.3	27.3	31.5	14.6	24.0	396.0	alive
	-2*	22.3	11.6	16.0	14.6	5.3	35.3	alive
	-3*	24.3	12.0	4.0	11.0	14.0	—	alive
0	G-1	19.0	49.3	23.5	20.3	47.0	80.6	alive
	-2	50.3	60.0	68.0	78.3	70.3	60.0	alive

Animals were sacrificed 6 weeks after the first injection in group E and 11 weeks after in groups F and G.
* Animal died in the course of experiment.
im=intramuscular; ip=intraperitoneal injection.

jection at 50 mg/kg for 5 consecutive days. Slight and transient reduction of mf density of *D. viteae* was observed in the jirds while treating with metrifonate (Table 3).

The method to evaluate macrofilaricidal activity of a compound was examined in the jirds infected with *L. carinii* or *D. viteae* by observing mf density after injecting a candidate macrofilaricide at first and metrifonate as the microfilaricide. With *L. carinii*, jirds were treated intrathoracically with Mel-W at a daily dose of 20 mg/kg for 5 consecutive days, then treated intraperitoneally with metrifonate at a daily dose of 25 mg/kg for 5 consecutive days. Macrofilaricidal action of Mel-W was clearly demonstrated by this method (Table 4). As macrofilaricidal effect of the test compound is satisfactory, no recovery of mf density was observed in the peripheral blood as in animal nos. H-1, -2 and -4 in Table 4.

With *D. viteae*, effect of Mel-W was also examined by intramuscular injection at a daily dose of 100 mg/kg for 5 consecutive days followed by subcutaneous injection with metrifonate at a daily dose of 50 mg/kg for 5 consecutive days. In this case, since microfilaricidal action of metrifonate was not marked, macrofilaricidal action was not proven.

Discussion

In the present study, usefulness of an animal filaria, *D. viteae*, in the jird was examined using existing macro- and microfilaricides to find out if this model can be utilized for testing macrofilaricides.

Antifilarial action of suramin had been reported in various filariasis (Denham, 1979; Van den Bossche, 1981). However, no effect was observed by suramin on adult worms and mf of *D. viteae* in the jird (Table 1, Worms *et al.*, 1961).

Arsenics have also been known as the antifilarial drug, and their macrofilaricidal action was reported on *D. viteae* in the jird (Worms *et al.*, 1961). In the present study, however, macrofilaricidal action of Mel-W was not clearly demonstrated (Tables 2 and 4).

Metrifonate, an organophosphorus compound, has been known as a microfilaricide (WHO, 1974). In the present experiment, although excellent microfilaricidal action of metrifonate was found on *L. carinii* in the jird (Table 4), less activity was observed against mf of *D.*

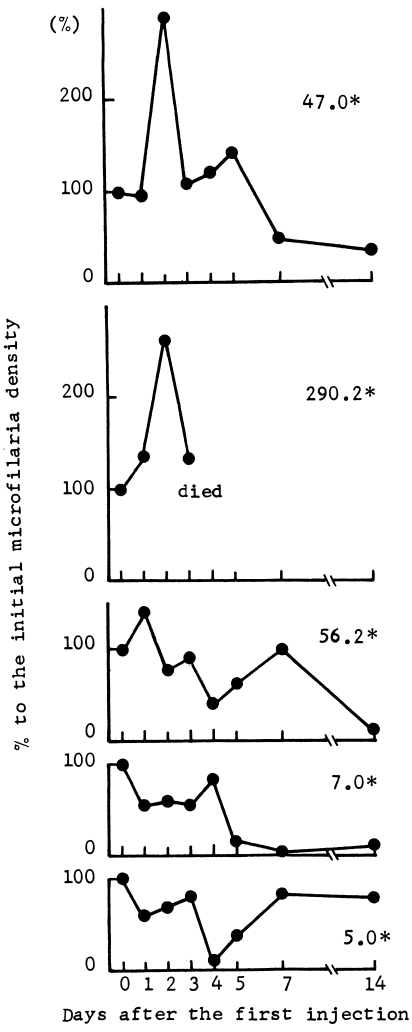

Fig. 1 Effect of diethylcarbamazine (DEC) on the microfilaremia in *Dipetalonema viteae* infected jirds. Treatment with DEC at a daily dose of 50 mg/kg for 5 consecutive days by intraperitoneal injection.
* Mf count per 5 μl of blood before treatment.

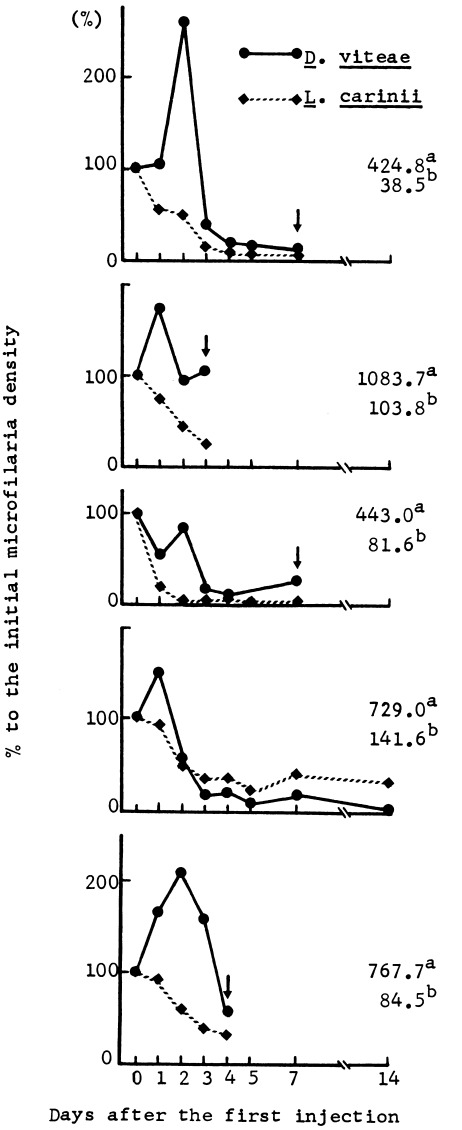

Fig. 2 Effect of diethylcarbamazine (DEC) on microfilaremia in jirds infected with *Dipetalonema viteae* together with *Litomosoides carinii*. Treatment with DEC at a daily dose of 50 mg/kg for 5 consecutive days by intraperitoneal injection. Mf count per 5 μl of blood before treatment ; a=*L. carinii*, b=*D. viteae*. ↓ died

viteae (Tables 3 and 4, Collins, 1974).

Since DEC is the most effective drug in human filariasis, the chemotherapeutic response to DEC is said to be important in the experimental animal filaria. The fact that low response of *D. viteae* in the jird to DEC reported by many researchers (Cavier *et al.*, 1971 ; Hawking, 1973 ; Matsuda *et al.*, 1976 ; Worms *et al.*, 1961) was the main shortage of this system for the chemotherapeutic study. It was reported that there was a wide variation of the response to DEC among jirds due to special physiological characteristics of the jird (Hayashi *et al.*, 1983 ; Matsuda *et al.*, 1976 ; Sasa,

Table 3 Effect of metrifonate on microfilariae of *Dipetalonema viteae* in jirds by intraperitoneal injection for 5 consecutive days

Daily dosage (mg/kg)	Animal no.	Number of microfilariae in the peripheral blood*					
		Days after the first injection					
		0	2	4	7	14	21
100(ip)	A-1§	18.0	8.5	4.0	17.0	—	—
	-2†	152.5	146.5	76.0	—	—	—
	-3	59.5	32.5	108.0	28.5	74.0	—
	-4	104.0	24.5	76.0	44.5	43.0	—
25(ip)	B-1	10.6	2.3	2.7	10.3	18.0	20.0
	-2	3.3	3.0	1.6	8.9	8.7	15.3
	-3	16.3	10.4	5.7	9.3	10.7	15.0
50(sc)	C-1	17.0	11.7	12.3	15.6	53.3	39.7
	-2	21.0	9.7	13.6	10.3	30.3	25.6
	-3‡	6.0	1.6	2.7	—	—	—
	-4	6.7	4.3	7.7	16.0	15.7	10.3
0	D-1	3.0	3.5	3.5	2.5	8.0	11.5
	-2	6.3	3.3	6.1	10.3	9.3	9.7
	-3	2.7	0.7	1.3	1.0	1.7	6.0
	-4	4.3	4.3	2.7	2.3	6.0	4.0

* Number of microfilariae in 5 μl in group A, and in 2.5 μl in groups B, C and D.
Animals died 5 days (†), 6 days (‡) and 8 days (§) after the first injection.
ip=intraperitoneal ; sc=subcutaneous injection.

1976 ; Tanaka *et al.*, 1981). In the present study, anti-microfilarial effect of DEC was also observed in some treated jirds when DEC was successively administered (Sturm and Henry, 1974). *D. viteae* in some jirds did not respond to DEC in contrast to marked anti-microfilarial effect against *L. carinii* coexisting in the jirds (Fig. 2). These present results indicate that the parasite, *D. viteae*, *per se* is also responsible to the resistance to DEC as well as the host so far reported.

Simplicity of the maintenance of *D. viteae* in the jird is an advantage, while the difficulty in finding out adult worms, especially when they were killed by the drug is a big disadvantage with this parasite (Hawking, 1973 ; Sasa, 1976). When only mf is killed by a microfilaricide, mf density is returning sooner or later to the initial level by the unhurt fecundity of living females. If the effective macrofilaricide is given prior to the microfilaricide, the recovery of mf density will be suppressed (Denham, 1974 ; Duke, 1962). By this method, *L. carinii* was considered to be useful in testing macrofilaricide because of the availability of reliable microfilaricide. However, this indirect method for evaluating the macrofilaricidal action was not successfuly woking with *D. viteae* in the jird, because the effect of existing microfilaricide was not satisfactory in this system.

In conclusion, although *D. viteae* responded unevenly to DEC, *D. viteae* in the jird dose not appear to be an appropriate model for evaluating the macrofilaricide in any experimental designs.

Summary

An animal filaria, *Dipetalonema viteae*, in the jird was examined to be useful as a screening model of macrofilaricides using existing drugs. *D. viteae* in the jird did not respond to a large dosage of human filaricides such as suramin, Mel-W and metrifonate. Anti-microfilarial effect of diethylcarbamazine (DEC) was

Table 4 Effect of treatment with Mel-W (Trimelarsan) followed by metrifonate on *Litomosoides carinii* or *Dipetalonema viteae* in jirds

Parasite		No. of microfilariae in the peripheral blood (Mf/2.5 μl)								
		Litomosoides carinii				*Dipetalonema viteae*				
Treatment		Mel-W (20 mg/kg×5 days, it) & metrifonate (25 mg/kg×5 days, ip)				Mel-W (100 mg/kg×5 days, im) & metrifonate (50 mg/kg×5 days, sc)				
Jird no.		H-1†	H-2	H-3	H-4	K-1	K-2	K-3	K-4	K-5
Days after the first treatment	0	528.0	784.5	2975.0	1323.0	9.3	6.0	11.0	8.0	11.7
	7*	855.5	1139.5	2577.0	1134.0	6.3	4.7	11.0	5.7	7.3
	8*	373.0	961.0	1606.5	1042.0	2.3	0.3	8.3	9.0	11.7
	9*	246.5	260.0	945.5	88.5	1.7	1.7	9.7	14.7	8.0
	10*	61.0	11.5	33.0	1.0	3.3	0.3	7.7	11.7	3.0
	11*	19.0	0.5	11.5	0.5	12.0	1.3	6.3	15.0	3.0
	12	0.5	0.0	3.5	0.0	4.3	2.7	9.0	5.3	2.7
	14	0.5	0.0	19.5	0.0	7.3	1.7	16.3	0.7	9.3
	21	3.0	0.5	112.0	7.5	24.3	4.7	17.3	1.3	10.0
	28	—	1.5	169.5	19.5	8.3	1.3	16.7	3.0	8.0
	35	—	1.0	275.5	10.0	3.0	1.0	21.0	1.0	13.7
	42	—	—	—	—	1.7	1.0	54.7	5.7	16.3
Effect on adult worms		killed	killed	almost‡ killed	killed	alive	alive	alive	alive	alive
Mf in pleural cavity		dead	dead	active	active	—	—	—	—	—

* metrifonate treatment. † Animal died 23 days after the first injection.
‡ Four adult females survived. it=intrathoracic; ip=intraperitoneal; im=intramuscular; sc=subcutaneous injection.
Animals were sacrificed 5 weeks after the first treatment in *L. carinii* group and 6 weeks in *D. viteae* group.

uneven on this filaria when jirds were administered with DEC successively for 5 days. On the other hand, *L. carinii* in the jird responded well to DEC, metrifonate and Mel-W. *D. viteae*-jird model showed a different susceptibility to drugs from that of human and was considered to be an inappropriate model for evaluating the filaricides.

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スナネズミ寄生 *Dipetalonema viteae* の抗フィラリア剤に対する低反応性

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スナネズミ寄生 *Dipetalonema viteae* が抗フィラリア剤の薬剤検定系として利用しうるかどうかを検討した．ヒトのフィラリア治療薬各種に対する *D. viteae* の感受性を検討した結果，suramin, Mel-W, metrifonate の大量投与では明確な効果は認められなかつた．Diethylcarbamazine (DEC) では50mg/kg量の5日間連続投与によつて，*D. viteae* 感染スナネズミの約半数例で抗仔虫効果が示された．一方スナネズミに感染させた *Litomosoides carinii* に於ては，Mel-W, DEC, metrifonate に感受性が示された．薬剤感受性がヒトのそれと近似していない点から，*D. viteae*—スナネズミ系は抗フィラリア剤検定系として好ましくないと思われた．