[Jap. J. Parasit., Vol. 27, No. 1, 41-45, 1978]

Mode of Action of Diethylcarbamazine on Microfilariae of *Setaria cervi* Implanted into White Rats

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(Received for publication ; November 25, 1977)

Introduction

Diethylcarbamazine citrate (Hetrazan) remains the drug of choice for the control of human and animal filariases. The administration of the drug results in drastic reduction in the microfilarial level of infected animal, while having no significant effect on adult worms at the therapeutic levels. The mode of action of the drug on the microfilariae is not very clear. Hawking *et al.* (1950) for the first time put forward the hypothesis that Hetrazan removes the microfilariae of *Litomosoides carinii* from peripheral blood circulation by modifying them in some way, so that they are held in the liver and destroyed by phagocytosis.

The present paper describes the antifilarial action of Hetrazan on microfilariae of *Setaria cervi*, with special reference to the mechanism underlying its action in the liver of implanted rats.

Materials and Methods

Five adult worms were implanted intraperitoneally into white rats according to the method described elsewhere (Baqui and Ansari, 1976). The study was conducted on 30 infected rats divided into two batches. In the first batch, 20 microfilaria positive rats were treatad with Hetrazan orally at 7 A. M. (100 mg/kg/day) ; and 15 of them comprising 5 rats each, were killed at 1-, 5-, and 20-hour intervals after drug administration. The liver was removed and fixed in Bouin's solution. Blood (1 mm^3) was taken from the tail immediately before the treatment and after 1-, 5- and 20-hour postmedication periods for counting microfilariae. Five rats were maintained to study the nature of reappearance of microfilariae after discontinuation of the drug. The second batch consisted of 10 untreated rats and served as control. Microfilarial density was also recorded in all these rats. Five of 10 control rats were maintained to observe the daily changes in microfilarial density for 26 days after infection, and rest of the rats were sacrificed for histological examination of the liver.

The organ was sectioned at 4 μ and stained in haematoxylin-eosin stains. The sections were searched systematically and number of microfilariae were counted. Usually 100 sq. mm. of section was examined. The count included only those microfiariae which were in sinusoids of the liver and microfilariae lying in tissues were excluded for it was sometimes difficult to identify. Only those microfilariae were counted which were cut obliquely or longitudinally to the plane of the section. Those cut transversally were almost impossible to distinguish and identify, hence excluded. The area of section was measured by using ocular micrometer. Immersion oil was used to identify the type

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Table 1 The distribution of microfilariae in control and Hetrazan treated rats

Organ	Control rats	Treated rats				Reappearance of mf (in days)		
		Before treatment	Hours after treatment					
	mf range		1	5	20	8	9	10
			mf range			mf range		
No. of mf/mm³ of blood	6~10 (7.8±0.3)	6~9 (8±0.2)	4~6 (4.8±0.3)	3~5 (4±0.4)	3~6 (4.4±0.5)	0~1 (0.2±0.1)	0~1 (0.2±0.1)	0~3 (1.6±0.5)
No. of mf/100 dsq. mm. of liver section	1~5 (2.8±0.7)	—	10~18 (13.8±1.6)	6~11 (8.6±1.0)	2~7 (4.6±1.0)	—	—	—

Figures in parentheses are mean microfilarial density.

of phagocytes adhering to or around the microfilariae. Photographs of the microfilariae were taken at a high power magnification.

Results

Table 1 presents the distribution of microfilariae in peripheral blood and liver of control and Hetrazan treated rats. Immediately before the drug administration (7 A. M.), microfilarial concentration in blood on 12th day of infection was noted to be between 6–9 (mean 8.0/mm³). At 1 hour post-treatment, microfilarial density in blood declined to 40.0%. By 5–20 hours, the decrease was noted to be between 50–45% (Table 1). Microfilariae during these post-treatment periods remained constantly low ranging from 3–6/mm³ of blood. The number of microfilariae present in liver section in control rats were ranging from 1–5 with an average of 2.8/100 sq. mm area. This figure was comparable with that of treated groups. At 1 hour post-treatment, there was a sharp increase (392.8%) in microfilarial density in liver of treated rats. This increase was followed by a relative decrease by 5 hours and tended to come to almost normal figure by 20 hours post-treatment (an increase of only 64.2%). Maximum microfilarial density in the liver was noted between 1–5 hours post-treatment (Table 1).

Five infected rats of the first batch were continuously treated with Hetrazan at a dosage of 100 mg/kg, and noted the time of complete disappearance and reappearance of microfilariae in blood. After 5 consecutive days of medication in these rats, microfilariae were found to have completely disappeared from peripheral blood circulation but reappeared after a lapse of 7 days of discontinuation of drug. The microfilariae thus appeared were found to be sheathed ones. Figure 1 shows the daily changes in microfilarial density of treated rats and untreated control rats. The microfilarial population in untreated control group was found to be smoothly increasing whereas in treated group

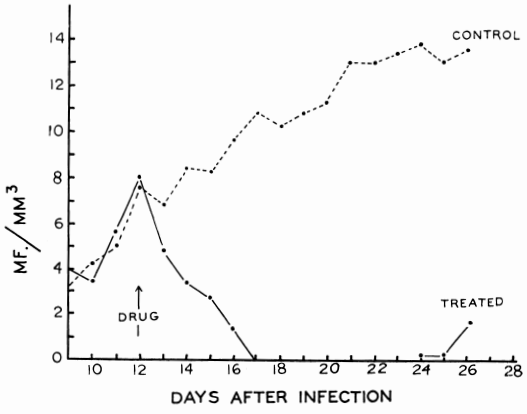

Fig. 1 Effect of diethylcarbamazine on microfilariae of *Setaria cervi*.

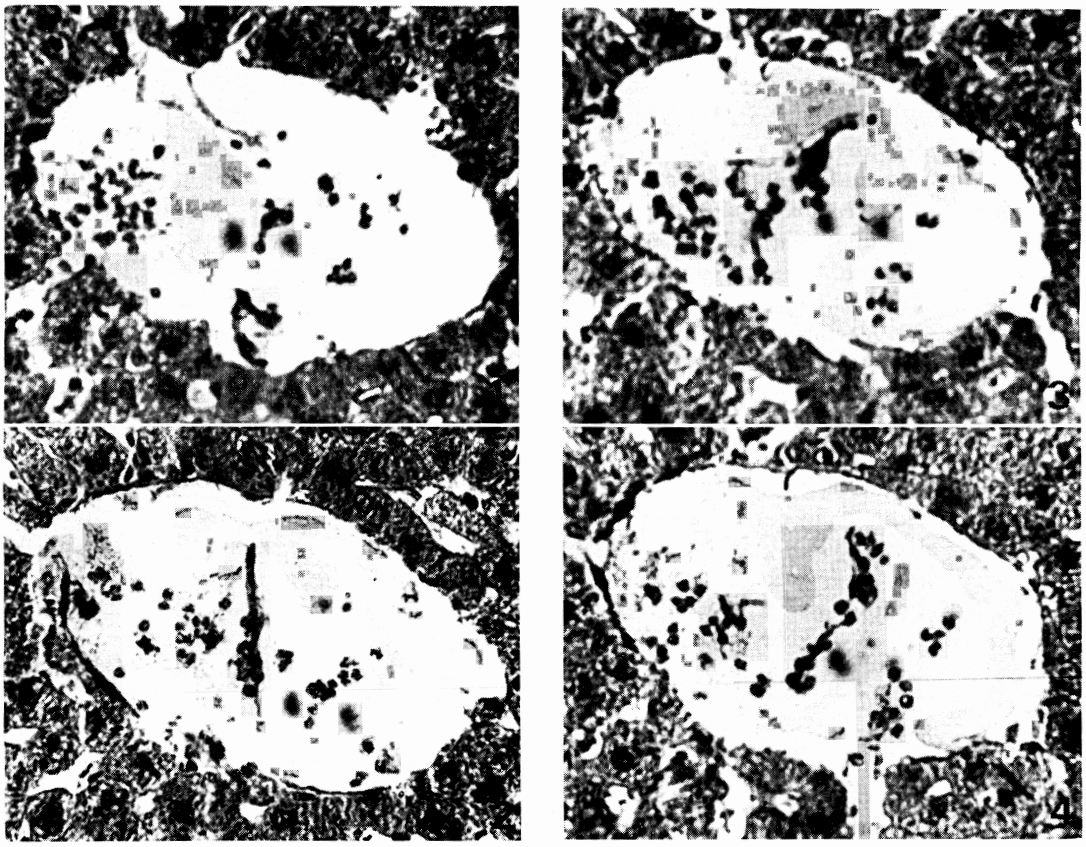

Photo. 1-4 Microfilariae in the liver of white rats ×675.

Photo. 1 Liver of control rat ; no phagocytosis.
Photo. 2 Liver 1 hour after Hetrazan treatment, some phagocytes near the microfilaria.
Photo. 3 Liver 5 hours after treatment, partial destruction of microfilaria.
Photo. 4 Liver 20 hours after treatment, microfilaria completely destroyed.

microfilarial density tended to decline following drug administration, and reached to zero by 17th day of infection. The microfilariae reappeared in blood circulation in these rats after a lapse of 7 days of discontinuation of drug but their number was very low and ranged from 0-3/mm^3 (Table 1 ; Fig 1).

Photograph 1 shows the normal microfilaria in liver section of control rat with no sign of phagocytosis. Photogrraphs 2-4 show the gradual destruction of microfilaria in the liver through a process of phagocytosis under the influence of the drug. Photograph 2 shows the crowding of phagocytic cells on and around the microfilaria after an hour of drug administration. Photograph 3 shows the partial destruction of microfilaria by phagocytes after 5 hours. By 20 hours, microfilaria was completely destroyed by phagocytes (Photograph 4). The cells surrounding the microfilaria were found to be polymorphs and Kupffer cells of the liver.

Discussion

Hetrazan has no significant lethal action *in vivo* on adult worms of *Setaria cervi* at therapeutic levels. However it causes rapid disappearance of microfilariae from peripheral blood circulation (Baqui and Ansari, 1976). It has been observed that under the influence

of the drug microfilariae leave blood circulation and get collected in the liver. Consequently, the level of microfilaraemia is considerably increased in the liver as compared to that of control group. This increase is, however, followed by a decline after a lapse of 20 hours which could be accounted for the destruction of microfilariae in the liver through a process of phagocytosis. This view is further strengthened by the fact that the microfilariae leaving peripheral blood circulation under the influence of the drug never return to it. Consequently, the level of microfilaraemia in peripheral blood never reaches to normal level even after 20 hours post-medication period. The cells mainly taking part in the phagocytic process were found to be polymorphs and Kupffer cells of the liver. These observations are in accordance with the earlier investigations carried out in cotton rats infected with *Litomosoides carinii* (Hawking *et al.*, 1950; Kobayashi *et al.*, 1969; Nand and Sen, 1976).

The factor initiating the phagocytic process following drug treatment is not clear. Hawking *et al.* (1950) have suggested that the drug exerts opsonin-like action on microfilariae and as a result they are finally removed through a process of phagocytosis in the liver. Their experiments have shown that under the influence of the drug maximum number of microfilariae get accumulated in the liver and to a minor degree in the spleen and marrow where they are phagocytized by polymorphs and Kupffer cells; and the time required for complete removal of microfilariae of *Litomosoides carinii* in the liver of cotton rats is 18–23 hours. These observations were further strengthened by Taylor (1960) in cotton rats infected with *Litomosoides carinii*.

Elaborate studies on the mode of action of Hetrazan have been carried out by Schardein *et al.* (1968) in *Litomosoides carinii* infection in gerbils. They have reported that within 20 minutes of the Hetrazan treatment given intravenously, the blood circulating microfilariae get collected in the sinusoids or localised within the hepatic cells. And the drug exerts its effect on microfilariae through a process of lysis perhaps through loss of sheath, and are finally removed by phagocytosis. He has further reported that those microfilariae localised within the hepatic cells apparently escape phagocytosis and may possibly be considered as a source of infection and reappearance.

Latest studies have shown that certain immunological factors are also involved which leave the microfilariae susceptible to drug's action and phagocytosis. Kobayashi *et al.* (1969) have reported that the effectiveness of diethylcarbamazine on microfilariae of *Litomosoides carinii* in cotton rats is closely associated with the presence of humoral antibodies. Matsuda *et al.* (1976) have also reported similar results in jirds infected with *Litomosoides carinii*, and have shown the presence of high antibody titer detectable by hemagglutination test. Their experiments have further shown that diethylcarbamazine not only removes the microfilariae of *Litomosoides carinii* in jirds but it also in some way increases the level of antibody titer. Rao *et al.* (1977) have reported that the microfilariae of *Litomosoides* freshly infused into normal animal remain unaffected by the drug, hence sensitization is a prerequisite for the drug to exert its effect.

Characteristic reappearance of microfilariae in the present study after discontinuation of the drug may be explained on the basis of earlier observations made by Hawking *et al.* (1950). It is the result of supply of fresh brood from adult worms which remain unaffected by the drug. This view is further strengthened by the fact that sheathed microfilariae were observed. Hence, these microfilariae may not be intracellular forms as suggested by Schardein *et al.* (1968).

Summary

White rats experimentally infected with *Setaria cervi* were treated with diethylcarbamazine citrate. Under the influence of the drug most of the circulating microfilariae leave the blood stream and get collected in the liver where they are phago-

cytized by polymorphs and Kupffer cells. Reappearance of the microfilariae after discontinuation of the drug supports the concept of fresh supply from adult worms which apparently remain unaffected by the drug.

Acknowledgement

We are grateful to Prof. S. M. Alam, Head, Dept. of Zoology for providing laboratory facilities.

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Setaria cervi のミクロフィラリアに対する Diethylcarbamazine の作用機構

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Litomosoides carinii に対する Diethylcarbamazine citrate (DEC) の作用機構については先人の研究があるが，まだ充分に解明されたわけではない．著者らは *Setaria cervi* のミクロフィラリア (Mf) を腹腔内接種したラットに DEC (1日 100 mg/kg) を経口投与し，投与直後および 1, 5. 20時間後の血中 Mf 数を調べ，投与 1, 5, 20時間後の肝 Mf 数を調べた結果，DEC 投与後の時間の経過とともに血中 Mf は減少し，肝 Mf は増加したが，肝 Mf は多形核白血球およびクップェル細胞に捕捉され，5時間後には一部に破壊がみられ，20時間までに完全に破壊された．また5日間の DEC 連日投与により血中 Mf が完全に消失した後，DEC 投与は中断した結果，7日後に血中に Mf が再出現した．これは DEC によって影響を受けなかった成虫から新生されたものと思われる．