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Comparative Studies on the Chemotherapy of Experimental *Setaria cervi* Infection

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Introduction

Setaria cervi, the cosmopolitan filariid worm, has a high rate of incidence among buffaloes and cattle population of India. The parasite is known to produce tissue fibrosis, penetration and obstruction in the intestine of the hosts (Sarwar, 1945). Evidences are also available that infective stages in strange circumstances may enter the central nervous system of the natural hosts and develop to partial maturity causing severe pathological condition known as 'lumbar paralysis' (Blazeck *et al.*, 1968; Pachauri, 1972). Accidental infection of this worm is also very common among sheep and goats which may result into 'cerebro-spinal nematodiasis'. Suggestions have also been put forward by Innes (1951), Innes and Shoho (1952), Kadenatsii (1956), Nelson (1965) that many neurological syndromes of unknown aetiology in man and animals in tropics are due to *Setaria* sp. infection.

In view of the heavy incidence of this worm and its involvement in serious diseases, the present work of its control by anthelmintics was undertaken. Survival of adult worms for a long duration in alternate hosts (Williams, 1955; Nelson, 1962; Ansari, 1964) stimulated the screening of anthelmintics in rat-cervi system. The drugs used as test chemicals are diethylcarbamazine citrate (Hetrazan), tetramisole and thiabendazole.

Materials and Methods

Adult worms collected from the peritoneal cavity of buffaloes, *Bos bubalis* from the abattoir, were washed several times in physiological saline to remove extraneous matter. Implant of the worm into peritoneal cavity of white rats was made via laparotomy. Each rat received three female and two male worms.

Blood of each rat was tested for microfilariae, and microfilarial count was done each day at 6-8 p.m. The amount of blood taken was always maintained as 1 mm^3. The drugs were administered orally after three days of appearance of microfilariae, and continued till the microfilariae disappeared from peripheral blood circulation. Prior to the administration of the drugs, weight of each rat was taken.

After a week of the discontinuation of drug, the blood of rats was checked again to find any possiblity of reappearance of microfilariae. At the close of the experiment, the rats were autopsied. A batch of ten rats non-medicated as control was also kept in the laboratory, and a record on microfilaraemia was maintained.

Results

Microfilariae appeared in the peripheral blood circulation of all rats with a latent period of 8 ± 2 days and continued to exist for 65 ± 5 days in control rats. An average maximum density of 19 microfilariae/mm^3 was recorded in these rats.

Use of diethylcarbamazine citrate in the experimental rats brought speedy disappearance of microfilariae from peripheral blood circulation. All rats cleared of microfilariae in 3-6 days at the dose of 100 mg/kg body

Table 1 Effect of various anthelmintics against microfilariae of *Setaria cervi* in white rats

Drug	Dose mg/kg body weight	Time of disappearance of mf. (days)	Duration of medication (days)	Rats cleared of mf.	Response	Recovery of live adult worm on autopsy
Diethyl-carbamazine citrate	25.0	10–13	12–15	5/10	50%	36%
	50.0	7–9	9–11	9/10	90%	40%
	100.0	3–6	5–8	10/10	100%	48%
Tetramisole	7.5	3–5	5–7	10/10	100%	32%
	15.0	2–3	4–5	10/10	100%	28%
Thiabendazole	25.0	Nil	20	0/10	0%	52%
	50.0	Nil	20	0/10	0%	48%
	100.0	Nil	20	0/10	0%	48%
Untreated control	—	—	—	0/10	—	40%

weight given twice daily presenting a 100% response. The onset of antifilarial action, however, was delayed by 7–9 days and 10–13 days at the dosages of 50 mg/kg and 25 mg/kg with 90% and 50% response in respective cases (Table 1). No side effect of any kind was evident at these dosages.

Tetramisole, a broad spectrum anthelmintic, was tested for the first time in the present experiment. The drug showed potent microfilaricidal property. The drug when administered orally in 7.5 mg/kg and 15.0 mg/kg body weight (single dose/day) produced complete elimination of microfilariae from peripheral blood circulation of all rats (response 100%). Disappearance time of microfilariae at 15.0 mg/kg varied from 2–3 days while slightly delayed by 3–5 days at the dose of 7.5 mg/kg. The chemical also showed no side-effect of any kind.

The drugs not only brought microfilarial elimination but the level of microfilaemia also reduced considerably. Maximum microfilarial density in Hetrazan and tetramisole treated cases, on an average, varied between 3.8 and 4.5, and microfilarial population showed a declining tendency (Fig. 1). Microfilariae reappeared after a lapse of one week of discontinuation of drug in all Hetrazan treated cases whereas no microfilariae were traced in groups of rats treated with tetramisole.

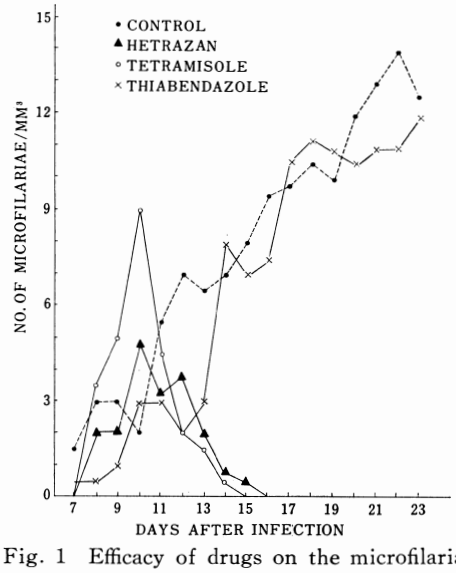

Fig. 1 Efficacy of drugs on the microfilarial density in the blood of rats.

Thiabendazole, another broad spectrum anthelmintic, when tested in the present experiment at various dosages administered twice daily, proved completely ineffective (Table 1). The microfilarial population continued to increase even at higher doses and followed the microfilarial curve pattern of control rats (Fig. 1).

The rats were autopsied after the treatment was over. Hetrazan treated groups

of rats were sacrificed over a period of 22–30 days from initial infection. Average recovery of live worms in these rats was 36%, 40% and 48% at the doses of 25 mg/kg, 50 mg/kg and 100 mg/kg respectively. The remaining worms were either dead, exhausted or untraceable. Tetramisole treated groups were taken up earlier between 22–24 days because of its extreme efficacy. Both of its doses being used produced sufficient mortality, and only 32% and 28% live worms, on an average, were recovered (Table 1). In case of thiabendazole treated groups, 48–52% of live worms, on an average, were recovered from rats even after 20 days of continued medication. Average recovery of live worms in untreated control group had been 40% after 30 days of initial infection.

Discussion

Diethylcarbamazine citrate has been a drug of choice in most of the animal and human filariases. Burch and Ashburn (1951), Duke (1957, 1968) reported the filaricidal action of Hetrazan on microfilariae of *Onchocerca volvulus* but remained ineffective on adult worms. Cherry (1960) reported Hetrazan as a superior and most effective drug in onchocerciasis. The allergic reaction, he reported, could be successfully controlled by prednisolone. Taylor and Terry (1960) observed Hetrazan to be ineffective against embryos and adult worms of *Litomosoides carinii* during *in vivo* and *in vitro* studies. However, they reported the drug to be effective against microfilariae of *Litomosoides carinii*.

Singhal *et al.* (1972, 1973) tested diethylcarbamazine citrate against *Setaria cervi in vivo* and *in vitro*, and found it quite effective against the microfilariae only. They also observed a decrease in the reproductive potential of the adult worms and attributed it to be the beneficial effect of the drug. The drug, they reported, was capable of producing 100% mortality of adult worms *in vitro* at a very high dose (750 μg/ml concentration). The present study supports the above observation in part. The drug has shown lethal effect on microfilariae, but remained quite ineffective against the adult worms in *in vivo* condition even at higher doses. The views expressed by Singhal *et al.* (1973) regarding the decrease in the reproductive potential as indicated by the absence of microfilariae from the blood circulation even in the presence of live adult worms, is based purely on imagination. Hawking (1950), Hawking *et al.* (1950) have already shown that microfilariae of *Litomosoides carinii* in the presence of Hetrazan move away from the blood circulation and get collected in the liver, and which are later destroyed by opsonin-like action. The characteristic reappearance of microfilariae in the peripheral blood circulation after a week of discontinuation of the drug in the present study is indicative of the fact that the adult worms have never lost the reproductive potential but continued to produce it throughout, and as the influence of the drug was over, the microfilariae reappeared in the peripheral blood circulation after a brief lapse.

Elaborate studies on the mode of action of Hetrazan have been carried out in *Litomosoides*, *Onchocerca* and *Wuchereria* infections. Hawking (1950) reported that the drug rapidly destroys the microfilariae of these worms by an opsonin-like action. Observations of Taylor (1960) on *Litomosoides* microfilariae provide perhaps the best possible explanation on the mode of action of Hetrazan. She observed that microfilariae under the influence of Hetrazan stick to the walls of the capillaries of the liver by their tails with few leucocytes adhering to their tails. The microfilariae, she reported, are probably eliminated by way of phagocytosis from the peripheral blood circulation. Although diethylcarbamazine appears to be quite effective against microfilariae, but there exists a dose dependent relationship. With the decrease of the dosage, the response also decreases proportionately.

The other test chemical, tetramisole, used in this experiment has been in extensive use

as a broad spectrum anthelmintic against a wide variety of nematode infections (Thienpont *et al.*, 1966; Campbell and Cuckler, 1967; Bossche and Janssen, 1967; Ross, 1968; Thienpont *et al.*, 1969; Moreau and Lagraulet, 1972). The drug showed pronounced and miraculous efficacy on the microfilariae of *Setaria cervi*. The microfilariae, unlike Hetrazan cases, disappeared completely from peripheral blood circulation at all dosages of tetramisole administered. Also, the onset of microfilaricidal action was recorded much earlier than diethylcarbamazine. All rats treated cleared of microfilariae presenting a 100% response at all dosages, while in Hetrazan treated groups the response at various dosages was 50%, 90% and 100%. It is also noteworthy that even lower dose of tetramisole (7.5 mg/kg) was as much effective as the higher dose (100 mg/kg) of Hetrazan, and in both cases response was 100%. Although diethylcarbamazine and tetramisole are both effective against the microfilariae but the latter has definite advantage because of its effectiveness even at lower doses. The drug has also shown to have some effect on adult worms, and by virtue of which the percentage of live worms recovered on autopsy was only 28–32%, a condition comparable to untreated control group. Further, non-reappearance of microfilariae after a week of discontinuation of drug reveals the persistence of drug's influence in the host body. The drug has been reported to be tolerable even at several times recommended dose of 2.5 mg/kg (Thienpont *et al.*, 1969). Ozcan (1967) reported that higher dose of tetramisole such as 20, 30 mg/kg body weight produced certain transient side-effects in experimental trichinosis in mice. *In vitro* study made by Thienpont *et al.* (1966) reveals that the drug exerts a rapid paralysing action on various species of nematode. Moreau and Lagraulet (1972) tested the *in vitro* activity of L. tetramisole on the third stage larvae of *Angiostrogylus cantonensis* and found this chemical very active even at very low concentration.

Thiabendzole, another broad spectrum anthelmintic, when tested on the experimental rats, proved completely ineffective. None of the rats cleared of microfilariae even for 20 days of continued medication. Further, the recovery of adult worms on autopsy was also 48–52% which are a fairly high figures indicating the inefficacy of the drug in such infection. However, Singhal *et al.* (1973) conducted *in vitro* studies and observed positive action of thiabendazole against adult *Setaria cervi* and reported 100% mortality at a concentration of 300 μg/ml. The ineffectiveness of thiabendazole as observed in the present experiment is undoubtedly in accordance with the earlier report by Nnochiri (1966) in patients with *Loa loa*, *Acanthochielonema* and *Onchocerca* infections. He tested thiabendazole on patients in a dose of 25 mg/kg for 5–10 days and found it to be ineffective. Larger doses increased the frequency of toxic effects in patients.

In vivo screening of diethylcarbamazine, tetramisole and thiabendazole using *Setaria cervi* as test organism has been very satisfactory. Diethylcarbamazine and tetramisole proved to be chemicals of considerable importance. Both the chemicals have shown the capability of destruction of circulating microfilariae in the blood of experimental host. These could be safely recommended as useful drugs against natural *Setaria cervi* infection; and a breakthrough could be achieved reducing the risk of transmission. Since tetramisole has shown its effectiveness against the adult worms and restricted their life span within the host body, this may be taken as drug of choice. A dual purpose could be served in this way: (1) like Hetrazan it may clear off the microfilariae from blood stream preventing its further transmission and (2) may cure the afflicted animals by killing the adult worms.

Summary

Three important anthelmintics namely diethylcarbamazine citrate, tetramisole and thiabendazole were tested on white rats experimentally infected with *Setaria cervi*. Diethylcarbamazine and tetramisole showed

positive microfilaricidal property. Tetramisole has shown detrimental effect on adult worms also which was characterised by the low percentage of live adult worms recovered on autopsy. Diethylcarbamazine citrate did not affect adult worms, and also had very temporary microfilaricidal influence hence there was a reappearance of microfilariae in peripheral blood circulation. Thiabendazole proved completely ineffective on microfilariae as well as adult worms.

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