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journal homepage: www.elsevier.com/locate/apjtbOriginal article <http://dx.doi.org/10.1016/j.apjtb.2015.05.019>Effects of filaricidal drugs on longevity and enzyme activities of the microfilariae of *Setaria cervi* in white ratsHaytham Ahmed Zakai¹, Wajihullah Khan^{2*}¹Faculty of Applied Medical Sciences, King Abdulaziz University, P.O. Box 80216, Jeddah 21589, Saudi Arabia²Section of Parasitology, Department of Zoology, Aligarh Muslim University, Aligarh 202002, U.P., India

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ABSTRACT

Objective: To analyse the efficacy of diethylcarbamazine (DEC), tetramisole and chlorpromazine on the longevity and activity of glucose-6-phosphatase and succinate dehydrogenase in the microfilariae recovered from the peripheral circulation of the rats before and after the treatment.

Methods: *Setaria cervi* worms were implanted in white rats via laparotomy and microfilaraemic rats were divided into 4 groups. Groups 1, 2 and 3 were treated with DEC, tetramisole and chlorpromazine respectively, while Group 4 served as infected control. Longevity of microfilariae and differential leucocyte counts were recorded till the disappearance of microfilariae from peripheral blood. Glucose-6-phosphatase and succinate dehydrogenase enzymes were localized in the microfilariae recovered from normal and treated rats.

Results: The microfilariae survived for 48 days in untreated rats while survival was reduced to 15, 21 and 27 days after treatment with DEC, tetramisole and chlorpromazine, respectively. Eosinophils and neutrophils increased during 2nd and 3rd weeks, whereas the lymphocytes increased during 4–7 weeks. DEC treatment resulted in slight decrease in the localization of succinate dehydrogenase but not in glucose-6-phosphatase. Tetramisole and chlorpromazine treatment did not show any appreciable change in the localization of both the above enzymes.

Conclusions: DEC proved the most effective drug which cleared the microfilaraemia within 15 days and reduced the activity of succinate dehydrogenase to some extent followed by tetramisole and chlorpromazine which took more time for the clearance of microfilariae and had no effect on the localization of both glucose-6-phosphatase and succinate dehydrogenase.

1. Introduction

A thorough understanding of physiological aspects of the host-parasite relationship is only possible if a careful study is made on the biochemical nature of the parasite and its host. Since human filariids are not available for conducting biochemical, chemotherapeutic and enzymatic studies, the bovine filariid, *Setaria cervi* (*S. cervi*) which resembles human species in having

microfilarial periodicity, is widely used in such studies. *S. cervi*, a cosmopolitan bovine filariid, is found in the peritoneal cavity of cattle and buffaloes causing peritonitis. In rare circumstances, it causes cerebro-setariasis which results in lumbar paralysis in cattle [1]. Fragmentary reports are available regarding the effects of anthelmintics on the longevity and enzyme activities of the adult worms and the microfilariae of different species of *S. cervi* *in vitro* and *in vivo* [2–5]. Generally, the presence of microfilariae in the peripheral blood circulation is taken as a tool for routine diagnosis of filariasis. Morphological and histochemical features have been widely employed in differential diagnosis of microfilariae by several investigators but strangely, reports on histochemistry of microfilariae and its use as a diagnostic tool in the identification of the species are scanty. In view of the above fact, the present study was aimed at assessing the effect of

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diethylcarbamazine (DEC), tetramisole and chlorpromazine on the longevity and enzyme activities of the microfilariae of *S. cervi* in white rats.

2. Materials and methods

2.1. Worm transplant and treatment in white rats

In the present study, forty laboratory-bred white rats (*Rattus norvegicus*), weighing 125–150 g were used for the transplantation of *S. cervi* worms. Worms were collected from freshly slaughtered buffaloes and three female and two male worms were transplanted intraperitoneally in each rat. Infected rats were divided into four groups. Groups 1, 2 and 3 were treated with DEC, tetramisole and chlorpromazine, respectively from the 9th day when microfilariae appeared in the peripheral circulation. DEC was given orally at a dose of 100 mg/kg per day, whereas tetramisole and chlorpromazine were given at the dosage of 15 mg/kg per day for 3 consecutive days. Group 4 served as untreated infected control. Survival of microfilariae after treating rats with DEC, tetramisole and chlorpromazine was recorded from microfilaraemia rats every second day till microfilariae disappeared from the peripheral circulation. Longevity of microfilariae was recorded in both treated as well as untreated rats so that effect of the drugs could be assessed.

2.2. Fixation and enzyme localization in microfilariae

Thick blood smears were prepared from treated and untreated microfilaraemic rats by making a slight cut on the tail. Smears were allowed to dry and then fixed in buffered-sucrose-formalin (pH 7.2) for 15 min at 4 °C. Fixed slides were rinsed and stored in gum-sucrose solution overnight. These slides were rinsed in cold saline and incubated in following incubation mediums for the localization of glucose-6-phosphatase and succinate dehydrogenase as described by Pearse [6].

2.3. Glucose-6-phosphatase

The slides were incubated overnight at 32 °C in a substrate mixture containing 20 mL of 0.12% solution of potassium glucose-6-phosphatase, 20 mL of 0.2 mol/L tris buffer (pH 7.6), 3 mL of 2% lead nitrate and 7 mL distilled water. Incubated sections were washed in distilled water and developed in yellow ammonium sulphide and treated with 6% neutral formaldehyde and mounted in glycerine.

2.4. Succinate dehydrogenase

The slides were incubated for 45 min at 37 °C in 20 mL of incubating medium consisting of 10 mL stock succinate solution (0.2 mol/L phosphate buffer, pH 7.6 and 0.2 mol/L sodium succinate, 1:1) and 10 mL aqueous solution of Nitro BT (1 mg/mL). After incubation slides were washed in saline, fixed in 10% formol-saline for 15 min, rinsed in 15% alcohol for 5 min and mounted in glycerine.

2.5. Differential leucocyte counts of infected rats

Differential leucocyte counts were recorded every third day after the appearance of microfilariae in peripheral blood of

infected rats to see the changes in the blood picture in normal as well as treated rats during the course of infection.

2.6. Statistical analysis

Statistical analysis was performed by using software SPSS (version 17.0, SPSS inc., Chicago, IL, USA). Significance of data was assessed by analysis of variance (ANOVA) and the level of significance was set at $P < 0.05$. Tukey's test was used to compare means, and results were expressed as mean \pm SD.

3. Results

Effect of DEC, tetramisole and chlorpromazine on the longevity of microfilariae in treated and untreated rats are shown in Table 1. Microfilariae appeared in peripheral circulation after (8 \pm 2) days in the rats infected with *S. cervi* via laparotomy. Microfilarial density recorded on 8th day was low which ranged from (0.50 \pm 0.53) to (1.00 \pm 0.81) mm³. Maximum microfilarial density of (14.50 \pm 3.00) mm³ was recorded on the 28th day in normal control which declined slowly to (0.50 \pm 0.75) mm³ on 56th day of implant.

Significant variations were seen in the density as well as longevity of microfilariae when data from normal rats were compared with that of treated rats. Microfilarial densities recorded in rats treated with DEC, tetramisole and chlorpromazine were (4.00 \pm 1.52), (5.00 \pm 1.00) and (6.42 \pm 1.27) mm³ after 9 days of loading dosage, exhibiting significant differences when compared with (8.00 \pm 2.13) mm³ of normal control (Table 1). Decrease in the microfilarial density in DEC, tetramisole and chlorpromazine treated rats was highly significant, being (1.28 \pm 0.75), (3.57 \pm 0.53) and (5.71 \pm 1.49) mm³ compared to (9.50 \pm 1.77) mm³ of normal control after 13 days of initial dose. As for the efficacy of these drugs, DEC was found to be the most effective drug which cleared microfilariae from the peripheral circulation within 15 days of treatment

Table 1

Effect of DEC, tetramisole and chlorpromazine on the longevity of the microfilariae of *S. cervi* in treated and untreated rats.

Days after treatment	Density of microfilariae of <i>S. cervi</i> (mm ³)			
	Control	DEC	Tetramisole	Chlorpromazine
8	0.50 \pm 0.53 ^a	0.71 \pm 0.75 ^a	0.57 \pm 0.53 ^a	1.00 \pm 0.81 ^a
10	2.00 \pm 0.75 ^a	1.00 \pm 0.81 ^a	1.00 \pm 0.81 ^a	1.00 \pm 0.57 ^a
12	3.50 \pm 0.92 ^a	2.42 \pm 1.27 ^a	3.00 \pm 0.81 ^a	3.57 \pm 0.78 ^a
14	5.50 \pm 1.41 ^a	3.00 \pm 1.41 ^b	4.57 \pm 1.51 ^{ab}	5.00 \pm 1.41 ^{ab}
16	7.00 \pm 2.39 ^a	4.42 \pm 1.78 ^a	5.71 \pm 1.60 ^a	6.00 \pm 1.29 ^a
18	8.00 \pm 2.13 ^a	4.00 \pm 1.52 ^c	5.00 \pm 1.00 ^{bc}	6.42 \pm 1.27 ^{ab}
20	8.00 \pm 2.00 ^a	3.00 \pm 1.29 ^b	4.00 \pm 1.15 ^b	6.28 \pm 1.11 ^a
22	9.50 \pm 1.77 ^a	1.28 \pm 0.75 ^d	3.57 \pm 0.53 ^c	5.71 \pm 1.49 ^b
24	12.50 \pm 2.77 ^a	0.57 \pm 0.75 ^c	2.28 \pm 0.75 ^{bc}	4.42 \pm 0.97 ^b
26	12.00 \pm 2.61 ^a	0.00 \pm 0.00 ^c	2.00 \pm 0.81 ^c	4.28 \pm 0.95 ^b
28	14.50 \pm 3.00 ^a	0.00 \pm 0.00 ^c	1.28 \pm 0.75 ^{bc}	3.71 \pm 0.95 ^b
30	11.50 \pm 1.92 ^a	0.00 \pm 0.00 ^c	0.42 \pm 0.53 ^c	2.57 \pm 0.53 ^b
32	10.50 \pm 1.92 ^a	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	1.57 \pm 1.13 ^b
36	8.00 \pm 1.85 ^a	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.42 \pm 0.53 ^b
40	5.00 \pm 1.30 ^a	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b
44	4.00 \pm 0.75 ^a	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b
48	2.00 \pm 1.30 ^a	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b
52	1.00 \pm 0.75 ^a	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b
56	0.50 \pm 0.75 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a

Data are expressed as mean \pm SD. Mean followed by a different letter has significant difference (Tukey's test, $P < 0.05$).

followed by tetramisole which took 21 days and chlorpromazine, the least effective drug which could clear microfilaraemia after 27 days (Figure 1).

In normal rats, differential leucocyte count demonstrated the neutrophils 34%, lymphocytes 59%, eosinophils 3%, monocytes 3% and basophils 0.5%. Figure 2 represents the leucocyte response in white rats infected with adult *S. cervi* worms. On the 6th day after transplant differential leucocyte count was normal. Basophils and monocytes did not show any appreciable change throughout the study. In infected rats, rise in eosinophils was observed during 2nd and 3rd weeks which declined in the 4th week, disappeared during 6th and 7th weeks and regained its normal value again during the 9th week. As far as the lymphocytes are concerned, first there was a decrease in their count during the 2nd and 3rd weeks. After this period, a progressive increase was recorded which touched its peak by the end of 7th week followed by a decline in successive weeks and then attained normal count in 9th week. In DEC treated rats lymphocyte increase was higher and fluctuated between 65% and 82% during 5th-7th weeks compared to those treated with tetramisole and chlorpromazine, which did not show any marked change in lymphocyte counts. Conversely, neutrophils showed first an increase during 2-4 weeks and then declined consistently during next 5-7

weeks and attained its normal value in the 9th week in normal and treated infected rats.

Distribution of succinate dehydrogenase and glucose-6-phosphatase in the microfilaria of *S. cervi* is shown in Figures 3-5. As far as the succinate dehydrogenase activity is concerned, it was intense in nuclear column, cephalic cells, muscle cells and excretory pore. Nerve ring and G-cells showed moderate reaction while innenkorper and anal pore showed low activity. Intense glucose-6-phosphatase activity was noted in the

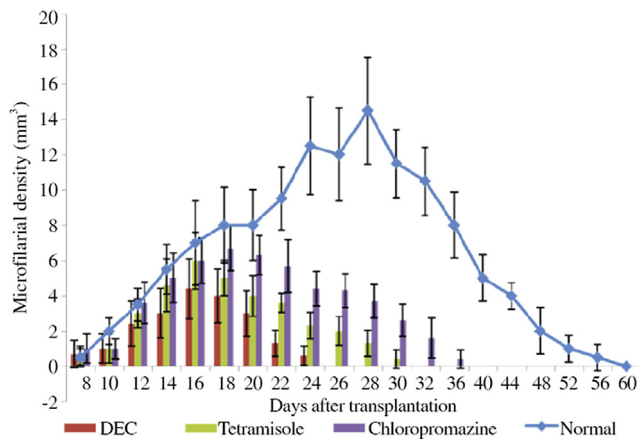


Figure 1. Longevity of microfilariae in rats treated with DEC, tetramisole and chlorpromazine and in untreated infected control.

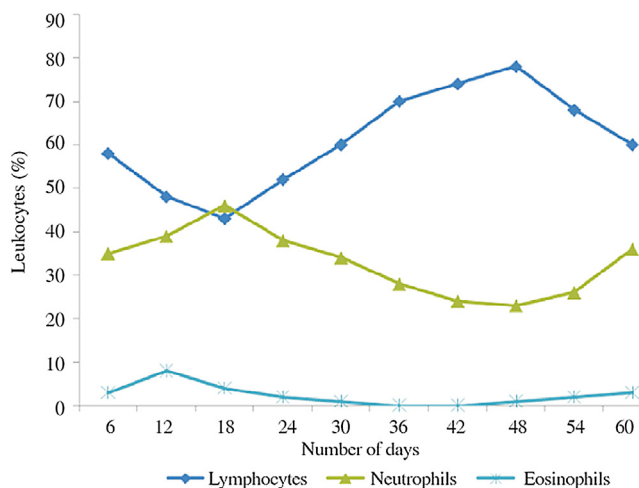


Figure 2. Leucocyte response in rats infected with *S. cervi*.

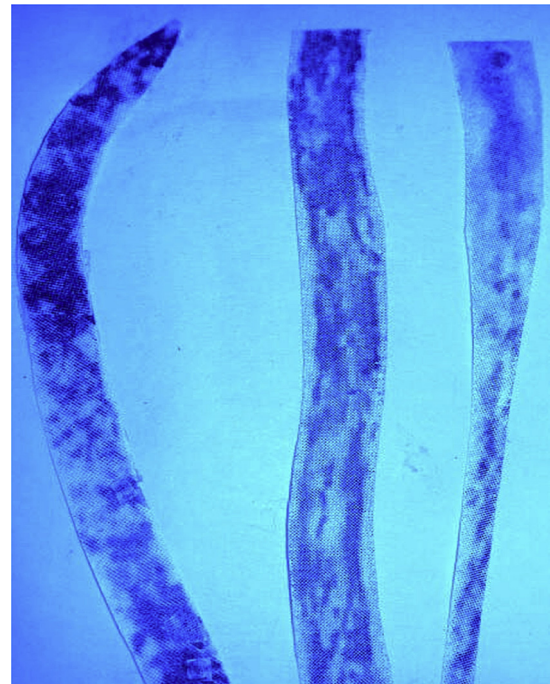


Figure 3. Distribution of succinate dehydrogenase in the microfilaria of *S. cervi* recovered from untreated infected rats.

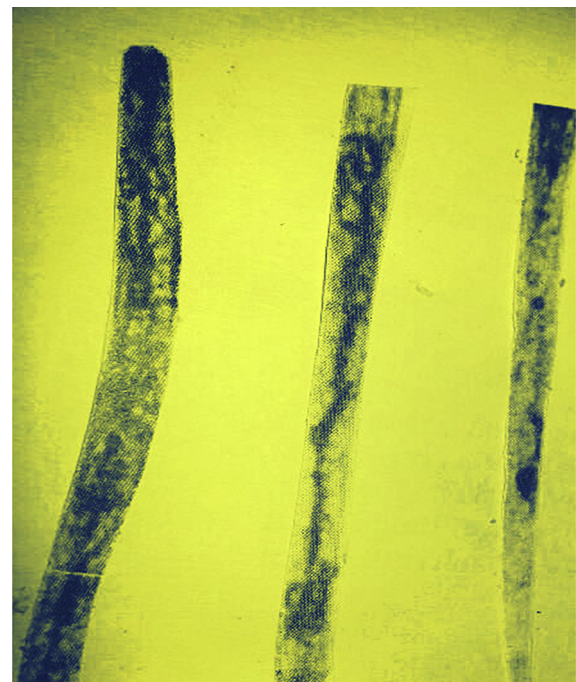


Figure 4. Distribution of glucose-6-phosphatase in the microfilaria of *S. cervi* recovered from untreated infected rats.



Figure 5. Distribution of succinate dehydrogenase in the microfilaria of *S. cervi* recovered from DEC treated rats.

cephalic cells, inner body thread, G-cells, and anal pore, while muscle cells nuclear column, nerve ring and excretory pore showed moderate activity. There was no appreciable change in the localization of these enzymes in the microfilariae recovered from treated rats except those treated with DEC where a slight decrease in the activity of succinate dehydrogenase was noticed.

4. Discussion

The maximum microfilarial density of $(14.50 \pm 3.00) \text{ mm}^3$ was recorded on 28th day which declined to a minimum of $(0.50 \pm 0.75) \text{ mm}^3$ after 56 days post-transplant, is in agreement with the earlier finding where almost similar peak and longevity were recorded for *S. cervi* in white rats [7]. In contrast, much lower survival periods of 23 days for the same parasite was recorded in guinea pigs [8]. In the present study the survival record of microfilariae in treated rats showed DEC to be the most effective drug which cleared microfilaraemia in rats within 15 days. It is known that DEC interferes with the metabolism of arachidonic acid, which blocks the production of prostaglandins, resulting in capillary vasoconstriction and infringement in the passage of microfilariae. DEC is also known to interfere with cyclooxygenase and lipoxygenase pathways, inhibiting the production of thromboxane, prostacyclin, prostaglandin and leukotriene [9–11]. Sharp increase of lymphocytes during 4–7 weeks post-implant coinciding with the clearance of microfilariae from peripheral circulation in our study, indicated the synergistic action of DEC and humoral immunity. The results are in conformity to earlier studies where quick clearance of microfilariae due to their adherence with granulocytes and endothelial wall was reported when DEC was administered in conjunction with host immune system [12–15]. Active immunization of microfilaraemic animals with methyl piperazine carboxylic acid and bovine serum albumin showed rapid clearance of microfilariae when the sub-

curative dose of DEC was administered in *Mastomys coucha* infected with *Setaria digitata* [16]. These observations are indicative of the fact that host immune system played a key role in DEC mediated parasite elimination. A slow and steady decline was also observed in the microfilarial density of *Brugia malayi* and *Wuchereria bancrofti* (*W. bancrofti*) after treatment with DEC alone, but the decline was immediate after the treatment when DEC-albendazole combination was used [17,18]. Clearance of microfilariae after 15 days with DEC in conjunction with antibodies produced by an intraperitoneal transplant of adult *S. cervi* worms in our study get support with earlier findings indicating that DEC reduces microfilarial count by trapping through cellular adherence and killing them. Degenerated microfilariae stick to both leucocytes and kupffer cells in the sections of liver, lungs, kidneys and spleen [4]. Tetramisole being less effective than DEC, cleared microfilariae from peripheral circulation after 21 days. Chlorpromazine was the least effective during our study as it took 27 days to clear microfilariae. Chlorpromazine was reported to suppress release of microfilariae of *S. cervi* *in vitro* [19].

There were remarkable changes in differential leucocyte count in white rats infected with *S. cervi*, especially for eosinophils, neutrophils and lymphocytes. The increase in eosinophils and neutrophils during 2nd and 3rd weeks may be correlated with the presence of live worms within the peritoneal cavity of rats as earlier observed in mice [12]. A decrease in counts of eosinophils and neutrophils during 4–7 weeks can be attributed to destruction and elimination of the parasites by these cells during this period, as they might have been associated with piece meal clearance of the parasite, as earlier observed in bancroftian filariasis where large number of eosinophils were found associated with DEC-induced worm [20]. In the present study, we noted a decrease in circulating lymphocytes during the 2nd and 3rd weeks and thereafter, steady increase was recorded which touched its peak by the end of the 7th week and attained normal count by the end of the 9th week. This trend clearly indicates sensitization phase followed by transformation of lymphocytes in plasma cells which secrete antibody to eliminate the parasite from infected rats. Similar increase in lymphocyte proliferation was recorded in mice infected with *Nocardia braziliensis*, when treated with DEC [21].

Enzymes have been extensively studied in adult nematodes including filarial worms, but reports regarding their localization and distribution in microfilariae are very few. Anthelmintics are known to inhibit a variety of enzymes in adult nematodes. A few enzymes operating in glycolytic and oxidative pathways were localized in *S. cervi* and *Onchocerca fasciata* [22,23]. Localization of succinate dehydrogenase almost on entire body of microfilariae indicates the presence of PEP-succinate pathway in the microfilariae of *S. cervi*. However, they are much densely found on cephalic cells, muscle cells and the nuclear column of microfilariae. Inhibition of succinate dehydrogenase in the microfilariae treated with DEC suggests that it affects the PEP-succinate pathway. It appears that succinate production is linked with the metabolism of microfilariae for their survival, as the longevity of microfilariae gets reduced post DEC treatment as earlier observed in the microfilariae of *W. bancrofti* [24]. Similar inhibition in nicotinamide adenine dinucleotide (reduced) linked fumarate succinate system has been reported in adult *S. cervi* and *W. bancrofti* [25–28].

In the present study, an intense to moderate activity of glucose-6-phosphatase in cephalic cells, inner body, G-cells, anal pore, nuclear column and excretory pore of microfilariae of *S. cervi* indicates its accumulation and role in the hydrolysis of glucose-6-phosphate, resulting in the creation of phosphate group and free glucose. Tetramisole is a proven drug which causes irreversible paralysis and inhibition of glycolytic and tricarboxylic acid cycle enzymes in nematode parasites [21]. This drug reduced the longevity of microfilariae in the present study, however, no marked changes were observed in the localization of glucose-6-phosphatase and succinate dehydrogenase in the microfilariae recovered from rats treated with tetramisole. The probable explanation for this may be that the drug might have exerted its effect against other vital mechanisms essential for the survival of microfilariae *in vivo*, which manifested in the form of irreversible paralysis. Chlorpromazine suppressed survival of microfilariae to some extent although was less effective compared to DEC and tetramisole but chlorpromazine showed no appreciable change in the localization of both glucose-6-phosphatase and succinate dehydrogenase in the microfilariae of *S. cervi*. Earlier researchers noticed the anthelmintic action of this drug, but were unable to give the mechanism of its anti-parasitic action [29,30].

Results obtained in the present study indicated that glucose-6-phosphatase and succinate dehydrogenase enzymes were presented in the microfilariae of *S. cervi* and showed their active involvement in glycolysis and tricarboxylic acid cycle. DEC proved to be the most effective drug which cleared microfilaraemia within 15 days of treatment and reduced the localization of succinate dehydrogenase.

Conflict of interest statement

We declare that we have no conflict of interest.

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