Histochemical changes in *Setaria cervi* caused by certain anthelmintics

ABDUL BAQUI and HUMAIRA KHATOON

Department of Zoology, Section of Parasitology, Aligarh Muslim University,
Aligarh 202 002, India

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Abstract. The present study deals with the preliminary *in vivo* screening of suramin and levamisole in rat-*Setaria cervi* system with special reference to the histochemical changes in the adult worms caused by the drugs. Levamisole proved to be highly effective as a micro- and macro-filaricidal agent. It also appears to be interfering with the normal activity of alkaline phosphatase and glycogen of the adult worms with no apparent effect on its protein content. The drug also causes irreversible paralysis in adult worms. Suramin, though an active pharmacological agent, proved to be completely ineffective on microfilariae as well as on adult worms of *Setaria cervi*. Consequently, no notable alterations in the histochemistry of the parasite following suramin treatment were observed.

Keywords. White rats; *Setaria cervi*; histochemical observations.

1. Introduction

Numerous anthelmintics have been tried on nematode parasites in experimental studies and their efficacy has been established; but their mode of action on the worms and the consequent biochemical or histochemical alterations brought about by the drugs are least understood. Levamisole and suramin are known potent anthelmintics. Levamisole is the newly-discovered highly potent broad spectrum anthelmintic effective on a variety of nematodes. But the mode of action of these drugs on the biochemistry or histochemistry of the parasite is not fully known. The present study deals with the preliminary screening of suramin and levamisole in rat-*Setaria cervi* system with special reference to the histochemical alterations in the adult worms caused by the drugs.

2. Materials and methods

About 20 laboratory bred white rats almost of the same age group and weight were used in the present experiment. Adult worms (*Setaria cervi*), collected from the peritoneal cavity of freshly slaughtered buffaloes, were implanted surgically into the peritoneal cavity of white rats according to the method described by Baqui....
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and Ansari (1975). Each rat received five adult worms of both sexes. Infected rats were divided into two groups: one for the suramin and the other for levamisole treatment. The drugs were given to the microfilaria-positive rats after a week of initial infection at the higher tolerant dose determined earlier. Levamisole and suramin were administered orally and subcutaneously at 20 mg/kg/day and 9 mg/kg/day respectively. Administration of the drugs and microfilarial count were made for 5–10 consecutive days, thereafter the treated rats of both groups were autopsied to observe the condition of the worms and the apparent effect of the drugs on the worms.

Untreated normal worms (control) and those recovered from treated autopsied rats were fixed in Carney's fluid and cold acetone for histochemical observations of protein, glycogen and alkaline phosphatase activities. Fixed materials were cleared in benzene and paraffin blocks were made. Protein and glycogen were localized by Mercury-bromophenol blue and carmine stain methods respectively as suggested by Pearse (1960). Alkaline phosphatase was estimated by calcium cobalt technique as described by Gomori (1952).

3. Results

It was observed that all the rats treated with levamisole for 5 consecutive days cleared of microfilariae (response 100%) from peripheral blood circulation (table 1). Microfilarial density continued to drop after the administration of the very first dose of the drug. Further, rats autopsied after the disappearance of microfilariae on the 15th day of infection showed only 20% recovery of live active adult worms (table 1). The remaining worms were either completely exhausted or degenerate. Some of the worms were completely well organized in their architecture but remained immobile and inactive even after transfer to the normal saline showing the sign of doubtful viability. Such worms were also counted as dead. Posterior \( \frac{1}{2} \) part of some live adult worms (male and female both) was found to be completely shrunk and contracted which remained unchanged even after transferring into the normal saline indicating the paralysing action of the drug.

Histochemical observations of the levamisole-treated worms revealed that protein content of cuticle, body muscles, boundary walls of ovary, uterus, microfilariae and developing embryos remained unchanged as compared to that of normal control. However, a heavy concentration of alkaline phosphatase found in subcuticle body muscles, lateral cords, embryos and microfilariae in control worms (figure 1) was noted to have considerably decreased in treated worms (figure 2). Similarly, glycogen content appreciably localized in muscles, boundary walls of uterus and developing embryos of control (figure 3) was also found to have relatively decreased in treated worms (figure 4).

Another drug, suramin, was found to be completely ineffective on microfilariae as well as adult worms of *S. cervi*. Some of the rats (50%) treated for 10 consecutive days did not show any sign of effectiveness on circulating microfilariae, consequently microfilarial density continued to increase in the peripheral blood circulation (table 1). Treated rats autopsied at 5 and 10 days intervals did not show any apparent microfilaricidal effect either. Live worms recovered on autopsy ranged from 40–60%. Further, no notable changes, in all the three biochemical
Figure 1. Alkaline phosphatase activity in the control worm.
Figure 2. Alkaline phosphatase activity in the levamisole-treated worm.
Figure 3. Glycogen localization in the control worm.
Figure 4. Glycogen localization in the levamisole-treated worm.
Table 1. The effect of certain anthelmintics on microfilariae and adult worms of *Setaria cervi*.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage (route)</th>
<th>Mean microfilarial density/ mm³ of blood</th>
<th>Duration of medication (in days)</th>
<th>% Recovery of live adult worms on an autopsy</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levamisole</td>
<td>20 mg/kg (oral)</td>
<td>4.5</td>
<td>0</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Suramin</td>
<td>9.0 mg/kg (subcutaneous)</td>
<td>3.0</td>
<td>12-16.4</td>
<td>5-10</td>
<td>40-60</td>
</tr>
</tbody>
</table>
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constituents, i.e., protein, glycogen and alkaline phosphatase, were recorded histochemically as compared to the control.

4. Discussion

Levamisole, a broad spectrum anthelmintic, has been found to be highly effective on microfilariae as well as adult S. cervi worms like its dextro-isomer, tetramisole as earlier reported by Baqui and Ansari (1976). Complete disappearance of circulating microfilariae following a 5-day treatment with levamisole and low percentage of recovery of live adult worms on autopsy are indicative of the fact that the drug contains micro—as well as macrofilaricidal property against S. cervi. As earlier observed, the transplanted worms normally survive in the peritoneal cavity of white rats for 4–6 weeks (Baqui and Ansari 1975). Hence, disintegration of the worms at this early stage of infection could be solely attributed to the effects of the drug.

Studies regarding the histochemical changes in the nematodes following anthelmintic treatment are scanty. However, there are a few reports on the biochemical changes of the worms brought about by certain drugs. Van den Bossche and Janssen (1969), Van den Bossche (1972), Malkin and Camacho (1972) and Prichard (1973) have reported that fumarate reductase activity is considerably inhibited in Haemonchus contortus and Ascaridia galli following treatment with tetramisole, levamisole and thiabendazole. Tetramisole also inhibits the cholinesterase, aldolase and acid phosphatase of Ascaridia galli (Vertinskaya et al 1972; Chakraborty et al 1976). Piperazine has been reported to decrease glycogen value in Ascaris lumbricoides tissues (Abdulazizov 1975; Bogoyavlenski et al 1975) and histamine content in Ascaris suum (Phillips et al 1976).

The present study supports the above observations. Levamisole has shown pronounced effects on adult worms which are characterized by death or irreversible paralysis of the worms. Suramin, though an effective drug in other filarial nematodes such as Onchocerca and Dipetalonema (Burch 1955; Gayral and Pommies 1976) proved to be completely ineffective on S. cervi. Hence no notable alterations in the histochemistry of the worms were observed. However, it has been reported that suramin inhibits strongly, in vitro, a variety of enzyme system of trypanosomes (Von Brand 1966).

Levamisole appears to be interfering with the carbohydrate metabolism especially with the absorption of carbohydrates and their intracellular utilization. As a result glycogen value is diminished in different organs. According to Von Brand (1966) inhibition of glucose absorption results in decrease in the concentration of energy-rich phosphate bond; finally the energy required for survival becomes inadequate and the parasite dies.

The drug also in some manner, inhibits the normal alkaline phosphatase activity of the worms as a result of which considerable decrease in its concentration in various organs is observed. The protein value remains unchanged in treated worms. There is very little information available, concerning nematode parasites, as to whether anthelmintics attack the parasite proteins or interfere with some phase of its nitrogen metabolism. Levamisole appears to have a paralysing action on adult worms and probably acts as a neuromuscular blocking agent like its
dextro-isomer, tetramisole (Gaitonde 1971). The sustained contracture of the somatic muscles of *S. cervi* results in the irreversible paralysis of the worm—a condition similarly reported in another filarial worm, *Breinlia sergenti* and *Ascaris* following *in vitro* treatment with levamisole (Natarajan *et al.* 1974; Van den Bossche 1972).

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