Histochemical changes in *Setaria cervi* caused by certain anthelmintics

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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
Abdul Baqui and Humaira Khatoon (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 Carnoy'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 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