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The Effects of Holothurin on the Development of *Trypanosoma musculi* in the Orchiectomized and Nonorchiectomized FN (Fawn) Male Mice

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ABSTRACT

The effects of holothurin on *Trypanosoma musculi* development in orchiectomized (IHO) and nonorchiectomized (IH) FN mice indicated that the drug administered simultaneously with the inoculation of trypanosomes increased resistance in favor of the host. The resistance was measured by the level of parasitemias at the peak and throughout the course of infection. Orchiectomy along with the administration of holothurin resulted in lower parasitemias in the IHO group compared with nonorchiectomized holothurin treated IH group. Sex hormones play an important role in the development of parasitic infections. In the present study, orchiectomized mice (IHOT) received testosterone after inoculation with holothurin and trypanosome infection. The parasitemic level in the IHOT group was higher compared with nonhormone treated IH and IHO groups. The decreased host resistance in the IHOT group of mice could not be explained in the present situation. The mechanism of action of androgen and steroid saponin has yet to be determined. Further investigation will help to elucidate the nonspecific factor of resistance against trypanosomiasis.

Key Words: Holothurin; Testosterone; Orchiectomy; FN Mice; *T. musculi*.

INTRODUCTION

Trypanosoma musculi has been the subject of extensive studies here at the laboratory and elsewhere (Lincicome et al., 1965; Jackson and Farmer, 1970; Albright and Albright, 1982; Wechsler and Kongshavn, 1985; Sen et al., 1981a, 1981b, 1993; Dusanic, 1978; Roger and Viens, 1986). Other studies document the relationships between susceptibility and sex of animals (Frayha et al., 1971; Graff et al., 1969; Terres et al., 1968; Wunderlich et al., 1991; Yamamoto et al., 1991).

Orchiectomy has also been reported to influence the response of animals to antigenic stimuli and subsequent production of immunity (Castro, 1974; Grossman, 1984). Finally, holothurin has been reported to have some biological effects on free living as well as on parasitic protozoa (Nigrelli and Zahl, 1952; Styles, 1970; Sen et al., 1981a).

The proposed study has been designed to determine the effects of steroid (holothurin and testosterone) and saponin (holothurin) on *Trypanosoma musculi* development in orchiectomized and nonorchiectomized FN (a new strain) male mice.

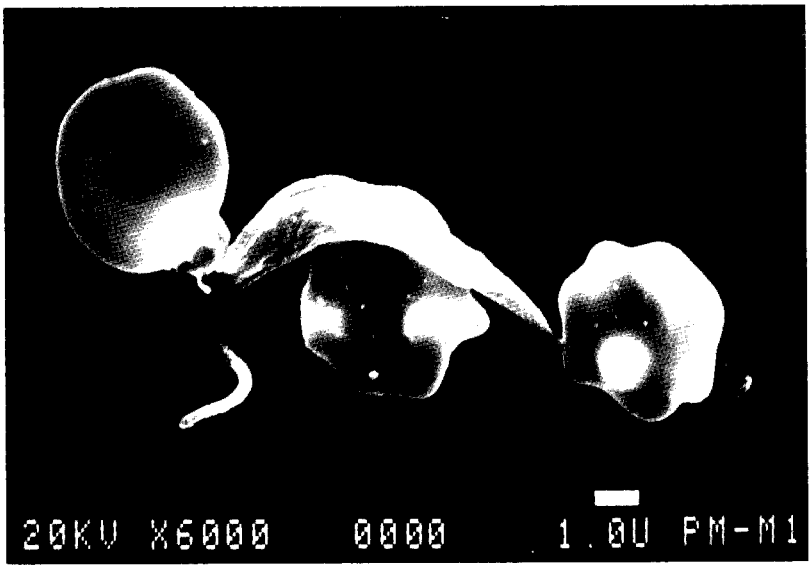

FIGURE 1. SEM micrograph of fresh isolate of *T. musculi* from peripheral blood of infected mouse.

METHODS

The FN (fawn) strain of male mice used in this study was originated and maintained in the Parasitology Laboratory of Virginia State University. These were the offsprings of a cross between a highly inbred NIH BG (Beige) strain and SW (Swiss Webster) mice. FN mice were inbred in the laboratory for over 20 generations. The initial weight of all mice ranged between 24 and 28 g at the onset of the experiments. Each mouse was housed individually under standard laboratory conditions and allowed free access of water and Purina Lab. Chow$^{(R)}$.

Trypanosoma musculi (H strain), Fig. 1., obtained from Howard University, Washington, D. C., was used in this study. The stercorarian hemoflagellates were passed at weekly intervals in SW male mice by intraperitoneal (i.p) inoculation of parasites washed in 1% sodium oxalate. All mice were infected using a standard i.p. inoculum of 5×10^4 trypanosomes prepared as suspensions of washed hemoflagellates (Sen et al., 1993).

The blood was collected, by cardiac puncture from a donor animal, in a syringe containing 1% oxalated 0.15 M NaCl solution and was centrifuged (548 X g) at room temperature for 15 min. Following centrifugation, the central buffy layer containing protozoan parasites was pipetted off and washed with NaCl solution. Further dilutions were made with 0.15 M NaCl solution until the desired concentration (5×10^4) of trypanosomes was obtained for inoculation in the experimental and control mice.

Parasitemia in FN mice was determined by counting trypanosomes in peripheral blood taken from the tail at 2-day intervals during days 9 to 19 post inoculation (PI) days and is expressed as trypanosomes/mm^3 of blood. Numbers of trypanosomes were estimated by standard technique (Lincicome and Watkins, 1963). Nembutal (sodium pentobarbital) was administered i.p. at a dose rate of 40 g/kg of body weight to induce anesthesia for about 60 min. One aliquot of 0.25 ml was injected i.p. to each

TABLE 1. The effects of steroid and saponin on the development of *Trypanosoma musculi* in the orchiectomized and nonorchiectomized FN mice. Group means within any given sampling period that are followed by different letters are significantly different at $p < 0.05$. Mean separation by Duncan's New Multiple Range Test.
(IC - infected/control; IH -infected/holothurin/ Orchiectomy/testosterone; IHOT - infectected/holothurin/orchiectomy/testosterone; IHO - (infected/holothurin/orchiectomy)

Day	Group Mean (Trypanosomes in thousands/mm^3 blood ± S D) IC	IH	IHOT	IHO
	(12)**	(10)	(11)	(10)
9	4.4 ± 0.6a	3.6 ± 1.0ab	3.4 ± 0.8ab	2.6 ± 0.7bc
11	38.0 ± 11.6a	24.2 ± 6.6b	23.8 ± 6.4b	12.6 ± 4.2b
13	42.0 ± 11.1a	23.4 ± 7.6b	*45.8 ± 9.8ac	13.2 ± 6.2bd
15	*50.6 ± 12.6a	31.8 ± 9.4b	45.2 ± 10.3abc	*15.0 ± 6.2d
17	31.4 ± 12.7a	*35.6 ± 15.4ab	30.6 ± 8.0abc	10.2 ± 5.7d
19	9.0±4.6ac	10.2 ± 6.2ac	16.8 ± 5.6a	5.2 ± 4.1bc

* - peak infection
** -number of animals

experimental mouse following the method of Pilgrim and De Ome (1955). Orchiectomy was performed by the method of Chapman et al., 1975; Sen et al., 1983. The surgery was performed 2 weeks before the inoculation of *T. musculi.*

Crude holothurin, a steroid and a saponin of animal origin, is composed of 60% glycosides; 1% cholesterol; 5-10% insoluble proteins; 30% salts, polypeptides and free amino acids (Nigrelli and Zahl, 1952). The drug was found to inhibit growth of some free-living and parasitic protozoa (Nigrelli and Jakowska, 1960; Sen et al., 1981a). A standard solution of 1mg/ml holothurin (obtained from Dr. George D. Ruggieri, Director of Osborn Laboratories of Marine Sciences, Brooklyn, New York) was prepared by the method of Styles (1970). The dose of holothurin consisted of (a) a 250 μg administered to 10 mice (IH) simultaneously with a standard inoculum of 5×10^4 trypanosomes, (b) a 250 μg administered to each of 11 animals of other group (IHOT) of orchiectomized animals receiving 150 μg testosterone simultaneously with trypanosome inoculation, (c) a 250 μg administered to 10 mice of a third group (IHO) of orchiectomized mice simultaneously with trypanosome inoculation. Twelve untreated mice infected with a standard inoculum of trypanosomes served as controls (IC). The present study involved 43 FN male mice distributed in two separate experiments. Both the nonorchiectomized groups (IC and IH) were sham operated and received corn oil (placebo) with no testosterone. Statistical treatment of the data involved one-way ANOVA. A probability level of $p < 0.05$ was considered significant.

RESULTS

The simultaneous administration of 250 μg of holothurin and 5×10^4 trypanosomes affected the level of parasitemia during days 11 through 15 post inoculation (PI) days.

The holothurin treated infected (IH) group had significantly lower parasitemia of 24,000 trypanosomes/mm^3 of blood on day 11 PI, 23,400 trypanosomes/mm^3 of blood on day 13 PI, and 31,800 trypanosomes/mm^3 of blood on day 15 PI, as compared to an average of 38,000 trypanosomes/mm^3 of blood on day 11 PI, 42,000 trypanosomes/mm^3 of blood on day 13 PI, and 50,600 trypanosomes/mm^3 of blood on day 15 PI in the control (IC) group. Parasitemia peaked on day 15 PI for the IC group and on day 17 PI for the holothurin treated experimental (IH) animals (Table 1).

Orchiectomized (IHO) mice receiving holothurin simultaneously with trypanosome inoculation developed a significantly lower level of parasitemia of 15,000 trypanosomes/mm^3 of blood on day 15 PI and 10,200 trypanosomes/mm^3 of blood on day 17 PI, compared to 31,800 trypanosomes/mm^3 of blood on day 15 PI and 35,600 trypanosomes/mm^3 of blood on day 17 PI in the holothurin treated infected (IH) animals. The orchiectomized group (IHO) receiving holothurin simultaneously with *T. musculi* inoculation also developed a significantly lower level of parasitemia as compared to the untreated but infected (IC) controls during days 9 through 17 PI. Parasitemia peaked on day 15 PI for both the groups (Table 1).

The experimental animals (IHOT) underwent orchiectomy and subsequent inoculation with test drug holothurin simultaneously with *T. musculi* and were subjected to injection of testosterone to replace the missing androgen. The parasitemic levels in IHOT group did not decline when compared with control (IC) and were not significantly different from each other during days 9 through 19 PI. The IHOT group showed a higher level of parasitemia during days 13 through 15 PI as compared to the IH group. No explanation could be found for such difference in parasitemic levels between these experimental groups. The holothurin treated orchiectomized (IHO) and holothurin treated nonorchiectomized (IH) group of animals were less susceptible to *T. musculi* infection as compared to the untreated controls (IC) and testosterone treated orchiectomized (IHOT) mice receiving holothurin simultaneously with trypanosomes. Levels of parasitemia in the IHOT group were significantly higher than IHO group during days 13 through 19 PI. The IHOT group developed a significantly higher level of parasitemia with 45,800 trypanosomes/mm^3 of blood compared to 13,200 trypanosomes/mm^3 of blood in the IHO on day 13 PI. Parasitemia peaked on day 13 PI for the IHOT group and on day 15 PI for the IHO group (Table 1). The missing androgen in IHO group may account for the decline in parasitemias as compared to the IHOT group.

DISCUSSION

The sex of the host appears to play an important role in predisposition to protozoal infection (Sen et al., 1983; Chapman et al., 1975). Evidence suggests that testosterone renders male mice more susceptible to parasitic infection than female mice (Alexander and Stimson, 1988; Hublart et al., 1988). The concentration of testosterone in the peripheral plasma of the laboratory mouse is extremely variable. Studies have suggested that in the laboratory mouse, testosterone is produced and released in an episodic fashion. Elevations in testosterone levels in peripheral plasma of mice are greater than those observed in other species, and testicular secretory episodes are interspersed with periods of minimal steroidogenic activity (Bartke and Dlterio, 1975). The susceptibility due to plasma testosterone can be altered by orchiectomy and hormone replacement therapy. Orchiectomy had been reported to effect the response of animals to antigens

and results in changes in antibody production (Wechsler & Kongshavn 1985). Other studies suggested that removal of gonadal steroids by orchiectomy could stimulate the cell mediated immune responses as well as alter the structure of the related immunological tissues (Castro, 1974; Grossman, 1984).

Due to endocrine and immune interaction, trypanosomiasis has proven to be particularly difficult to prevent or to even treat effectively. Antigenic variation allows the parasite to avoid the host's immune response and presents it with an endless barrage of antigens (D'Alesandro, 1970).

The result of the present investigation shows that holothurin administered simultaneously with an inoculation of *Trypanosoma musculi* increased the host resistance against trypanosome infection in both nonochiectomized (IH) and orchiectomized (IHO) FN mice. The resistance was measured by the number of trypanosomes at the peak and throughout the course of the infection. The parasitemic levels of both the experimental (IH and IHO) groups were lower during days 9 through 15 PI, when compared to the untreated but injected control (IC) counterparts. Testosterone replacement in the IHOT group made the experimental animals more susceptible as compared with the IHO group. Levels of parasitemia in the IHO group were lower as compared to the IH group. The only speculation is that the missing androgen in the IHO group failed to suppress the host immune system resulting in further lowering the level of parasitemia which was further augmented by the presence of holothurin.

Styles (1970) experimented with holothurin in relation to *T. lewisi* in rats. It was found that rats treated with holothurin simultaneously with an infection of trypanosomes had lower parasitemias than did the controls. The author proposed that the inhibitory effects of holothurin was exerted directly on the trypanosomes as a toxic factor. It is interesting to note that this biotoxin, a steroid saponin of animal origin, has almost the identical effects of those of bacterial endotoxin. According to Singer et al. (1964), the bacterial endotoxins were suspected of exerting their effects by means of some alteration of the reticuloendothelial (RE) system in the host. Endotoxin were taken up by the circulating leukocytes and a leukopenia developed. At that time, endotoxin began to accumulate in the RE system and was cleared from the circulation in a short time. In the present study, the increased activity of the RE system might account for the increased resistance of holothurin treated mice to trypanosome infection. The other speculation is that holothurin may act by interfering with the transport of essential substances across the *T. musculi* plasma membrane. Patton (1972) showed that ouabain inhibits the reproduction of *T. lewisi* and that the effect is indistinguishable from reproduction inhibition produced by ablastin (reproduction inhibiting antibody) *in vitro*. The mechanism by which holothurin is able to influence resistance against *T. musculi* in mice is unknown, but it affects the *in vivo* development of the stercorarian hemoflagellates.

It is probable that factors such as the age, agression, sex and strain etc. determine the resistance or susceptibility of the host to trypanosomiasis. Other factors that may affect host resistance are: nutrition, season, external environment of the host, temperature, animal agression etc. (Ali and Sweatman, 1966; Barkley Goldman, 1977; Jackson and Farmer, 1970; Lincicome et al., 1965; Hanek and Fernando, 1978; Sen et al., 1981b). It is hoped that further studies of the previously untested experimental model (*Trypanosoma musculi* and orchiectomized and nonorchiectomized FN mice) with and

without steroid and saponin treatments will enable to elucidate the mechanisms involved.

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