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A NEW APPROACH FOR SCREENING PROPHYLACTIC AGENTS IN SCHISTOSOMIASIS

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SUMMARY

Schistosomula were obtained in the peritoneal cavity of mice previously injected intraperitoneally with cercariae of *Schistosoma mansoni*. The number of recovered larvae from peritoneal washings was about 30% after 1, 2, 5 and 8 days of injection. Drugs were given to groups of 20 mice 3 hours after the cercarial injection (oxamniquine) and continuing for 4 consecutive days for triostam. Animals were sacrificed 8 days after starting treatment. Schistosomula were counted under a dissecting microscope. Oxamniquine as well as triostam were used to evaluate the screening method. Oxamniquine-treated mice showed no larvae; triostam group presented 5% of recovered larvae against 45% showed by the control. The results obtained agree with previously described effects of these drugs on early developing stages of *S. mansoni*.

INTRODUCTION

During World War II, the necessity of conducting military operations in endemic areas of schistosomiasis stimulated the search on protective measures for avoiding schistosome infection. Systematic studies began early in 1945 and were chiefly undertaken by the U.S. Army Commission on Schistosomiasis (WRIGHT et al. [17, 18]; HUNTER et al. [3]). The general problem of protection against schistosomiasis has been considered from several aspects: 1) destruction of snails; 2) elimination of cercariae by cercaricides; 3) immediate protection by use of uniforms made of protecting fabrics and impregnated clothing; 4) application of cercaricidal and/or cercarial repellents to the skin (KUNTZ et al. [4]; MOON & HUNTER [5]).

Objection on the suitability of the methods above mentioned have been raised by CUSHING [3], who suggested that research is needed to develop a prophylactic drug for the prevention of schistosomiasis, of low toxicity and effective by oral administration.

Up to now, only a limited effort has been directed toward the search for prophylactic agents active against *S. mansoni* (SCHUBERT [13]; STANDEN [14]; STOHLER & FREY [15, 16]; MOUSA et al. [8]; MORS et al. [7]). According to RADKE et al. [12], this situation may be attributed in part to the need for more convenient methods of screening for prophylactic activity. These same Authors described a mouse mortality test system for mass screening for prophylactic compounds: mice

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were exposed by tail immersion to about 3,000 cercariae, per animal, for 30 min. Drug administration consisted of single doses (640 and 1920 mg/kg) 2 days after cercarial exposure. The survival of treated mice beyond 30 days would suggest antischistosomal activity. All mice surviving 49 days were killed for worm collection.

MOORE & MELENEY [6] described the development of *Schistosoma mansoni* in the peritoneal cavity of mice and hamsters. PEREIRA et al. [11], injecting schistosomula into the abdominal cavity of mice, observed that some larvae remained in this site up to 90 days.

A simple and rapid method for mass screening of prophylactic agents, using peritoneal schistosomula, will be presented in this paper.

MATERIAL AND METHODS

Intraperitoneal inoculation — S. mansoni cercariae (LE strain, Belo Horizonte), shed by experimentally infected *Biomphalaria glabrata*, were concentrated as described by PELLEGRINO & MACEDO [10] to a final concentration of about 900 larvae per ml of spring water. The larvae were then injected (0.8 ml) intraperitoneally (i.p.) into 36 adult male mice with a Cornwall syringe provided with a 20 x 10-gauge needle. In order to avoid loss of liquid through the skin perforation, the needle was passed subcutaneously for about 1 cm before penetrating the peritoneal cavity.

Collection of schistosomula — Groups of 9 animals were killed by cervical fracture 1, 2, 5 and 8 days after i.p. inoculation to check the reproducibility in larval counts. The skin was removed from the abdomen and 5 ml of isotonic saline injected into the peritoneal cavity. The testes were pushed into the abdomen and gentle massage was applied to the abdominal viscera. Finally the peritoneum was opened with a pair of scissors and the liquid collected in a Petri dish. An additional 10 ml of saline was used to wash the peritoneal cavity and the liquid was then transferred to centrifuge tubes. Larvae were concentrated on the bottom of the tubes by centrifugation for 1 min at 1.000 rpm. The supernatant was discarded. the remaining fluid (about 1 ml) was transferred to slides and schistosomula were counted under a dissecting microscope.

Drugs tested — Three groups of 20 mice were injected i.p. with about 180 cercariae per animal. Three hours after i.p. injection. oxamniquine (6-hydroximethyl-2-isopropylamino-methyl-7-nitro-1,2,3,4-tethahydroquinoline, Pfizer Laboratories) was injected intramuscularly, single dose (200 mg/kg). Triostam (sodium antimonyl gluconate, Wellcome Laboratories) was injected subcutaneously in five daily doses of 35 mg/kg. The third group served as control. Mice were killed one week after cercarial inoculation and schistosomula counts performed as described.

In routine screening of compounds of unknown activity, treatment is given for 5 consecutive days at the daily dose level corresponding to about 1/5 of the LD_{50}.

RESULTS AND COMMENTS

The number of schistosomula recovered from the peritoneal cavity after i.p. injection of cercariae is shown in Table I. As

TABLE I

Schistosomula collected after i.p. cercarial inoculation (about 700 larvae per mouse) at different time intervals

Days after i.p. injection of cercariae	Schistosomula per animal									Mean and standard error	Mean recovery (%)
1	230	293	166	243	181	367	325	138	274	246 ± 25.3	35.1
2	427	193	296	184	323	285	124	237	203	252 ± 30.0	36.0
5	135	134	228	385	238	321	291	337	202	252 ± 29.3	36.0
8	336	265	294	219	227	389	130	243	179	254 ± 26.0	36.2

TABLE II

Schistosomula recovered from the peritoneal cavity of mice injected with about 180 cercariae (*S. mansoni*) and treated with antischistosomal drugs

Drugs	Route of administration	Dose	Number of schistosomula mean and standard error	Mean recovery (%)
Oxamniquine	Intramuscular	Single (200 mg/kg)	0.0	0.0
Triostam	Subcutaneous	5 × 35 mg/kg	10.2 ± 4.6	5.6
Control	—	—	81.8 ± 11.2	45.4

can be seen, about 30 percent ob injected larvae were recovered in all groups inoculated with 700 cercariae. The time between the inoculation and the necropsies (1 to 8 days) did not alter the mean number of recovered schistosomula.

The activity of known antischistosomal compounds (oxamniquine and triostam) on peritoneal schistosomula is summarized in Table II.

Oxamniquine is known to display activity on developing schistosomes (PELLEGRINO & KATZ [9]). Antimonials (triostam) are known to protect mice when given in repeated doses just after exposure (SCHUBERT [13]) or shortly before or after the time of infection (BRUCE et al. [1]; STOHLER & FREY [15, 16]). As can be seen in Table II, no schistosomula were found in the group of mice treated with oxamniquine. The mean number of schistosomula recovered from mice treated with triostam differed significantly ($p < 0.01$) from that observed in the control group (partial activity).

Some advantages of the method here described are: a) the need of a small number of cercariae (200 against 3,000 used for the mouse mortality test); b) simple way to infect mice (it requires no previous anesthesia or animal immobilization prior to tail exposure to cercariae); c) it is less time-consuming, requiring only 8 days from infection to necropsy, in contrast with 49 days needed in the mortality test; d) it uses a simple peritoneal washing instead of portal system perfusion.

The described method as well as the mouse mortality test are fitted to screen only drugs with systemic activity.

The results here presented are, of course, not sufficient to evaluate the sensitivity of the method. Further investigations, including tests with known antischistosomal agents, are being done.

The idea to inoculate cercariae i.p. into mice with a previous experimental schistosome infection in order to screen curative and/or prophylactic compounds at the same time is very atractive. Work on this line is in progress.

RESUMO

Novo método para testar compostos com atividade profilática na esquistossomose

Esquistossômulos foram obtidos na cavidade peritoneal de camundongos previamente inoculados intraperitonealmente com cercárias de *Schistosoma mansoni*. A recuperação de larvas na salina usada para lavar a cavidade abdominal foi cerca de 30%. As drogas foram administradas a grupos de camundongos 3 horas após a infecção cercariana e os animais sacrificados 8 dias a partir do início do tratamento. Os esquistossômulos foram contados com a ajuda de um estereomicroscópico. Oxamniquine e triostam (de conhecida atividade profilática) foram usados para a avaliação do método. O grupo de camundongos tratados com oxamniquine não apresentou larvas; o grupo tratado

com triostam apresentou 5% de larvas recuperadas, contra 45% presentes no grupo controle.

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