In vivo cercaria-schistosomulum transformation: inhibitory effect of oxamniquine*

A. L. MELO, L. H. PEREIRA and M. C. R. CORRÊA
Grupo Interdepartamental de Estudos sobre Esquistossomose, Universidade Federal de Minas Gerais, Caixa Postal 2486, 30-000 Belo-Horizonte, Brazil

Summary
High doses of oxamniquine (given intramuscularly) produced inhibition of cercaria-schistosomulum transformation following intraperitoneal injections of cercariae into mice. Cercariae, tail-less cercarial bodies, and schistosomula were recovered from the peritoneal cavity of drug-treated mice in numbers significantly different from those recovered from untreated mice. Since untreated animals induced transformation of almost all the injected cercariae, the data suggest the compound is active during the process of host-larvae adaptation.

Introduction
Oxamniquine, now the drug of choice for the treatment of schistosomiasis mansoni in Brazil, also had chemoprophylactic properties which prevent infection of laboratory animals after exposure to cercariae (Foster, 1973; PELLEGRINO et al., 1977). Different routes of experimental infection do not alter the results, and activity against early developing forms of the parasite has been confirmed after exposure of skin to Schistosoma mansoni cercariae (OLIVEIRA et al., 1976) and after inoculations into the peritoneal cavity of mice (PELLEGRINO et al., 1974, 1976a, 1976b; PEREIRA et al., 1974, 1975). With standard doses (50 to 400 mg/kg), drug action was found to be directly against schistosomula.

There has been no report of drug activity against the parasite during the short period when cercariae change into schistosomula; the results of such studies are presented in this paper.

Materials and methods
The method described by PEREIRA et al. (1974) to study the chemoprophylactic action of compounds on schistosomula recovered from the peritoneal cavity of mice was used in all experiments. Albino mice (males, weighing 20 ± 2 g) were inoculated intraperitoneally with Schistosoma mansoni cercariae (L. E. strain) shed by laboratory-reared-and-infected Biomphalaria glabrata (Belo-Horizonte strain). The organisms were concentrated by the method of PELLEGRINO & MACEDO (1955) and 0.5 ml of well water containing about 450 larvae were injected by a Cornwall syringe with a 20 x 10-gauge needle.

Appropriate doses of oxamniquine* were mixed with polyethylene-glycol in a mortar and injected (0.1 ml) intramuscularly in the hind leg. The drug was administered one hour before the cercarial injections and doses of 2,000, 1,000 or 500 mg/kg were given to three groups of five animals each. Untreated mice served as controls.

Three hours after the inoculations, the mice were killed by cervical fracture, and the parasites, recovered from the peritoneal cavity with saline, were centrifuged, and counted under a dissecting microscope (PEREIRA et al., 1974). The larvae were classified initially as cercariae and tail-less organisms (living or dead). To separate cercarial bodies, which remain alive in fresh water, from schistosomula, which die in such conditions, 5 ml of distilled water was dropped into the Petri dish containing the larvae and after 10 minutes the live and dead larvae were recounted. In addition to the absence of motility, dead larvae showed herniation of the acetabulum.

Results
The data are summarized in Fig. 1. About 30% of the inoculated cercariae were recovered from the peritoneal cavity of all mice. Marked differences were observed between larvae recovered from the control mice and the animals treated with oxamniquine at 1,000 or 2,000 mg/kg.

Percentages of living cercariae from larvae recovered from treated and control mice were appropriated changed to arc sin √(X/n) and the Student's t test was performed; groups treated with 1,000 and 2,000 mg/kg differed significantly from their respective controls showing t values of 11.73 and 18.64 respectively (P < 0.005).

Discussion
Oxamniquine is a compound active intramuscularly at 200 mg/kg against mature schistosomes and young larvae in laboratory mice; its action against older schistosomula at the same dose is less efficacious (PELLEGRINO et al., 1976a). By using doses of 50 to 400 mg/kg, no action was demonstrated on the transformation of cercariae into schistosomula. However, as can be seen in Fig. 1, doses of 1,000 and 2,000 mg/kg of the drug produced some delay in the transformation process; from mice exposed to 2,000 mg/kg, 25.08% of

* This work was supported, in part, by grant number 4696/75 from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and by the World Health Organization, Switzerland. The authors are indebted to Dr. P. Williams for reviewing this manuscript, and to Dr. I. Sampaio for helping with the statistical analysis.

Contribution Number 96 from the Schistosomiasis Research Unit.

* "Mansil", Pfizer Laboratories.
living cercariae were recovered as against 0.22\\% from control mice, and mice exposed to 1,000 mg/kg produced 3.34\\% living cercariae as against nil in the controls. A dose of 1,000 mg/kg also resulted in the recovery of more living cercarial bodies, 16.72\\% against 0.78\\% in controls, suggesting partial inhibition of the loss of the glycocalyx.

As shown by HOWELLS et al. (1974), the cercariae-schistosomula transformation begins with the loss of the cercarial tails followed by the loss of the glycocalyx of the tail-less organism. Thus, the oxamniquine induced inhibition of transformation could be explained by: (i) inhibition of the mechanism of tail loss, and (ii) lowering of cercarial activity as a whole, leading to a delay in transformation. HOWELLS et al. (1974) believe the loss of the cercarial tail is a mere consequence of muscular activity of the organism; if oxamniquine affects the cercariae by reducing their movements, the detachment of the tail would be less likely to occur.

Although well described by EVELAND (1972), the cercaria-schistosomulum transformation in the peritoneal cavity of mice needs additional study of the factors that trigger the process. Possibly, in vivo mechanisms could differ in some aspects from in vitro processes.

References


Accepted for publication 11th November, 1977.