0022-3565/78/2062-0382$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 206, No. 2

Printed in U.S.A.

Evaluation of Oral Therapy of Mansonial Schistosomiasis in the Sudan Using Single and Multiple Doses of Hycanthone and Oxamniquine Drugs[1]

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Accepted for publication February 24, 1978

ABSTRACT

Magzoub, M. and S. E. I. Adam: *Evaluation of oral therapy of mansonial schistosomiasis in the Sudan using single and multiple doses of hycanthone and oxamniquine drugs.* J. Pharmacol. Exp. Ther. **206:** 382–387, 1978.

Single doses of 20 mg/kg of hycanthone or oxamniquine or a combination of 20 mg/kg of each drug, given to Nile rats (*Arvicanthus niloticus*) infected with *Schistosoma mansoni,* did not produce significant changes in the parasitic fauna or in the hepatic damage induced by the parasite. Three daily doses of 20 mg/kg of these drugs alone or in combination reduced considerably the intensity of parasitic infection and caused a marked recovery from the tissue damage induced by parasitic invasion. The proportions of immature eggs, as compared with the mature ones, were 1 to 11.5 in presence of hycanthone and 1 to 8 in presence of oxamniquine. There were no signs of immature eggs when the two drugs were used in combination. All living worms and nearly almost all immature eggs disappeared from the tissues of the treated animals examined 10 and 20 days after completion of treatment with hycanthone and oxamniquine alone as well as in combination. Gross and microscopic examination of the animals given both drugs in combination revealed the absence of worms and eggs in the portal tracts and reduced hepatocellular necrosis and schistosome pigment in the Kupffer cells. In the treated rats there was less of a decrease in the glycogen content of the hepatocytes but aggregation of mononuclear cells was found in the portal areas. Splenomegaly associated with hyperplasia and granulomatous splenitis was seen in the untreated infected rats, but not in the infected rats given a combination of hycanthone and oxamniquine.

The present experiments were concerned with the treatment of intestinal schistosomiasis in the Nile rat (*Arvicanthus niloticus*) with single, multiple and mixed doses of hycanthone and oxamniquine. The criteria used for the evaluation of these antischistosomal agents included the susceptibilities to drugs displayed by adult schistosomes and the effect of treatment on pathological changes in different organs caused by bilharzia infection.

Materials and Methods

One hundred Nile rats (*A. niloticus*) of both sexes and weighing 40 to 60 g each were used for the experimental study. These were randomly grouped. a) Four uninfected rats were killed by a blow on the head during the 9th week after the start of the experiment. b) Ten rats, each infected percutaneously with 200 cercariae of our local (Sudan) strain of *Schistosoma mansoni,* were killed in subgroups of at least 3 at 49

Received for publication January 5, 1977.
[1] This work was supported by a grant from the National Council for Research, Khartoum.

days (b1), 56 days (b2) and 63 days (b3) from the date of infection. c) Twenty-eight infected rats were dosed orally, 8 weeks after infection, with hycanthone given in a dose of 20 mg/kg. Hycanthone was dissolved in distilled water and a single dose was given to 3 rats (c1); three daily doses to 25 rats (c2), killed after 3 (c2a), 10 (c2b) and 20 (c2c) days after the end of treatment. d) Twenty-eight infected rats were treated orally, 8 weeks after infection, with oxamniquine (20 mg/kg) which was prepared in the same way as hycanthone; a single dose was given to 3 rats (d1) and three daily doses were given to 25 rats (d2) killed after 3 (d2a), 10 (d2b) and 20 (d2c) days after the completion of treatment. e) Thirty infected rats were treated, 8 weeks after infection, with a combination of hycanthone and oxamniquine, each in a dose of 20 mg/kg; a single dose of the combined drugs was given to 4 rats (e1), one of which died during treatment. Twenty-five other rats were given three daily doses of the combined drugs (e2) and killed after 3 (e2a), 10 (e2b) and 20 (e2c) days from the end of treatment.

Histological and histochemical methods. Specimens of liver, intestines, spleen, kidneys, lungs and heart were fixed in 10% formol-saline, embedded in paraffin wax and sectioned at 5 μm. All sections were stained with hematoxylin and eosin (H&E) and selected sections were stained by Prussian blue, periodic acid-Schiff (PAS) with and without prior incubation with diastase and Schmorl's method for lipofuscin.

Results

Parasitological findings. Ten rats were sacrificed, three 49 days, four 56 days and three 63 days after exposure to 200 cercariae of *S. mansoni*. In all rats examined, the number of the immature eggs in the tissues of the intestine was greater than the mature ones. Forty-nine days after infection, the immature eggs represented 76% and the mature eggs 15% of the total; 56 days after infection, the immature eggs represented 77% and the mature eggs 15% of the total; 63 days after infection, the percentages of immature and mature eggs were 71 and 17%, respectively. There were 9% dead eggs 49 days after infection, 8% 56 days after infection and 22% 63 days after infection.

The worms from the liver and mesenteric vessels of the infected and treated animals were recovered by the perfusion technique of Smithers and Terry (1965) and were counted during postmortem examination of each rat. The data are presented in table 1. The average number and sex of the worms found in the body of each infected rat in the first group was 20 males and 16 females in the mesenteric vessels and 14 males and 10 females in the liver; in the second group 18 females and 22 males were recovered from the mesenteric vessels and 16 males and 14 females from the liver; in the third group, killed 63 days after infection, 22 females and 26 males were recovered from the mesenteric vessels and 12 females and 18 males from the liver.

Hycanthone experiments. Three days after the completion of treatment, 8 rats were killed and examined for the presence of adult worms and eggs of *S. mansoni* in the tissues of the intestine; the average number of worms found in the liver of each rat was 2 males and 1 female and in the mesenteric vessels 4 males and 3 females; of the eggs found, 2% were immature, 23% were mature and 75% were dead.

Ten days after completion of treatment, 7 rats were examined and the results revealed a complete absence of living worms in the liver and intestines of the treated animals. Examination of the eggs showed 1% were immature, 53% were mature and 46% were dead.

Ten infected rats were examined for living worms and eggs of the schistosome parasite 20 days after treatment. No worms were found in the liver and intestines. Examination of the eggs in the tissues of the intestine revealed 53% were mature and 47% were dead; there was a complete absence of immature eggs.

Oxamniquine experiments. Three days after the completion of the treatment with oxamniquine, 8 rats were examined for living worms and eggs of the schistosome parasite. Fourteen males and 10 female worms were found in the liver and intestines of these animals. Ten days after completion of treatment, 5 males and 3 females were detected in the tissues of the liver and intestines of 8 rats. Twenty days after the treatment, 9 rats were examined; living worms in the liver and intestines were absent.

Examination of eggs in the intestines of these animals 3 days after the end of treatment showed 3% were immature, 23% were mature and 74% were dead. Ten and 20 days after the end of treatment, the percentages were 1 and 0% immature, 34 and 31% mature and 65 and 69% dead, respectively.

Combined administration of hycanthone and oxamniquine. Three days after the end of treatment with the two drugs in combination, 5 rats were examined for living worms and eggs of the schistosome parasite. Four males and 2 females were picked from the liver and intestines of these rats. Ten and 20 days after the treatment, no living worms were found in the liver and intestines of the 20 animals examined.

The oogram study showed 0% immature, 29% mature and 71% dead eggs 3 days after the end of treatment; 10 and 20 days after completion of

treatment the immature eggs were absent, the mature were 18 and 1% and the dead eggs 82 and 99%, respectively.

Pathological changes. *Liver.* The effects of hycanthone and oxamniquine treatments on the liver changes produced by *S. mansoni* infection in the Nile rat are summarized in table 2.

Infected rats. There were irregular nodules and fatty change. Many enlarged portal tracts showed egg granulomata (fig. 1). The cellular infiltrations consisted of round cells, eosinophil leukocytes, mononuclear cells and fibroblasts. There was fatty cytoplasmic vacuolation, focal necrosis and depletion of glycogen in the par-

TABLE 1

Efficacy of Hycanthone and Oxamniquine in the treatment of *S. mansoni* infection in the Nile rat

Group	No. of Rats per Group	Age of Infection	Dose of Drug	No. of Daily Doses of Drug	Average No. of Worms in				
					Mesenteric veins		Liver		
					♂	♀	♂	♀	
		days	*mg/kg*						
Uninfected control									
A	4								
Infected control									
B1	3	49			60	48	42	30	
B2	4	56			88	72	64	56	
B3	3	63			78	66	54	36	
Hycanthone									
C1	3	56	20	1	46	28	26	18	3 days post-treatment
C2a	8	56	20	3	32	24	16	8	3 days post-treatment
C2b	7	56	20	3					10 days post-treatment
C2c	10	56	20	3					20 days post-treatment
Oxamniquine									
d1	3	56	20	1	42	24	24	16	
d2a	8	56	20	3	8	6	6	4	3 days post-treatment
d2b	8	56	20	3	3	2	2	1	10 days post-treatment
d2c	9	56	20	3					20 days post-treatment
Hycanthone + oxamniquine									
e1	4	56	20	1	20	12	8	6	
e2a	5	56	20	3	4	1	2	1	3 days post-treatment
e2b	10	56	20	3					10 days post-treatment
e2c	10	56	20	3					20 days post-treatment

TABLE 2

Assessment of hepatic damage in infected Nile rats treated with hycanthone and oxamniquine

Drug	Dose of Drug	No. of Daily Doses	Liver Changes*				
			Granulomas	Fatty change	Necrosis	Loss of glycogen	Schistosomal pigment
	(mg/kg)						
Infected control			+++	++	+	++	++
Hycanthone	20	1	++	+	+	+	++
Hycanthone	20	3	+	+	(−)	+	(−)
Oxamniquine	20	1	+++	+	+	+	+
Oxamniquine	20	3	+	+	(−)	+	(−)
Hycanthone + oxamniquine	20	3	+	+	(−)	+	(−)

* +→+++, increasing severity of lesions; (−), absence of lesions.

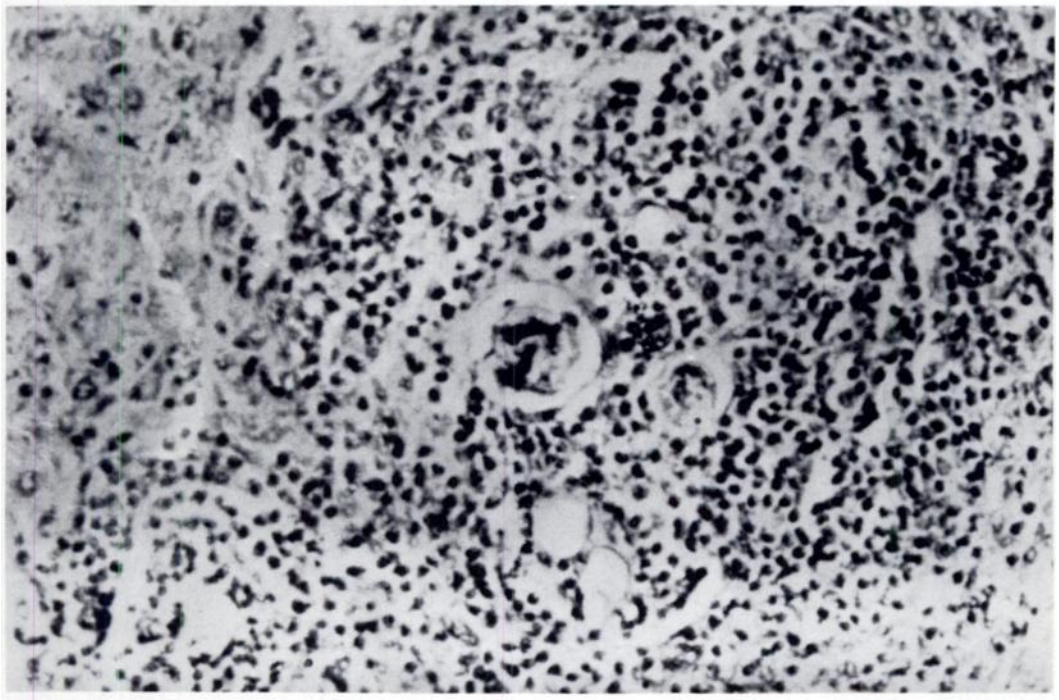

Fig. 1. Rat liver 9 weeks after infection with 200 cercariae of *S. mansoni*: egg granuloma. (H&E, ×235.)

enchyma. Kupffer cells in the periphery of the lobule and the subcuticle of the worms contained schistosomal pigment which did not stain with either Schmorl's or Prussian blue methods.

Hycanthone-treated rats. The numbers of granulomas in the portal vessels and the levels of glycogen and fatty change were slightly reduced in the liver of rats which received single doses of hycanthone. After three doses, there was no evidence of schistosomal pigment in Kupffer cells. The number of granulomas in the portal areas was considerably reduced and many portal tracts showed neither worms nor ova. There was infiltration of mononuclear cells and eosinophil leucocytes in the portal tracts (fig. 2). Focal necrosis disappeared and the glycogen content was increased in the parenchyma.

Oxamniquine-treated rats. The livers of rats in the single dose group did not differ significantly from the infected controls. After three doses, there was neither hepatic necrosis nor deposition of schistosomal pigment in Kupffer cells. The number of granulomas was reduced and the hepatocytes showed an increase in glycogen content.

Hycanthone and oxamniquine treatments. The lesions in the liver resembled those found previously in rats given three doses of either hycanthone or oxamniquine. The numbers of granulomas in the portal areas and of the worms and ova were very low compared to the infected controls.

Other tissues. The findings in other tissues of infected rats were infiltration of the intestinal lamina propria with inflammatory cells, pulmonary congestion and/or edema and splenomegaly (fig. 3). Splenomegaly was also seen in rats which received single doses of oxamniquine. After three doses of hycanthone or oxamniquine, there were few such changes.

Discussion

To find more suitable anthelminthics for use against *Schistosoma mansoni,* attention was turned to orally administered drugs. Magzoub and Maegraith (1969) showed that niridazole was very active in reducing the motility of the Egyptian strain of *S. mansoni* through the inhibition of the exogenous glucose uptake from blood and the subsequent depletion of glycogen in the tissues of the treated worms. Magzoub and Adam (1973) tested lucanthone hydrochloride for its activity against the Sudan strain of *S. mansoni* in albino mice and found that the drug was most active in killing the worms and preventing the development of hepatic damage when given in 10 daily doses of 100 mg/kg.

In this paper, we tested hycanthone and oxamniquine against *S. mansoni* infection in Nile

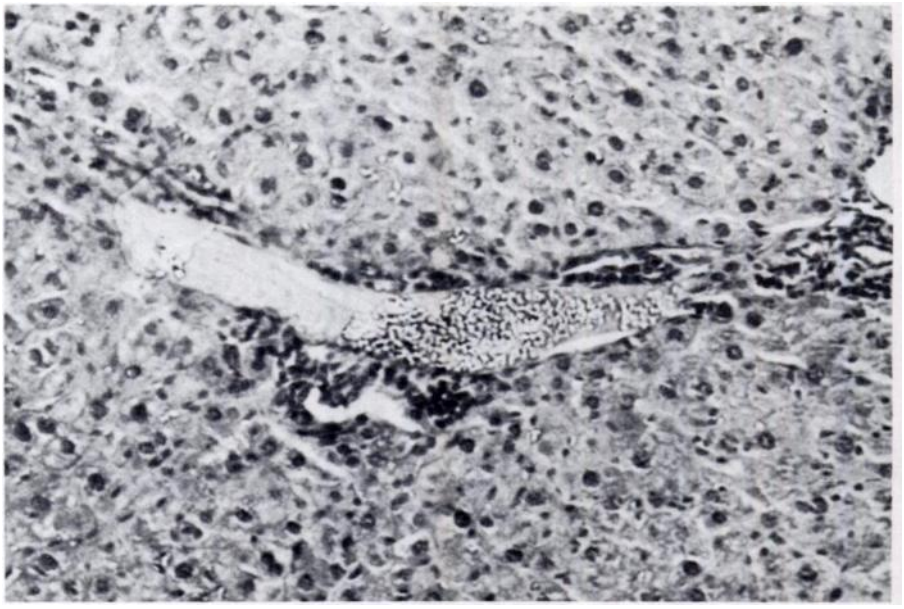

Fig. 2. Rat liver after treatment with three daily doses of hycanthone: absence of egg granuloma and infiltration of mononuclear cells in the portal tracts. (H&E, ×235.)

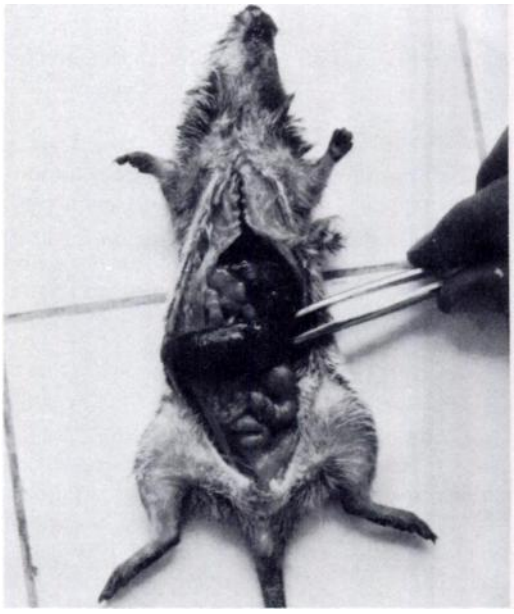

Fig. 3. Splenomegaly in a Nile rat 9 weeks after infection with 200 cercariae of *S. mansoni*.

rats (*Arvicanthus niloticus*). After single doses of either hycanthone or oxamniquine, there was no significant reduction in the number of adult worms in the liver and the intestines, no change in the oogram of the intestines and no significant decrease either in the number of granulomas or of hepatocellular necrosis. These findings indicate that the administration of both drugs at single oral doses did not produce any significant effect on the worm load and on the hepatic damage induced by the parasite.

The oral administration of hycanthone, oxamniquine and their combination in three daily doses of 20 mg/kg proved to be more effective. In the presence of hycanthone, infected rats (killed after 10 days from treatment) showed no living worms in the tissues of the liver and intestines and a complete absence of immature eggs in the intestines. Ten days after the end of treatment with oxamniquine, no living worms were seen in the tissues of the treated animals and the oogram of the intestines showed the presence of 1% immature eggs as compared with 77% in the absence of treatment. Animals treated with mixed doses of both drugs and examined 10 days after treatment showed no living parasites and no immature ova in the tissues of the intestines.

However, our pathological investigations showed that the administration of hycanthone, oxamniquine or their combination in three daily doses proved to be an effective treatment for the removal of *S. mansoni* as indicated by the absence of the parasites and ova from the portal tracts, of hepatocellular necrosis and schistosome pigment from Kupffer cells. The glycogen content was increased in the hepatocytes and aggregates of mononuclear cells were found in the portal areas. Schiller and Haese (1973) re-

ported persistence of the granulomata for many weeks after elimination of the worms by treatment. The carcinogenicity of hycanthone in Swiss-Webster strain of mice has been demonstrated by Haese and Beuding (1976).

Hepatosplenomegaly is a constant finding in *S. mansoni* infection in man and is accompanied by marked eosinophilia (Schiff, 1963). We did not observe splenomegaly in albino mice infected with a Sudan strain of *S. mansoni* (Magzoub and Adam, 1973, 1974a,b). The results recorded in this paper demonstrated the presence of splenomegaly in the Nile rat infected with a Sudan strain of *S. mansoni* and this was associated with hyperplasia and granulomatous splenitis.

Acknowledgments

We thank Mr. H. Ibrahim and Mr. A. H. Saad for technical assistance and Mr. E. M. Abu Ganaya for photography.

References

HAESE, W. H. AND BUEDING, E.: Long-term hepatocellular effects of hycanthone and of two other anti-schistosomal drugs in mice infected with *Schistosoma mansoni*. J. Pharmacol. Exp. Ther. **197:** 703–713, 1976.

MAGZOUB, M. AND ADAM, S. E. I.: Effect of lucanthone hydrochloride on the hepatic lesions induced by Sudan strain of *Schistosoma mansoni*. J. Comp. Pathol. **83:** 429–435, 1973.

MAGZOUB, M. AND ADAM, S. E. I.: Experimental infection and hepatic changes in mice infected with *Schistosoma mansoni* (Sudan strain). J. Pathol. **113:** 47–52, 1974a.

MAGZOUB, M. AND ADAM, S. E. I.: A comparative study of the liver damage and worm burden following infection with *Schistosoma mansoni* from northern and southern Sudan. Br. J. Exp. Pathol. **55:** 260–276, 1974b.

MAGZOUB, M. AND MAEGRAITH, B. G.: Glycolytic activities of *Schistosoma mansoni* and the mode of action of antischistosomal agents (Ambilhar and Astiban). Ann. Trop. Med. Parasitol. **63:** 377–391, 1969.

SCHIFF, L.: Diseases of the Liver, 2nd ed., Pitman Medical Publishing Comp. Ltd., Philadelphia, 1963.

SCHILLER, E. S. AND HAESE, W. H.: Histologic processes of dealing in hepatic injury due to ova of *Schistosoma mansoni* in mice following curative chemotherapy. Amer. J. Trop. Med. Hyg. **22:** 211–214, 1973.

SMITHERS, S. R. AND TERRY, R. J.: The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of the adult worms. Parasitology **55:** 695–700, 1965.

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