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ACTIVITY OF THE ARTEMETHER IN EXPERIMENTAL SCHISTOSOMIASIS MANSONI

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The action of the ether of artemisinin (artemether) on Schistosoma mansoni *in mice and hamsters experimentally infected with the LE strain was studied. In mice, the drug showed high schistosomicidal activity using a single intramuscular dose of 100 mg/kg/day. By the oral route, this dose showed a low activity. Mice treated with a single intramuscular dose of 200 mg/kg/day, and examined 15 days after treatment, presented 100% alteration of the oogram; when examined 45 days after treatment, the oogram was normal. With doses of 100 mg/kg/day, i.m., during 3 or 5 consecutive days, the death rate of mice was very high. Morphologic analysis of the worms collected by perfusion of mice treated with a single dose of 100 mg/kg/day, i.m., detected a marked decrease in the length of male and female worms, degenerative alterations in the parenchyma and in the reproductive system of the females, with the reduction of vitellinic material and in ovary volume; the intestinal contents presented a marked despigmentation. In the male worms significant alteration was not apparent by optical microscopy.*

Key words: *Schistosoma mansoni* – artemether – experimental therapeutic – schistosomicide

Artemisinin is an isolated compound of *Artemisia annua* L. Compositae. This compound in China was called ginghaosu in 1972 and since then it is being used in the clinical treatment of malaria with satisfactory results (China Cooperative Research Group, 1982 a, b).

The action of artemisinin was experimentally demonstrated in parasite such as: *Clonorchis sinensis, Schistosoma japonicum, S. mansoni, Plasmodium berghei* and *P. falciparum*, including those resistant to chloroquin (Klayman, 1985).

The objective of the present paper was to study the action of artemether (ether of artemisinin) in mice and hamsters experimentally infected with *S. mansoni*.

MATERIALS AND METHODS

The strain of *S. mansoni* used was the LE, maintained for over 25 years in our laboratories with sucessive passages in *Biomphalaria glabrata* and mice or hamsters. Swiss albino mice were experimentally infected, by the subcutaneous route, with 100 ± 10 cercariae and hamsters infected, in the same way, with 200 ± 20 cercariae. Treatment was performed with injectable artemether (an oily solution of 12-methyl ether of the hydro-artemisinin), produced by the Kunning Pharmaceutical Factory-Yunnan, People's Republic of China.

Forty-five days after infection, groups of mice were treated with artemether; 200 mg/kg/day x 1, 100 mg/kg/day x 5, 100 mg/kg/day x 3, 100 mg/kg/day x 1 or 50 mg/kg/day x 1, by the intramuscular or oral routes and were sacrificed 1, 3, 7, 15 or 45 days after treatment.

Two other experiments were undertaken in mice: administering artemether, 100 mg/kg/day x 3, i.m., the animals having been exposed at the 2 nd day of treatment, or 100 mg/kg/day x 1, i.m., with infection 48 h after treatment. In both experiments, infection was with 100 ± 10 cercariae during 50 min, by the tail imerson method (Pellegrino & Katz, 1968).

The treatment of hamsters was undertaken 45 days after the infection and the therapeutic schemes were: single doses of 200, 100, 50, 25 and 12.5 mg/kg by the intramuscular route. The animals were sacrificed 3 days after treatment.

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For evaluation of drug activity, the animals were sacrificed and perfused to recover the worms which were in the mesenteric veins and in the liver. The liver was crushed between two glass plates and examined under the sterioscopic microscope, looking for dead worms. Fragments of the small intestine were pressed between two glass slides to observe the oogram. Alterations of the oogram were considered significant when one or more developmental stages of immature eggs were absent (Pellegrino & Katz, 1968).

The worms recovered by perfusion, were fixed with 10% neuter formaldehyde, stained by alcoholic hydrocloric carmine, dehydrated in the alcoholic series, made transparence with creosate of Faia and installed with balm of Canada of proceed to the morphologic study. The measurements were made with a Leitz microscope with micrometric ocular (Kohn et al., 1979).

At the same time as the experiments were performed, control groups of animals which, excepting the treatment, were maintained in identical conditions as the treated groups.

RESULTS

Mice treated with artemether (100 mg/kg/day) during 3 or 5 days, by the intramuscular route, presented a high death rate; with a single dose of 100 mg/kg i.m., the antiparasitic activity was accentuated (100% alteration of the oogram and 61.5% of dead worms in the liver – Table I). This same dose, orally, showed only 22.6% of dead worms, without alteration of the oogram.

Mice treated with 200 mg/kg/day x 1, i.m. and sacrificed after 15 days, presented 51.1% dead worms in the liver and a 100% alteration of the oogram while, when sacrificed 45 days after treatment, the oogram was normal, even though 38.6% of the worms were found dead in the liver (Table II).

In mice treated with 100 mg/kg/day x 3, i.m. and exposed to the cercariae at the second day of treatment or with 100 mg/kg, single dose, i.m., and exposed 48 later, protection against infection was not observed.

In hamsters, only those treated with a single dose of 50 mg/kg/day, i.m., could be evaluated, since, with the other dosages used the death rate of the animals was 100% in two repeated experiments (Table III).

The worms did not presented significative structural alterations as evidenced by optical microscopy, when examined 24 h after treatment. Three days after treatment a pronounced decrease in the length of male and female worms was observed. In the females, degenerative changes of the parenchyma, which became slack were observed; the reproductive system presented a reduction of viteline material and in the volume of the ovary, the intestinal contents presented a pronounced despigmentation. In the male worms, significative alteration was not observed by optic microscopy.

DISCUSSION

The results in the present paper demonstrated the activity of artemether in mice and hamster experimentally infected with *S. mansoni.* A single dose of 100 mg/kg, by the intramuscular route, caused cessation of egg laying in 100% of the infected mice and about 50% of the worms were found dead in the liver. Nevertheless, this activity was found to persist for less than 45 days, when no alterations of the oogram were found, although more than half of the worms were still in the liver.

It is important to emphasize the high death rate of the treated hamsters. Fifteen days after artemether treatment (50 mg/kg, i.m.) oogram alterations were found in 100% of the treated hamsters, with about 50% of the worms in the liver, 35% of them dead worms.

Only the female worms showed degenerative changes in the parenchyma, as well as in the reproductives system.

Prophylatic schemes were not succesful showing that the drug had a partial curative action, but not a prophylatic one.

Results referring to the activity of the artemether applied in mice experimentally infected with *S. mansoni* are to be found in Klayman (1985) who mentioned an 80% reduction of the number of worms when compared with a control group.

TABLE I

Activity of artemether on mice experimentally infected with *Schistosoma mansoni*

Schedule of treatment mg/kg/day-route	Number of animals			Mean number of worms	Distribution of worms %		Dead worms in the liver %	Oogram alteration %
	Treated	Dead	Examined		Mesenteric vessels	Liver		
100 x 5-i.m.	5	4	1	27.0	3.7	96.3	96.3	100.0
Control	–	–	11	31.6	92.5	7.5	0.0	0.0
100 x 3-i.m.	10	4	6	16.0	18.8	81.2	76.6	100.0
Control	–	–	7	37.0	92.7	7.3	0.0	0.0
100 x 1-i.m.	10	2	8	4.3	26.9	73.1	61.5	100.0
50 x 1-i.m.	10	4	6	17.0	75.5	24.5	8.8	0.0
Control	–	–	7	12.1	90.6	9.4	0.0	0.0

TABLE II

Activity of artemether on mice experimentally infected with *Schistosoma mansoni*

Schedule of treatment mg/kg/day-route	Examined at days after treatment	Number of animals			Mean number of worms	Distribution of worms %		Dead worms in the liver %	Oogram alteration %
		Treated	Dead	Examined		Mesenteric vessels	Liver		
200 x 1-i.m.	15	12	7	5	35.6	43.8	56.2	51.1	100.0
200 x 1-i.m.	45	12	7	5	22.8	37.7	62.3	38.6	0.0
Control	–	–	–	5	34.6	95.4	4.6	0.0	0.0
100 x oral	15	11	7	4	36.5	66.4	33.6	22.6	0.0
100 x 1-i.m.	15	11	3	8	24.4	44.6	55.4	44.6	100.0
Control	15	–	–	10	26.0	87.3	12.7	0.0	0.0

TABLE III

Activity of artemether on hamsters experimentally infected with *Schistosoma mansoni*

Schedule of treatment mg/kg/day-route	Number of animals			Mean number of worms	Distribution of worms %		Dead worms in the liver %	Oogram alteration %
	Treated	Dead	Examined		Mesenteric vessels	Liver		
50 x 1-i.m.	5	3	2	48.0	50.0	50.0	38.5	100.0
Control	–	–	4	114.5	98.7	1.3	0.0	0.0
50 x 1-i.m.	10	4	6	103.6	50.7	49.3	30.0	100.0
Control	–	–	10	101.2	86.6	13.4	0.0	0.0

All hamsters treated with single, i.m. doses of 200, 100, 25 and 12.5 mg/kg died before perfusion.

Yue et al. (1984) studied the action of the artemether in mice experimentally infected with *S. japonicum* and noted that three to seven days after a dose of 100 mg/kg x 3, subcutaneously, 100% of the worms had shifted to the liver on the seventh day, and 74% of them remained in the liver on the 14th day. The majority of the surviving worms, however, returned to the portal system 19 to 29 days after treatment. Alterations were observed in worms chiefly in the reproductive organs of the females. Three to five days after the treatment, eggs were not found in the uterus of females or they were degenerate. Normal eggs were noted again after 11 days. Oograms showed that, once the effect of the drug had finished, the laying and eclosion of eggs became normal. Wu et al. (1983) noted that

artemether in mice, experimentally infected by *S. japonicum*, in doses of 225 to 435 mg/kg/day x 3, subcutaneously produce significative histological changes. Shuhua & Catto (1989) studied the activity of the arthemether against *S. mansoni in vitro* and *in vivo* and noted that the drug caused a decrease of 30% to 50% in the length of worms when observed 14 days after treatment. After 56 days the dimensions of worms returned to normal. Alterations, also reversible, were met in the testis and ovaries of the worms. With a single oral dose of 300 mg/kg, there was a displacement of the worms to the liver after 8 h of treatment. The therapeutic activity of the drug, when applied on the 56th day of the infection, was partial. In fact, doses of 200 mg/kg x 6, by oral administration, presented 39% of reduction of the number of worms; however, mice treated in the 2nd or 3rd week after infection, presented a reduction of 83 to 98%.

Trials in monkeys and in humans must be undertaken to better understand the possible value of artemether in schistosomiasis infection.

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