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In Vitro and In Vivo Studies of the Effect of Artemether on *Schistosoma mansoni*

XIAO SHUHUA† AND BRIAN A. CATTO*

Section of Infectious Diseases, Department of Medicine, Veterans Administration Medical Center/Medical College of Georgia, 15th Street, Augusta, Georgia 30912

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To determine whether artemether, a derivative of the antimalarial agent qinghaosu, is therapeutically active against *Schistosoma mansoni*, we determined the in vitro, in vivo, and histopathologic effects of the drug on *S. mansoni* worms. In vitro, toxic effects of artemether on *S. mansoni* were not seen at concentrations of less than 100 μg/ml. However, in vivo, 30 and 50% reductions in the lengths of male and female worms, respectively, were observed 14 days after treatment. By 56 days worm dimensions had returned to control values. Similar reversible effects on male testes and female ovaries were seen. In vivo, a single oral dose of artemether (300 mg/kg) induced a shift of worms towards the liver within 8 h after treatment. By 3 and 14 days after treatment, 99 and 76%, respectively, of worms were still in the liver. In vivo, the therapeutic effect of artemether on adult *S. mansoni* treated on day 56 after infection was modest. Doses as high as 1,200 mg (200 mg/kg per day, six doses) resulted in a worm reduction rate of only 39%. However, in infected mice treated on day 14 or 21 after infection, worm reduction rates of 83 to 98% were obtained. Thus, artemether exhibited modest in vitro and in vivo activities against adult *S. mansoni* but was twofold more active against 2- to 3-week-old liver-stage parasites.

Qinghaosu (artemisinin) is a new antimalarial drug derived from a traditional Chinese herbal remedy, qinghao or *Artemisia annua* L. (7). To enhance the antimalarial activity and solubility of qinghaosu (Fig. 1A), a series of chemical derivatives of qinghaosu, e.g., artemether and artesunate, have been synthesized and studied in laboratory and clinical trials (8, 13). As a result of these studies, qinghaosu, artemether (Fig. 1B), and artesunate were found to be active against malarial parasites as well as effective in the treatment of *Schistosoma japonicum* infections (9, 10). Thus, qinghaosu (2) and its derivatives appear to have antischistosomal as well as antimalarial chemotherapeutic potential. Recently, it was suggested (3) that resistance might be developing to praziquantel, the current drug of choice for the treatment of all species of human schistosomiasis (5). Thus, continued identification and evaluation of new antischistosomal agents are important in the event that clinical resistance to praziquantel therapy develops. Using *Schistosoma mansoni*-infected mice as a model of infection as well as for a source of parasites for in vitro studies, we investigated the potential usefulness of artemether in the treatment of *S. mansoni* infections.

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MATERIALS AND METHODS

Animals. Male C3H/HenNCr (C3H) and DBA/2NCr inbred mice weighing 18 to 22 g were obtained through a Veterans Administration contract with the National Cancer Institute, Bethesda, Md. In addition, female CF1 outbred mice, obtained from Jackson Laboratory, Bar Harbor, Maine, were used in early studies on the induction of the hepatic shift of *S. mansoni* by artemether in infected mice. Animals (C3H and DBA/2NCr mice) were maintained with rodent Blox (Wayne Pet Food Division, Continental Grain Co., Chicago, Ill.) and water ad libitum in the animal care facility of the Veterans Administration Medical Center/Medical College of Georgia, Augusta, Ga.

Drug. Artemether (Fig. 1B), a methoxy derivative of reduced qinghaosu, was prepared semisynthetically in the Institute of Parasitic Diseases, Shanghai, People's Republic of China. The drug preparation used in these studies appeared chromatographically as a single peak under the following high-pressure liquid chromatography conditions: mobile phase, 50% acetonitrile–water; flow rate, 2 ml/min; detector setting, 214 nm (model 160 detector; Beckman Instruments, Inc., Fullerton, Calif.); column, 10-cm Spheri-5 ODS-MP (5-μm diameter) plus 1.5-cm precolumn (Brownlee Labs) at ambient temperature. Further resolution of the peak with different solvent combinations and/or columns was not attempted. Under these conditions, with a chart speed of 10 mm/min, the peak eluted at 9.5 min. The drug was dissolved in polyethylene glycol (PEG 400; Fisher Scientific Co., Pittsburgh, Pa.) and administered to the mice by gavage in a volume of 0.4 ml/20 g of body weight. For in vitro studies, 5 mg of artemether was dissolved in 3 ml of PEG 400 and then diluted to 5 ml with Hanks balanced salt solution (HBSS; M. A. Bioproducts, Walkersville, Md.). Drug solution (50 to 200 μl) was added to 1.8 to 1.95 ml of medium in each assay dish to yield a final volume of 2 ml for in vitro studies.

Parasites. *S. mansoni* cercariae (KEB strain), obtained from infected *Biomphalaria glabrata* snails, were kindly provided by Raymond T. Damian, Department of Zoology, University of Georgia, Athens (4). C3H and DBA/2NCr male mice were infected subcutaneously with 90 and 150 cercariae, respectively, for in vivo efficacy studies. For hepatic shift studies, cercariae from a Puerto Rican strain of *S. mansoni* were kindly provided by A. Mahmoud, Division of

* Corresponding author.

† Present address: Institute of Parasitic Diseases, National Center for Preventive Medicine, Chinese Academy of Medical Sciences, Shanghai, People's Republic of China.

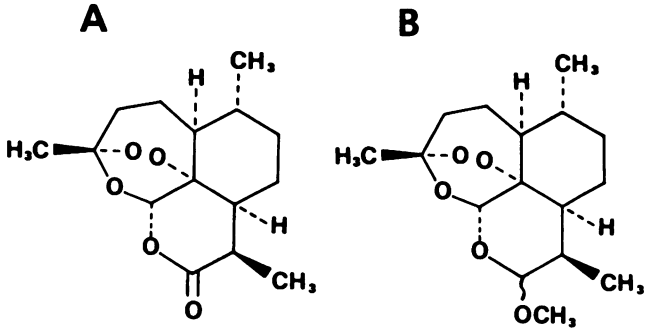

FIG. 1. Chemical structures of qinghaosu (A) and artemether (B).

Geographic Medicine, Case Western Reserve School of Medicine, Cleveland, Ohio.

In vitro studies. RPMI 1640 supplemented with 20% heat-inactivated fetal bovine serum (M. A. Bioproducts), 300 U of penicillin, 300 U of streptomycin, and 0.75 μg of amphotericin B (Fungizone) per ml was used to maintain schistosomes for in vitro studies. Three to five worm pairs obtained by perfusion from infected DBA/2NCr mice (infected with 150 or 300 cercariae for 8 to 15 weeks) were washed with ice-cold HBSS three times and then transferred to 1.8 to 1.95 ml of the above-described medium contained in 35- by 10-mm plastic petri dishes (Falcon; Becton Dickinson Labware, Lincoln Park, N.J.). The parasites were kept in an incubator at 37°C in an atmosphere of 95% air–5% CO_2. At 1 to 2 h after incubation, 50 to 200 μl of artemether solution (60% PEG 400, 40% HBSS) was added to the medium, yielding final drug concentrations of 25, 50, and 100 μg/ml. Final PEG 400 concentrations were 1.5, 3.0, and 6.0%, respectively. Medium containing no PEG 400 as well as medium containing the above-mentioned final PEG 400 concentrations served as controls. An inverted microscope (AO Scientific Instruments Microstar) was used to observe the appearance and motor activity of the parasites over a 5-day period of observation (15).

Measurement of worm size. Carmine-stained worms were mounted on microscopic slides with Permount (Fisher Scientific) and a cover slip. Worm measurements were obtained with an AO Spencer light microscope (American Optical Corp., Buffalo, N.Y.) equipped with an ocular micrometer. An average of 7 to 10 worms in each group was measured (see Table 1).

Hepatic shift. CF1 female mice infected with 250 *S. mansoni* cercariae (Puerto Rican strain) by tail immersion were treated orally with artemether at a single dose of 300 mg/kg 6 to 7 weeks after infection. Groups of six mice were killed at different times within 2 weeks after treatment. Schistosomes located in the mesenteric veins and liver were obtained by perfusion by the method of Yolles et al. (16). The percentage of schistosomes distributed in the above-mentioned locations was then calculated.

Therapeutic effect. C3H mice were individually infected with either 90 or 150 cercariae by subcutaneous injection. Seven weeks after infection, mice were treated orally with artemether at various doses (see Table 2). The animals were sacrificed 5 weeks after treatment, and the mesenteric veins and liver were perfused and then torn apart to recover the worms. Control mice were divided into two groups. One group of mice was sacrificed 7 weeks after infection to determine the average worm number. The second group of infected control mice was given a single dose of PEG 400 alone (0.4 ml/20 g of body weight per day) daily for 1 to 3 days to assess the effect of PEG 400 alone as well as to determine the survival time of untreated, infected mice. The therapeutic effect of the drug was quantitated by measuring the worm reduction rate.

Histopathologic studies. Infected mice were treated daily with a single oral dose of artemether (300 mg/kg) for 2 days and autopsied at different times within 8 weeks after treatment. Schistosomes lodged in the mesenteric veins and liver were obtained by perfusion with ice-cold HBSS and then fixed in 70% alcohol. These worms were stained with carmine for morphological studies and measurement of worm body, testis, and ovary size. A total of 16 groups containing 10 male and 10 female worms were studied at each time (see Table 1).

Effect of artemether on different stages of *S. mansoni*. C3H mice infected with 100 or 150 cercariae by subcutaneous injection were divided randomly into groups of 10 to 12 animals. In two different experiments (see Table 3), individual groups of mice were treated on days 1, 3, 7, 14, 21, 28, 42, and 56 after infection. Artemether dissolved in PEG 400 was given orally at a daily dose of 300 mg/kg for 2 days commencing on the days listed above. Mice in each experimental group were autopsied 5 to 6 weeks after treatment to determine the number of residual worms. The susceptibility of worms to artemether at different developmental stages was determined by counting residual worms at autopsy and calculating the worm reduction rate of each group versus untreated controls.

Statistics. The one-tailed Student *t* test was used to compare groups.

RESULTS

In vitro effect of artemether on adult schistosomes. No effect on adult worms was noted when the lowest concentrations of PEG 400 (1.5%) and artemether (25 μg/ml) were studied. However, when 10 worm pairs were exposed to a medium containing 3 or 6% PEG 400 alone, the worms contracted immediately and showed decreased motor activity. One to two hours later, all worms were no longer contracted but exhibited unnatural movements. When 12 worm pairs were exposed to a medium containing artemether at a concentration of 50 or 100 μg/ml dissolved in PEG 400 (final PEG 400 concentrations, 3 and 6%, respectively), the appearance of the worms in the 50-μg/ml artemether group was similar to the appearance of the worms in the corresponding PEG 400 control group. In the 100-μg/ml artemether group the worms appeared more elongated and less active, and a few male and female worms had some tegumental damage. Male and female worms in the 100-μg/ml artemether group began to die 1 day after incubation. By day 5, 11 of 12 male and 10 of 12 female worms were dead. Similar effects were not seen in the 20 worm pairs maintained in drug-free PEG 400-containing medium over the same period of incubation.

Morphological alterations in schistosomes induced by artemether. Infected mice were treated orally with artemether at a daily dose of 300 mg/kg per day for 2 days. Within 1 day after treatment, both male and female worms appeared to be decreased in length (Table 1). Three days after treatment, the width of the worms was also reduced. Pairs of worms appeared to shrink in length and become thinner within 3 to 21 days after treatment when compared with control worms ($P < 0.05$) (Table 1). By 28 days after treatment, the width of both male and female worms had returned to normal. How-

TABLE 1. Changes in the size of the worm body, testis, and ovary of *S. mansoni* after treatment of infected C3H mice with oral artemether at a dose of 300 mg/kg per day for 2 days

Time after treatment (day)	Worm sex[a]	No. of worms examined	Size (length × width in μm)[b] of:		
			Worm body	Testis	Ovary
None (control)	M	10	732 ± 126 × 31 ± 3	8.8 ± 1.4 × 4.9 ± 1.1	
	F	10	841 ± 112 × 13 ± 2		33 ± 4 × 9 ± 1
1	M	10	572 ± 62 × 29 ± 4	6.2 ± 0.8 × 3.6 ± 0.7	
	F	7	596 ± 78 × 14 ± 2		22 ± 5 × 8 ± 2
3	M	10	633 ± 92 × 25 ± 3	6.0 ± 0.9 × 3.5 ± 0.9	
	F	9	493 ± 116 × 11 ± 2		23 ± 5 × 6 ± 1
7	M	10	512 ± 102 × 25 ± 3	5.6 ± 1.2 × 3.1 ± 0.5	
	F	10	484 ± 89 × 12 ± 2		17 ± 6 × 6 ± 1
14	M	10	509 ± 64 × 26 ± 3	5.6 ± 0.8 × 3.2 ± 0.7	
	F	7	445 ± 84 × 11 ± 1		16 ± 5 × 4 ± 1
21	M	10	562 ± 88 × 26 ± 3	6.8 ± 1.5 × 3.6 ± 0.5	
	F	10	542 ± 91 × 13 ± 2		26 ± 7 × 8 ± 2
28	M	10	657 ± 59 × 32 ± 3	9.2 ± 1.0 × 4.8 ± 1.7	
	F	10	646 ± 95 × 14 ± 1		32 ± 5 × 9 ± 2
56	M	7	828 ± 140 × 29 ± 2	7.5 ± 1.0 × 6.3 ± 0.8	
	F	9	950 ± 239 × 13 ± 2		42 ± 11 × 7 ± 2

[a] M, Male; F, female.
[b] Mean ± standard deviation.

ever, the length of female worms but not that of male worms remained shorter than that of a corresponding control group of worms ($P < 0.05$) (Table 1). Fifty-six days after treatment, the dimensions of male and female worms were similar to those of control worms (Table 1).

Organ-specific damage was assessed by light microscopy with carmine-stained worms. The testes of male worms and the ovaries of female worms showed degeneration and atrophy which resulted in a significant decrease in their size within 14 days after treatment (Table 1 and Fig. 2A to D). By 28 days after treatment, the size of the testes and ovaries had returned to normal. In a few female worms an almost complete disappearance of the ovary was noted as early as 3 days after treatment, as only a trace of the gland could be detected. Seven days later, the majority of the female worms had only a trace of an ovary. Other morphological alterations included severe degeneration of the vitelline glands in female worms; extension, distortion, and depigmentation of the intestine in both male and female worms; and evidence of attachment of host cells to the surfaces of a few female worms.

Hepatic shift of *S. mansoni* induced by artemether. Six to seven weeks after female CF1 mice were infected with *S. mansoni*, 91.2% of male and female worms were located in the mesenteric veins, while 6.5% of the worms were found in the liver (Fig. 3). When infected mice were treated orally with a single dose of artemeter (300 mg/kg), a shift of the worms towards the liver was observed during the first 8 h after treatment (Fig. 3). The number of worms appearing in the liver gradually increased from day 1 after treatment, reaching a maximum (99.3%) by day 3. Worms remained in the liver until approximately day 7 and then gradually began to return to the mesenteric veins. However, 75.9% of the worms were still detected in the liver on day 14 (Fig. 3).

Therapeutic effect of artemether. Different therapeutic doses for artemether treatment were used (Table 2). Although the average number of worms in each treated group was significantly lower than that in the corresponding control group ($P < 0.01$), the rate of worm reduction only ranged from 23.8 to 41.2%. Two groups of 10 mice each were used as survival controls. Infected mice treated orally with PEG 400 alone at a daily dose of 0.4 ml/20 g for 1 to 3 days began to die 9 to 10 weeks after infection whether infected with 90 or 150 cercariae. By 11 to 12 weeks over 50% of the mice infected with 90 cercariae and 90% of the mice infected with 150 cercariae were dead. In mice treated with higher doses of artemether (400 or 500 mg/kg), 13.3% in the 400-mg/kg treatment group and 33.3% in the 500-mg/kg treatment group were dead within 1 week after treatment. No further deaths of treated animals at the higher doses were observed during the remaining 3 to 4 weeks of the study.

Effect of artemether on different developmental stages of *S. mansoni*. Table 3 shows the efficacy of oral artemether after a 2-day treatment course given to groups of mice at different times after infection. In two separate experiments, artemether treatment resulted in no significant reduction of worm burden in the groups treated 1 and 3 days after infection as compared with untreated control animals ($P > 0.05$) (Table 3). Seven-day-old parasites exhibited a moderate susceptibility to artemether which resulted in a significant decrease in average worm number, i.e., a 70% worm reduction rate as compared with that in the control group ($P < 0.01$). Fourteen- and twenty-one-day-old parasites were the most susceptible to artemether; worm reduction rates of 8.27 to 98.3% and 85.4 to 89.9%, respectively, were noted. In addition, 11 of 23 mice in the day-14 group and 7 of 23 mice in the day-21 group were free of female worms. The susceptibility of 28-, 35-, 42-, and 56-day-old parasites was markedly lower, as evidenced by a lower reduction of worm

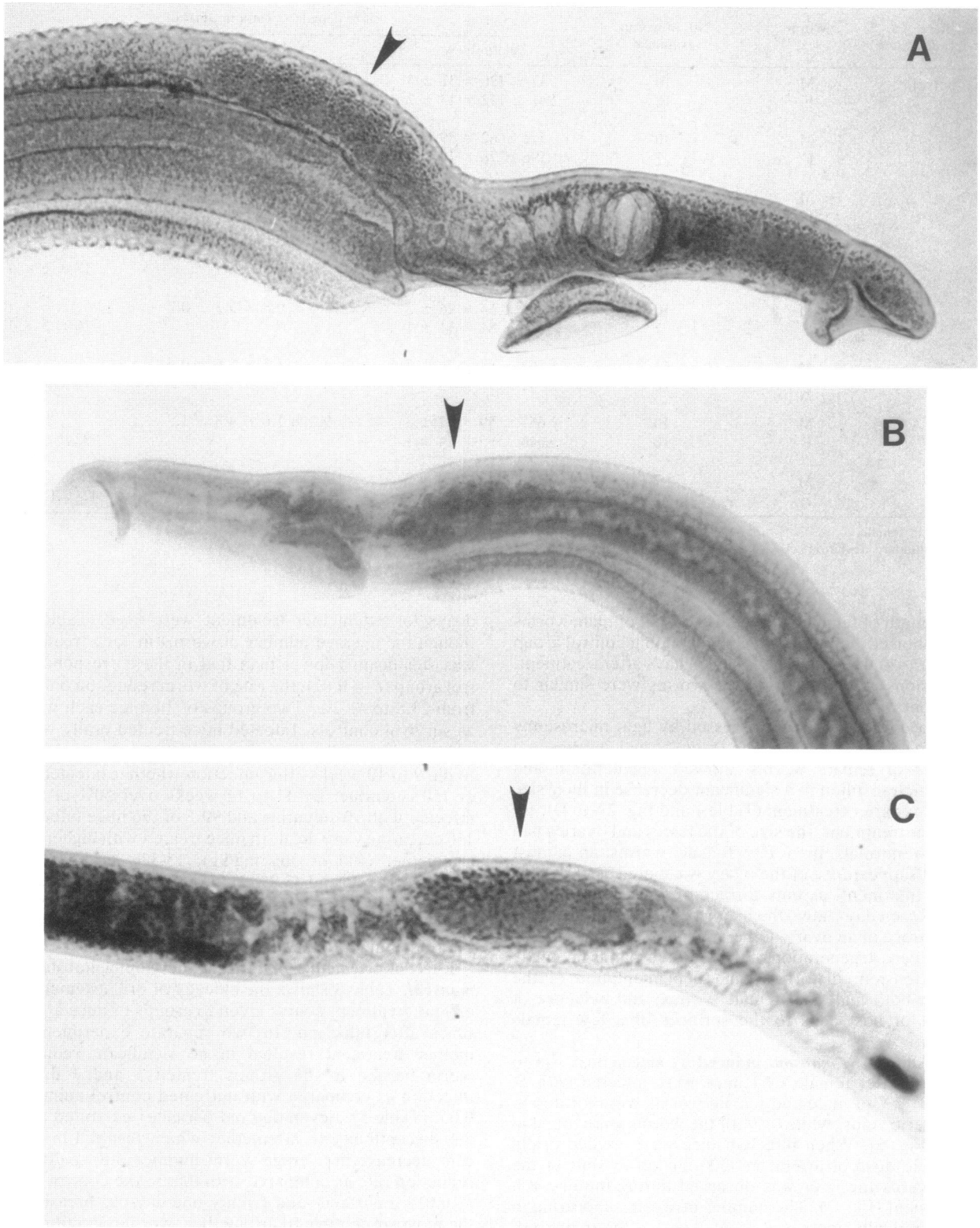

FIG. 2. Effect of artemether (300 mg/kg per day for 2 days) on *S. mansoni* testes and ovaries (see Materials and Methods for details). (A) Untreated control male *S. mansoni* worm. (B) Treated male *S. mansoni* worm 3 days after artemether treatment, showing atrophy of testes. (C) Untreated control female *S. mansoni* worm. (D) Treated female *S. mansoni* worm 7 days after artemether treatment, showing atrophy of ovaries. Magnification for all photomicrographs, ×200. Arrowheads indicate testes or ovaries.

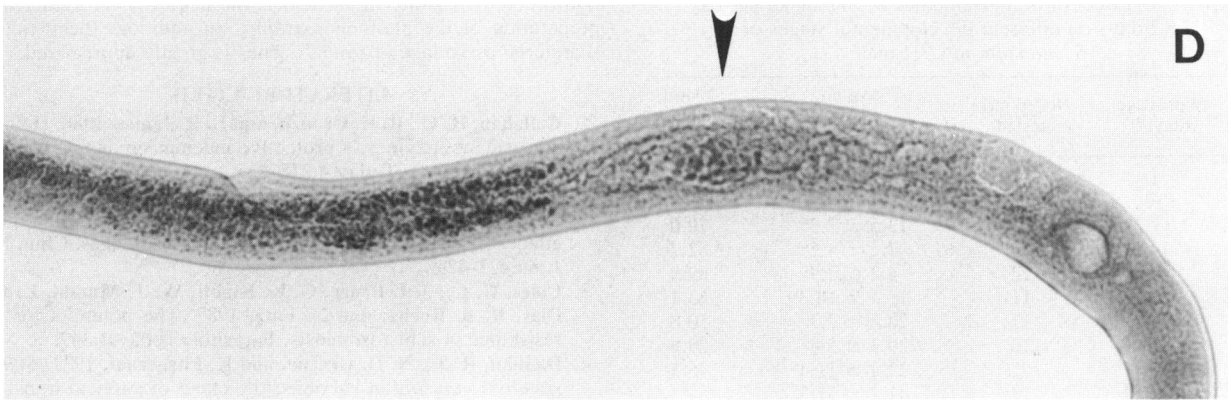

FIG. 2—*Continued*

burden as compared with that in groups treated 14 or 21 days after infection (Table 3).

DISCUSSION

It has been reported that artemether is effective against adult *S. japonicum* (10). When mice infected with adult *S. japonicum* were treated orally with artemether at a total dose of 400 to 800 mg/kg over a 1- to 4-day course of therapy, worm reduction rates of 55.3 to 79.9% were reported (10). In our studies of mice infected with *S. mansoni*, doses as high as 1,200 mg/kg over a 3- to 6-day course resulted in adult worm reduction rates of only 23.8 to 39.1% (Table 2). Thus, the therapeutic effect of artemether in *S. mansoni*-infected mice is not dose dependent and is lower against adult *S. mansoni* than against adult *S. japonicum*. This observation is particularly interesting because adult *S. mansoni* worms in general are more susceptible to antischistosomal drugs than are adult *S. japonicum* worms (5). Similar differences between species were also seen when stage-specific effects of artemether were studied. In *S. mansoni* infections, 14- and 21-day-old worms were more susceptible to artemether, while 7-, 28-, and 35-day-old or older adult worms exhibited less susceptibility to the drug. In *S. japonicum* infections, 7-day-old parasites but not 14- or 21-day-old parasites were reported to be particularly susceptible to artemether (10), indicating that these two species of schistosomes appear to be affected differently by the drug.

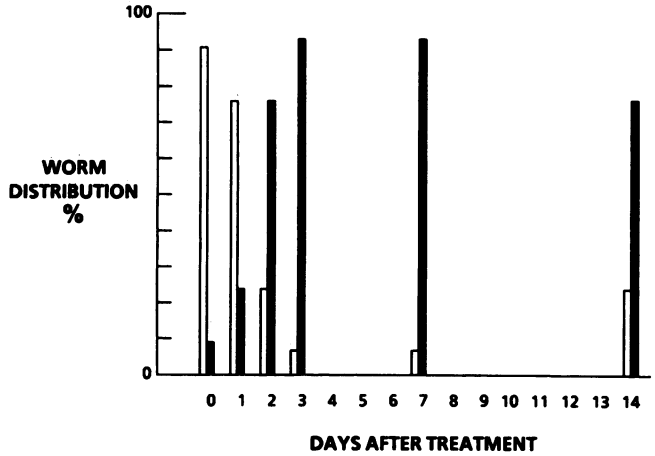

FIG. 3. Distribution of adult worms in mesenteric veins (□) and liver (■) after a single oral dose of artemether (300 mg/kg).

Artemether has a slow therapeutic effect on adult *S. mansoni*. This effect is characterized by a shift of the worms to the liver within 8 h after drug administration, an apparent reduction in size of both male and female worms, and degeneration of reproductive organs. Although all of these drug-induced alterations may last for 3 to 4 weeks after the cessation of treatment (Table 1), only a small percentage of adult worms are killed, as determined by efficacy studies. Analysis of the effect of artemether on adult worms in vitro indicates that the drug has a lethal effect only when worms are maintained in a medium containing 100 μg of artemether per ml and 6% PEG 400. In vitro, therefore, artemether has little direct effect on adult *S. mansoni*. Similar studies have not been reported with *S. japonicum*. Whether adult worms that survive treatment regain functional integrity and resume egg laying is not known.

Artemether, a semisynthetic derivative of the naturally occurring sesquinterpene lactone qinghaosu, is characterized by having an endoperoxide bridge within the molecule (Fig. 1B). Whether the endoperoxide bridge is essential for antischistosomal activity, as it is for antimalarial activity (8), is not known. In *Plasmodium falciparum*, qinghaosu and its derivatives appear to inhibit protein synthesis more effectively (6) than nucleic acid synthesis (11). More recent data suggest that quinghaosu-induced oxidant stress may be more important (8). Under in vivo conditions, the metabolism of the endoperoxide linkage of qinghaosu and its derivatives by malarial or schistosomal parasites may generate oxygen radicals which are detrimental to parasite macromolecules.

TABLE 2. Efficacy of oral artemether in C3H mice infected with adult *S. mansoni*

Expt	Dose (mg/kg per day × no. of doses)	No. of mice treated	No. of mice autopsied	Avg no. of worms (mean ± SD)	Worm reduction rate (%)
1	200 × 4	18	18	14.3 ± 5.7[a]	41.2
	300 × 2	19	19	16.9 ± 5.2[a]	30.5
	None (control)		15	24.3 ± 6.4	
2	200 × 6	16	15	21.0 ± 9.5[a]	39.1
	300 × 3	15	15	24.8 ± 12.8[a]	28.1
	400 × 3	15	13	26.3 ± 10.7[a]	23.8
	500 × 2	18	12	22.3 ± 14.0[a]	35.4
	None (control)		10	34.5 ± 7.8	

[a] $P < 0.01$ as compared with the corresponding control group.

TABLE 3. Effect of oral artemether at 300 mg/kg per day for 2 days on different developmental stages of *S. mansoni* in C3H mice

Expt	Worm age (days)[a]	No. of mice tested	Avg no. of worms (mean ± SD)	Worm reduction rate (%)
1	1	12	38.7 ± 16.7[b]	7.2
	3	12	37.2 ± 8.3[b]	10.8
	7	13	12.5 ± 7.3[c]	70.0
	14	13	7.2 ± 4.5[c]	82.7
	21	12	4.2 ± 2.9[c]	89.9
	28	11	15.4 ± 10.3[c]	63.1
	35	11	20.5 ± 7.7[c]	50.8
	42	10	29.3 ± 5.4[c]	29.8
	56	7	22.4 ± 11.4[c]	46.3
	Control	14	41.7 ± 11.3	
2	1	10	22.8 ± 9.2[b]	5.0
	3	10	24.8 ± 8.9[b]	0
	7	10	7.3 ± 4.1[c]	69.6
	14	10	0.4 ± 0.7[c]	98.3
	21	11	3.5 ± 0.8[c]	85.4
	28	10	8.9 ± 5.0[c]	62.9
	35	10	14.7 ± 5.9[c]	48.7
	42	11	14.8 ± 8.8[c]	48.3
	Control	10	24.0 ± 7.3	

[a] First day of 2-day treatment course after infection.
[b] $P > 0.05$ as compared with the corresponding control group.
[c] $P < 0.01$ as compared with the corresponding control group.

S. mansoni, known to possess a nitroreductase, is postulated to be killed by the antischistosomal drug niridazole through the generation of reactive drug intermediates which bind to parasite macromolecules (12). Since our studies suggest that the onset of artemether action is slow, metabolism of the drug by the parasite, not a direct toxic effect of the parent compound, may be more important. Why young liver stages of *S. mansoni* are more susceptible to qinghaosu than adult stages is unclear. Perhaps young liver stages do not possess adequate antioxidant protective mechanisms (1). Likewise, the potential usefulness of qinghaosu and its derivatives as antischistosomal agents is unclear, as demonstrable histopathologic changes in mouse liver (14) as well as dog intestine, kidney, liver, and adrenal glands (10) may limit their clinical usefulness in humans. However, further study of this interesting class of potential antiparasitic agents is warranted, as new derivatives may prove to be less toxic and more efficacious.

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LITERATURE CITED

1. **Callahan, H. C., R. K. Crouch, and E. R. James.** 1988. Helminth antioxidant enzymes: a protective mechanism against host oxidants? Parasitol. Today **4**:218–225.
2. **Cheng, D. G., L. F. Fu, P. P. Shao, F. Z. Wu, Z. Z. Fang, H. Su, Z. S. Ren, and S. L. Sen.** 1980. Studies on chemotherapeutic effect of qinghaosu in experimental schistosomiasis. Chin. Med. J. **60**:422–425.
3. **Coles, G. C., J. I. Bruce, G. K. Kinoti, W. T. Mutaki, L. C. S. Dias, R. S. Rocha, and N. Fatz.** 1987. The potential for drug resistance in schistosomiasis. Parasitol. Today **3**:349.
4. **Damian, R. T., N. D. Greene, and K. Fitzgerald.** 1972. *Schistosomiasis mansoni* in baboons: the effect of surgical transfer of adult *Schistosoma mansoni* upon subsequent challenge infection. Am. J. Trop. Med. Hyg. **21**:951–958.
5. **Davis, A.** 1986. Recent advances in schistosomiasis. Q. J. Med. **58**:95–110.
6. **Gu, H. M., D. C. Warhurst, and W. Peters.** 1983. Rapid action of *qinghaosu* and related drugs on incorporation of (3H) isoleucine by *Plasmodium falciparum in vitro*. Biochem. Pharmacol. **32**:2463–2466.
7. **Klayman, D. L.** 1985. Qinghaosu (artemisinin): an antimalarial drug from China. Science **228**:1049–1055.
8. **Krungkrai, S. R., and Y. Yathavong.** 1987. The antimalarial action on *Plasmodium falciparum* of qinghaosu and artesunate in combination with agents which modulate oxidant stress. Trans. R. Soc. Trop. Med. Hyg. **81**:710–714.
9. **Le, W. J., J. Q. You, and J. Y. Mei.** 1983. Chemotherapeutic effect of artesunate in experimental schistosomiasis. Acta Pharm. Sin. **18**:619–621.
10. **Le, W. J., J. Q. You, Y. Q. Yang, J. Y. Mei, H. F. Guo, H. Z. Yang, and W. W. Zhang.** 1982. Studies on the efficacy of artemether in experimental schistosomiasis. Acta Pharm. Sin. **17**:187–193.
11. **Li, Z. L., H. M. Gu, D. C. Warhurst, and W. Peters.** 1983. Effects of qinghaosu and related compounds on incorporation of [G-3H] hypoxanthine by *Plasmodium falciparum in vitro*. Trans. R. Soc. Trop. Med. Hyg. **77**:522–523.
12. **Tracy, J. W., B. A. Catto, and L. T. Webster, Jr.** 1982. Reductive metabolism of niridazole by adult schistosomes: correlation with covalent drug binding to parasite macromolecules. Mol. Pharmacol. **24**:295–299.
13. **World Health Organization.** 1981. The development of qinghaosu and its derivatives as antimalaria agents. Fourth meeting of scientific working group on the chemotherapy of malaria. Beijing, People's Republic of China, 6 to 10 October 1981. World Health Organization, Geneva.
14. **Wu, L. J., H. Z. Zang, and Y. Q. Yong.** 1983. Histological and histochemical changes of *Schistosoma japonicum* and host liver caused by artemether. Acta Pharm. Sin. **18**:7–14.
15. **Xiao, S. H., B. A. Catto, and L. T. Webster, Jr.** 1985. Effects of praziquantel on different developmental stages of *Schistosoma mansoni in vitro* and *in vivo*. J. Infect. Dis. **151**:1130–1137.
16. **Yolles, T. K., D. V. Moore, D. L. DeGuisti, C. A. Ripsom, and M. S. Meleney.** 1947. A technique for the perfusion of laboratory animals for the recovery of schistosomes. J. Parasitol. **33**: 419–426.