EXPERIMENTAL STUDIES ON EARLY TREATMENT OF SCHISTOSOMAL INFECTION WITH ARTEMETHER

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Abstract. An early treatment with artemether given in appropriate regimens was tested in mice, rabbits and dogs for prevention purposes. Artemether was administered intragastrically (ig) to the hosts on day 7 after infection with *Schistosoma japonicum* cercariae at a single dose, and the same dose of artemether was repeated every 1 or 2 weeks for 2-4 times. As a result, most of the female worms were killed before their oviposition with female worm reduction rates of 90-100%, resulting in protection of the host from damage induced by schistosome eggs. When rabbits were treated *ig* with artemether 10 mg kg^{-1} on day 7 after infection, followed by repeated dosing every week for 4 times, some parameters related to acute schistosomiasis, such as temperature, eosinophil count and eggs in the feces were negative, and low specific antigen and antibody levels in serum were seen. Further study showed that the appropriate regimens of Artemether were also effective in early treatment of reinfection with cercariae. When rabbits infected with 48-52 cercariae once every other day for 5 times were treated *ig* with artemether 15 mg kg^{-1}, followed by repeated dosing every 1 or 2 week for 2-3 times, the female worm reduction rates were 92.1-98.4%. Histopathological examination of the livers showed that the above-mentioned early treatment with Artemether exhibited a promising protective effect on dogs and rabbits. The major features included normal appearance of the liver resembling those of uninfected dogs and rabbits; few or no dispersed miliary egg tubercles appeared on the surface of the liver; the structure of the hepatic lobules was normal with normal arrangement of the liver bundles; few or no eggs appeared in the portal vein area and there was apparent diminution of total egg granuloma, comprising inflammatory, fibrous or scarred egg granuloma. On the basis of above-mentioned results, early treatment with Artemether could be recommended for field trial for controlling acute schistosomiasis, reducing infection rate and intensity of infection.

INTRODUCTION

In some endemic areas, especially in those of lake and mountain regions, the morbidity of acute schistosomiasis has increased drastically in recent years. Therefore, it is an urgent need to develop some ways to protect the people from infection with schistosome cercariae. A great deal of attention has been devoted to schistosomicidal drugs, with oral drugs used for prophylactic purposes being considered as one of the effective measures. For this study artemether (β-methyl ether artemisinin) was selected. Artemether is a derivative of artemisinin, which is not only effective against malaria (Gu *et al*, 1981), but also shows an effect on schistosomes (Le *et al*, 1982; Xiao and Catto, 1989; You *et al*, 1992). Further studies indicated that Artemether was more effective against 7-day-old schistosomules, less effective against 35-day-old adult schistosomes and much less effective against other developmental stages of schistosomes in mice (Xiao and Catto, 1989). Therefore, it is suggested that when Artemether is given to the host as an appropriate regimen after infection with schistosome cercariae, most or even all of the female worms will be killed before their oviposition. As a consequence, the host would be protected from schistosome egg infestation, which results in controlling acute schistosomiasis and reducing the infection rate and intensity of infection. This paper reports the effect of early treatment with artemether in mice, rabbits and dogs after infection with schistosome cercariae.

MATERIALS AND METHODS

Parasites

Schistosoma japonicum cercariae (Anhui isolate), released from infected snail *Oncomelania hupensis*, was provided by our Institute.

Animals

Male and female Kunming strain mice weighing 18-24 g were maintained on a rodent feed and water

ad lib in the animal care facilities of the Institute. Male and female New Zealand strain rabbits weighing 2.2-2.5 kg were also provided by the Institute. Male and female hybrid dogs with 6-9 kg body weight bought from market were used.

Drug

Artemether and its capsule (ArtC) were the products of Kunming Pharmaceutical Factory. Artemether was suspended in 1% tragacanth at concentrations of 10-30 mg ml^{-1}.

Infection and therapy

Mice, rabbits and dogs were infected each with 48-52, 198-202 and 198-202 cercariae *via* shaved abdominal skin. Appropriate regimens of Art used in early treatment were determined in mice. Generally, mice were first treated intragastrically (*ig*) with artemether in a single dose at various durations after infection, and repeated the dosing once at 1-3 week intervals for several times. In rabbits and dogs, appropriate regimens of artemether identified in mice were used, *ie* artemether or ArtC was given *ig* on day 7 after infection, followed by repeated dosing every 1 or 2 weeks for 2-4 times. The appropriate regimens of artemether were also used in rabbits reinfected with cercariae. In these experiments, rabbits were infected with 48-52 cercariae once every week for 6 times, or once every other day for 5 times. Artemether was given initially on day 7 after the first infection, followed by repeated dosing once every 1 or 2 weeks for 4-9 times.

Evaluation of efficacy

The above-mentioned mice, rabbits and dogs were killed 4-5 weeks after the last medication for collection of residual worms. The efficacy was represented by the average number of total and female worms, and the number of animals without female worm. The liver was evaluated as -: normal, fresh red color without egg tubercle; ± : the liver in fresh red color with few fine egg tubercles; +: the liver in light red color with some egg tubercles; ++: the liver in markedly red color with numerous small egg tubercles; +++: the liver in marked grey color with numerous large egg tubercles.

Effect on egg production of female worms

Mice infected with 48-52 schistosome cercariae were treated *ig* with at a single dose of 300 $mg.kg^{-1}$ on day 7 after infection. Groups of 2-3 mice were killed 2 and 3 weeks after treatment and the residual worms harbored in the host were perfused out with ice cold Hanks' balanced salt solution from the liver and mesenteric veins. The worms were fixed in 70% ethanol and stained with acid carmine. The reproductive organs of the female worms were examined under a light microscope and the eggs in the uterus were counted. Untreated 21-day-old and 28-day-old female worms were examined as control.

Determination of some parameters related to acute schistosomiasis

Six rabbits each infected with 198-202 cercariae were treated ig with Art 10 mg kg^{-1} on day 7 after infection, followed by repeated dosing once every week for 4 times. Four to 9 weeks after infection, the following parameters related to acute schistosomiasis (Yue *et al*, 1980) were determined weekly, *ie* rectal temperature, eosinophil count in blood withdrawn from the ear vein, and schistosome eggs in feces examined by hatching and light microscope. The above-mentioned parameters were also determined in 5 untreated control rabbits. Meantime, serum was examined weekly by circumoval precipitin test (COPT) (Anonymous, 1979) and dot-ELISA using monoclonal antibody (Yan *et al*, 1990) (n = 4 in each group) for schistosomal antibody and circulating antigen, respectively, during 5th-9th weeks after infection.

Histopathological observation

In autopsy of the above-mentioned groups of dogs, groups of rabbits reinfected with cercariae once every other day for 5 times, and corresponding control groups, the livers were removed and fixed in 10% neutral formalin. Three to five small pieces were cut off from each of the fixed liver, and tissue sections of each liver piece were prepared and stained with HE according to the routine method of pathological examination. In each group, 25 different sections with 0.5 cm^2 area were selected randomly. The number of schistosome egg granuloma in each section with 0.5 cm^2 was counted and the egg detected

inside the granuloma was estimated according to its morphological characteristics and compared with the control group.

Statistical methods

All data obtained from the experiments were analyzed with Student's *t*-test.

RESULTS

Appropriate regimens of artemether determined in mice

Mice treated *ig* with artemether 300 mg kg^{-1} on the day of infection (day 0) resulted in no apparent effect. When the same dose of the drug was given repeatedly 1-3 week intervals 4-5 times after the first dosing, the average numbers of total and female worms of each group were significantly less than those of the control and exhibited no apparent differences among these groups (Table 1). With the regimens used, none of the mice was free from female worm and only the group treated with Artemether at 1 week intervals showed mild change of the liver.

When mice were first treated ig with Artemether 300 mg kg^{-1} on day 7 after infection, followed by repeated dosing at 1, 2 or 3 week intervals for 1-4 times, the total and female worm reduction rates of these groups were higher than those of groups treated initially on day 0. Among these regimens, the most promising one was that the drug was administered every week for 5 times beginning from the first dosing on day 7 after infection, which resulted in about or over 90% of female worm reduction rate and part of the mice without female worms (Table 1).

In another experiment, mice treated *ig* with artemether 200 or 300 mg kg^{-1} showed similar average numbers of total and female worms in these 2 groups. When artemether 200 or 300 mg kg^{-1} was given to mice on day 7 after infection, and the same dose was administered repeatedly once every week for 4-5 times, the average numbers of total and female worm were less than of corresponding group treated only

Table 1

Mice infected with *Schistosoma japonicum* cercariae and treated intragastrically with artemether 300 mg kg^{-1} given once at different intervals after infection.

Group	Time of administration (d)	Mice without ♀ worm	Total worm	WRR (%)	Female worm	FWRR (%)	Liver alteration
1	Control	0/20	32.7 ± 8.7	-	15.9 ± 4.7	-	+ − + +
2	d_0	0/17	32.5 ± 9.1[c]	0.6	15.5 ± 4.8[c]	2.5	+ − + +
3	$d_0d_{14}d_{21}d_{28}d_{35}$	0/10	12.1 ± 8.1[c]	63.0	5.6 ± 4.2[b]	64.8	− − +
4	$d_0d_{14}d_{28}d_{42}$	1/15	16.4 ± 6.4[c]	49.8	7.1 ± 3.1[c]	55.3	± − + +
5	$d_0d_{21}d_{28}d_{35}d_{42}$	1/12	14.4 ± 8.0[c]	55.9	5.3 ± 2.6[c]	66.6	± − + +
6	$d_0d_{21}d_{35}d_{49}$	0/6	16.3 ± 6.0[c]	50.0	6.0 ± 1.7[c]	62.2	+ − + +
7	d_7	1/20	10.7 ± 4.2[c]	67.3	4.7 ± 2.0[c]	70.4	+ − + +
8	d_7d_{14}	0/13	6.9 ± 3.0[c]	78.9	3.0 ± 1.4[a]	81.1	± − +
9	d_7d_{21}	1/12	7.4 ± 4.7[c]	77.4	3.4 ± 2.3[a]	78.6	± − + +
10	$d_7d_{14}d_{21}d_{28}d_{35}$	1/13	4.2 ± 2.8	87.1	2.2 ± 1.2	86.2	− − ±
11	$d_7d_{21}d_{35}d_{49}$	1/13	7.0 ± 3.9[c]	78.6	2.7 ± 1.3[a]	83.0	− − +
12	$d_7d_{28}d_{49}$	3/11	7.5 ± 4.0[c]	77.0	3.3 ± 1.1[a]	79.2	± − +
13	Control	0/20	31.5 ± 8.9	-	14.1 ± 4.6	-	+ − + +
14	d_7	2/19	9.5 ± 4.4[c]	69.8	3.2 ± 2.0[c]	77.3	− − +
15	$d_7d_{14}d_{21}d_{28}d_{35}$	9/17	2.0 ± 2.3	93.7	0.9 ± 1.1	93.6	− − ±
16	$d_7d_{21}d_{35}$	2/15	6.6 ± 4.0[c]	79.0	3.0 ± 2.0[c]	78.7	± − +
17	$d_0d_{14}d_{28}d_{42}$	0/17	18.9 ± 6.8[c]	40.0	7.9 ± 3.3[c]	44.0	+ − + +

WRR = worm reduction rate; FWRR = female worm reduction rate
$x \pm s$; [a]$p = 0.05$; [b]$p > 0.05$; [c]$p < 0.01$ *vs* group 10 or group 15

once with the same dose of artemether. The average numbers of total and female worm of 300 mg kg^{-1} group were somewhat lower than those of 200 mg kg^{-1} group, but the differences was not significant.

However, in 300 mg kg^{-1} group the number of mice without female worms and cured mice were slightly higher than that in 200 mg kg^{-1} group. After early treatment with artemether, 1/4-1/3 mice showed normal appearance of livers similar to that of uninfected mice, while other mice showed some dispersed egg tubercles on the surface of their livers (Table 2).

Effect on egg production of female worms

Two weeks (*ie* 21 days after infection) after mice were treated ig with artemether 300 mg kg^{-1} on day 7 after infection, 23 residual female worms were examined and only 4 of them showed retarded ovary and sparse vitelline glands. Neither apparent ovary and vitelline glands nor any eggs were detected in the remaining female worms. In the control group, all 10 female worms examined showed sparse vitelline glands and ovaries with different sizes, although, no eggs were found in their uterus. Three weeks after treatment (*ie* 28 days after infection) 6 out of 12 residual female worms exhibited apparent development of ovary and vitelline glands, and 1-21 eggs were found in their uteri with an average number of 7 ± 7/per female worm. The remaining female worms showed poor development of reproductive organs and no egg was detected in their uteri. In the control group, 19 female worms examined showed good development of their reproductive organs and 24-170 eggs were found in their uteri with an average number of 84 ± 47/per female worm. Thus, the egg reduction rate in the Artemether group was 91.7%.

Early treatment in rabbits

Single infection with cercariae: Rabbits treated ig with artemether 30 mg kg^{-1} on day 7 after infection resulted in decrease of average numbers of total and female worm with reduction rate of over 90% as compared with the control (groups 25 and 26). In rabbits treated ig with Artemether at the same dose once every 1 or 2 weeks following the first dose for 3-4 times (groups 27 and 28), all 7 rabbits in group 27 were cured. In group 28, the average numbers of total and female worms were less than those in group 26, and 3/7 rabbits were cured. Only 1-2 female worms were found in each of the other 4 rabbits. In the 2 groups, the rabbit livers appeared normal (Table 3).

When the dose of artemether was decreased to 10 or 15 mg.kg^{-1} and given to the infected rabbits at above-mentioned regimens (groups 32 and 33), the efficacies were similar to that treated with 30 mg kg^{-1} (group 34), but much higher than those treated

Table 2

Mice infected with *Schistosoma japonicum* cercariae and treated intragastrically with artemether at various doses given once at different intervals after infection.

Group	Time of administration (d)	Dose (mg kg^{-1})	Mice without ♀ worm	Total worm	WRR (%)	Female worm	FWRR (%)	Liver alteration
18	Control	0	0/13	28.1 ± 11.5	-	13.9 ± 4.3	-	++
19	d_7	200	1/14	10.0 ± 4.7[a]	64.4	3.7 ± 2.2[a]	73.3	--++
20	d_7	300	0/10	8.2 ± 4.6	70.8	3.6 ± 2.2	74.1	--++
21	$d_7d_{14}d_{21}d_{28}$	200	6/13	3.1 ± 3.0[a]	89.0	1.2 ± 1.3[a]	91.4	--+
22	$d_7d_{14}d_{21}d_{28}$	300	7/14	2.4 ± 2.3	91.5	0.9 ± 1.1	93.5	--+
23	$d_7d_{14}d_{21}d_{28}d_{35}$	200	2/13	4.5 ± 3.2[a]	84.0	1.9 ± 1.3[a]	86.3	--+
24	$d_7d_{14}d_{21}d_{28}d_{35}$	300	5/15	3.3 ± 3.1	88.3	1.4 ± 1.4	89.9	--+

WRR = worm reduction rate; FWRR = female worm reduction rate

$x \pm s$; [a]$p > 0.05$; *vs* corresponding 300 mg kg^{-1} group

Table 3

Effect of artemether on rabbits infected with *Schistosoma japonicum* cercariae.

Group	Day of medication (d)	Dose (mg kg^{-1})	Rabbits without ♀ worm	Total worm	WRR (%)	♀ worm	♀ WRR (%)	Liver alteration
25	Control	0	0/9	127 ± 7	-	57 ± 6	-	+++
26	d_7	30	0/6	9.0 ± 4.9^b	92.9	4.3 ± 2.2^c	92.4	±−++
27	$d_7d_{14}d_{21}d_{28}d_{35}$	30	7/7	0	100.0	0	100.0	−
28	$d_7d_{21}d_{35}d_{49}$	30	3/7	0.9 ± 1.0^b	99.3	0.4 ± 0.5^b	99.3	±
29	Control	0	0/9	122 ± 15	-	49 ± 10	-	+++
30	d_7	10	0/7	31 ± 19^c	74.6	13 ± 7^c	74.2	+
31	d_7	15	0/6	22 ± 10^c	82.2	9.7 ± 5.7^c	80.3	+
32	$d_7d_{14}d_{21}d_{28}d_{35}$	10	3/8	3.1 ± 3.8^a	97.7	1.5 ± 1.8^a	97.0	−−±
33	$d_7d_{14}d_{21}d_{28}d_{35}$	15	4/8	1.8 ± 2.1^a	98.6	0.8 ± 0.9^a	98.4	−−±
34	$d_7d_{14}d_{21}d_{28}d_{35}$	30	2/4	2.0 ± 2.4	98.4	0.8 ± 1.0	99.3	−−±

WRR = worm reduction rate
x ± s; $^a p < 0.05$; $^b p < 0.05$; $^c p < 0.01$ *vs* group 27 or group 34

with only a single dose of 10 or 15 mg kg^{-1} (groups 30 and 31) (Table 3). In groups 32, 33 and 34 about half of the rabbits treated were free from female worms. No apparent changes in the livers were detected in most of the rabbits, but in each group 1-2 rabbits showed some fine egg tubercles in the liver with fresh red color and soft quality of the tissues.

When artemether was given to rabbits on day 7 at 10 mg kg^{-1}, followed by repeated dosing once every 1 week for 2-4 times (groups 38, 39 and 40), the efficacies were similar among the 3 groups with female worm reduction rates of over 98%, and half or even more of the rabbits were free from female worm and with no apparent changes in the livers detected (Table 4). In rabbits treated *ig* with Artemether 10 mg kg^{-1} on day 7 or day 7 and day 14 after infection for 1 or 2 administrations, lower reduction rates of total and female worms were seen, but female worms were also found in all rabbits (Table 4).

Table 4

Effect of artemether on rabbits infected and treated *ig* with at 10 mg kg^{-1} on day 7 after infection and repeated once weekly for 1-4 times.

Group	Day of medication (d)	Rabbits without ♀ worm	Total worm	WRR (%)	♀ worm	♀ WRR (%)	Liver alteration
35	Control	0/7	114 ± 10	-	54 ± 5	-	+++
36	d_7	0/4	28 ± 3^b	75.0	14 ± 1	74.2	+
37	d_7d_{14}	0/4	17 ± 19^b	85.0	8.0 ± 8.4^b	85.2	±−++
38	$d_7d_{14}d_{21}$	2/4	1.0 ± 1.2^a	99.1	0.5 ± 0.6^a	99.1	−−±
39	$d_7d_{14}d_{21}d_{28}$	2/4	2.0 ± 2.8^a	98.2	1.0 ± 1.4^a	98.1	−−±
40	$d_7d_{14}d_{21}d_{28}d_{35}$	5/8	1.6 ± 2.4	98.6	0.8 ± 1.2	98.5	−−±

WRR = worm reduction rate
x ± s; $^a p > 0.05$; $^b p < 0.01$ *vs* group 40

Rabbits infected with 198-202 cercariae were treated ig with artemether at a single dose of 15 mg kg^{-1} on day 3, 5, 7, 9, 11, 14, 17, 21, 28 or 35 after infection. All treated rabbits were killed 4 weeks after treatment for collection of residual worms. 5-, 7-, 9-, 11- and 14-day-old schistosomules exhibited similar higher susceptibility to artemether with worm reduction rates around 90%. Other stages of schistosomules showed less or much less susceptibility to the drug (Table 5).

eosinophils decreased, but was still much higher than that before week 4. No apparent change was found in the group treated with Artemether during the 4-10th weeks after infection (Fig 1B).

At the 6-9th week after infection, schistosome eggs in feces were found in all control rabbits, but not in all rabbits treated with artemether.

Table 5
Effect of Artemether on developmental stages of schistosomes harbored in rabbits treated ig with artemether at a single dose of 15 mg kg^{-1}.

Group	Age of worm (d)	No. of rabbits	Total worm	WRR (%)
41	Control	7	128 ± 11^b	-
42	3	5	109 ± 18^b	14.3
43	5	3	13 ± 13^a	90.0
44	7	5	9 ± 7	93.0
45	9	6	13 ± 13^a	90.0
46	11	5	8 ± 4^a	94.0
47	14	6	13 ± 13^a	90.0
48	17	5	31 ± 8^b	75.8
49	21	5	40 ± 6^b	68.7
50	28	5	89 ± 8^b	30.8
51	35	6	95 ± 8^b	25.8

WRR: worm reduction rate
$^a p > 0.05$; $^b p < 0.01$ *vs* group 44

Effect on temperature, eosinophils and eggs in feces

On the fourth week after infection, the average temperature of rabbits in the control group was 39.5°C, and reached its peak valves of 40.7°C 6 weeks later. Afterwards, the temperature of the control rabbits was sustained around 40°C up to the 9th week. In the group of rabbits initially treated *ig* with artemether 10 mg kg^{-1}, followed by repeated dosing once every week for 4 times, the temperature fluctuated between 38.5-39.4°C 4-10 weeks after infection (Fig 1A).

The number of eosinophils in rabbits in the control group increased gradually from week 5 after infection, and reached its peak value of $(11.7 \pm 4.1) \times 10^9$/l 8 weeks later. Thereafter the number of

Effect on antibody and antigen level in serum

The antibody level in serum was determined by COPT. Five weeks after infection, all rabbits in the control group showed positive reactions with a circumoval precipitin rate of 33 ± 5%, and then reached and sustained around 57-63% 6-9 weeks later. In all rabbits treated with artemether, positive reactions occurred in the 6th week after infection with a circumoval precipitin rate of 8%. Afterwards, the circumoval precipitin rates were sustained at around 15-21% (Fig 1C).

The antigen level in serum was determined by a dot-ELISA method using monoclonal antibody. Five to 9 weeks after infection, the average serum titers in the control group were 45-1,720, while those in groups treated with artemether were 3.4-38 (Fig 1D).

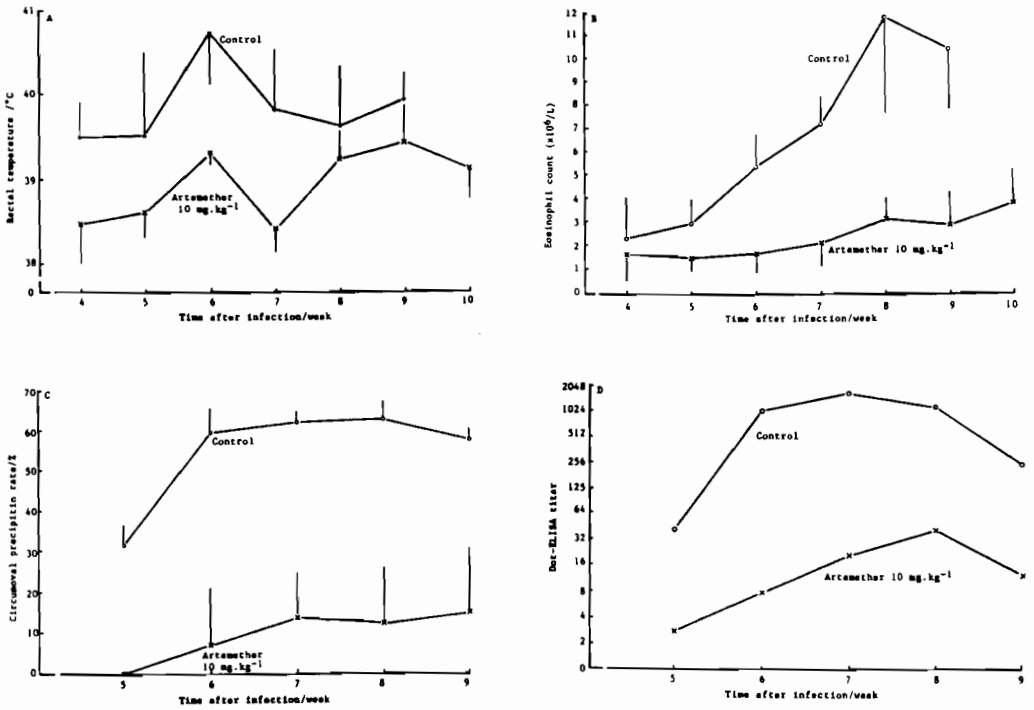

Fig 1–Rectal temperature (A, n = 5-6, x ± s); eosinophil count (B, n = 5-6, x ± s)); circumoval precipitin rate (C, n = 5-6, x ± s) and antigen level (D, n = 4, x) in infected rabbits treated *ig* with artemether 10 mg kg^{-1} (x). The untreated infected rabbits were the control (o).

Fig 1A–rectal temperature (°C)

Week	4	5	6	7	8	9	10
Artemether	38.5 ± 0.5	38.6 ± 0.3	39.3 ± 0.2	38.4 ± 0.3	39.2 ± 0.3	39.4 ± 0.4	39.1 ± 0.4
Control	39.5 ± 0.4	39.5 ± 1.0	40.5 ± 0.6	39.8 ± 0.7	39.6 ± 0.7	39.9 ± 0.3	-

Fig 1B–Eosinophil count (× 10^6/l)

Artemether	1,601 ± 634	1,542 ± 587	1,711 ± 873	2,143 ± 961	3,021 ± 1,683	2,878 ± 1,472	3,751 ± 1,498
Control	2,352 ± 1,792	2,983 ± 1,031	5,379 ± 1,391	7,161 ± 1,168	11,726 ± 1,168	10,336 ± 2,532	-

Fig 1C–Circumoval precipitin rate/%

Artemether	0	7.5 ± 16.5	14.8 ± 19.5	12.7 ± 14.7	15.7 ± 14.8	-	-
Control	33.4 ± 4.8	57.4 ± 5.7	62.4 ± 3.0	62.6 ± 6.4	57.2 ± 3.5	-	-

Fig 1D–Dot-ELISA (titer)

Artemether	3.4 ± 4.2	8.0 ± 3.5	22.7 ± 5.2	38 ± 3.3	13.5 ± 2.8	-	-
Control	45.2 ± 2.4	1024 ± 1.8	1722 ± 1.4	1218 ± 1.4	256 ± 1.8	-	-

Reinfection with cercariae

Weekly infection: When rabbits infected with cercariae once every week for 6 times were initially treated ig with artemether 10 mg kg^{-1} after the first infection, followed by repeated dosing every 1 or 2 weeks for 9 (group 53) or 4 (group 54) times, higher efficacy was seen in group 53 with total and female worm reduction rates of 97.4% and 97.8%, respectively. Group 54 also showed reduction in the numbers of total and female worms (Table 6).

Every other day infection

In the first experiment, rabbits infected with cercariae once every other day for 5 times were initially treated *ig* with artemether 10 mg kg^{-1}, followed by repeated dosing once every week. Although total and female worm reduction rates were over 95%, 2-7 female worms were found in each of 4 rabbits (group 56) with slight changes in their livers (Table 7). When the same dose of artemether was given every 2 weeks for 3 times since the first administration (group 57), the total and female worm reduction rates were 78.2-78.3% accompanied by moderate or even severe gross changes of livers (Table 7). In another group (group 58), the rabbits were treated *ig* with artemether at a single dose of 10 mg kg^{-1} on day 49 after the first infection; there was less severe damage of their livers (Table 7).

Table 6

Effect of artemether (Art) on rabbits infected with *Schistosoma japonicum* cercariae once every week for 6 times and treated ig with artemether started on day 7 after the first infection.

Group	Drug	Time of administration (d)	Dose (mg kg^{-1})	Rabbits without ♀ worm	Total worm	WRR (%)	♀ worm	FWRR (%)	Liver alteration
52	Control	-	-	0/8	166 ± 17	-	76 ± 10	-	+++
53	Art	$d_7d_{14}d_{21}d_{28}d_{35}$ $d_{42}d_{49}d_{56}d_{63}d_{70}$	10	2/6	4.3 ± 4.8[a]	97.4	1.7 ± 2.3[a]	97.8	−−±
54	Art	$d_7d_{21}d_{35}d_{49}d_{63}$	10	0/5	17.4 ± 6.8[a]	89.5	8.4 ± 3.5[a]	88.9	+

WRR : worm reduction rate; FWRR : worm reduction rate
x ± s; [a]$p < 0.01$ *vs* the control

Table 7

Effect of artemether (Art) on rabbits infected with *Schistosoma japonicum* cercariae once every other day for 5 times and treated with the drug started on day 7 after the first infection.

Group	Drug	Time of administration (d)	Dose (mg kg^{-1})	Rabbits without ♀ worm	Total worm	WRR (%)	♀ worm	FWRR (%)	Liver alteration
55	Control	-	-	0/6	180 ± 13	-	87 ± 7	-	+++
56	Art	$d_7d_{14}d_{21}d_{28}d_{35}d_{42}$	10	0/4	8.3 ± 4.2	95.4	4.0 ± 2.2	95.4	+
57	Art	$d_7d_{21}d_{35}d_{49}$	10	0/6	39 ± 10[b]	78.3	19 ± 5[b]	78.2	++−+++
58	Art	d_{49}	10	0/2	137 ± 13[b]	23.9	62 ± 1[b]	28.7	+++
59	Control	-	-	0/7	113 ± 12	-	51 ± 5	-	+++
60	Art	$d_7d_{14}d_{21}d_{28}d_{35}$	15	4/6	2 ± 3	98.2	0.8 ± 1.6	98.4	−−±
61	Art	$d_7d_{21}d_{35}d_{49}$	15	2/5	2.6 ± 2.4[a]	97.7	1.2 ± 1.1[a]	97.6	−−±
62	Art	d_{49}	15	0/7	59 ± 14[b]	47.8	26 ± 6[b]	49.0	++−++

WRR : worm reduction rate; FWRR : worm reduction rate
x ± s; [a]$p > 0.05$; [b]$p < 0.01$; *vs* corresponding groups 56 and 59

In the second experiment, the above-mentioned rabbits were treated *ig* with artemether by the same regimens but the dose of the drug was increased to 15 mg.kg^{-1} (group 60 and group 61), the female worm reduction rates being 97.6% and 98.4%. Meantime, in these 2 groups 4 out of 6 rabbits (group 60) and 2 out of 5 rabbits (group 61) were free from female worms (Table 7), and the other rabbits retained 1-2 female worms. Only slight gross change was seen in the liver surface (Fig 2).

Early treatment in dogs

Dogs were treated ig with Artemether 10 mg kg^{-1} on day 7 after infection, followed by repeated dosing every 1 week for 2 or 4 times (group 64 and group 65). The numbers of total and female worms of these 2 groups were much less than those of the controls with worm reduction rates of over 96% (Table 8). In another experiment, ArtC was given *ig* to the dogs on day 7 after infection at a single dose of 15 mg kg^{-1}, followed by repeated dosing once every 1 or 2 weeks for 3-4 times (group 67 and group 68). The efficacies were similar to those of group 64 and group 65 (Table 8).

Fig 2–Group 60 treated with artemether, showing normal appearance of the liver (right) as compared to the control (left) 10 weeks after infection.

Effect of early treatment on the liver

Histological observations: After dogs were infected with schistosome cercariae for 10 weeks (group 63), their livers showed softness and a dark red color with many yellow miliary egg tubercles distributed unevenly on the surface. Histological examination showed swelling and cloudy of liver cells, narrow sinusoids, deposition of eggs in the portal vein area, and formation of inflammatory and fibrous granulomas (Fig 3A). Kupffer's cells were swollen and contained many dark grey particles. After early treatment with Artemether or ArtC (groups 64, 65, 67 and 68), the livers of the dogs were red in color and nor or only a few miliary egg tubercles were scattered on the liver surface. In sections of the liver tissue, few eggs were found deposited in the portal vein area. No or much less inflammatory and fibrous egg granulomas were detected, whereas the structure of the hepatic lobules and the arrangement of the liver bundles remained normal (Fig 3B, 3C).

Table 8

Effect of artemether and artemether capsule (Art C) given orally at a single dose to dogs on day 7 after infection with *Schistosoma japonicum* cercariae and followed by a repeated dose once every week for 2-4 times.

Group	Drug	Day of medication (d)	Dose (mg kg^{-1})	Dogs without ♀ worm	Total worm	WRR (%)	♀ worm	FWRR (%)
63	Control	-	-	0/2	105 ± 3	-	41 ± 2	-
64	Art	$d_7d_{14}d_{21}$	10	1/3	3.3 ± 3.1[a]	96.9	1.0 ± 1.0[a]	97.5
65	Art	$d_7d_{14}d_{21}d_{28}d_{35}$	10	2/3	1.7 ± 1.5[a]	98.4	0.3 ± 0.6[a]	99.3
66	Control	-	-	0/3	100 ± 4	-	47 ± 2	-
67	ArtC	$d_7d_{14}d_{21}d_{28}d_{35}$	15	1/4	3.5 ± 4.4[a]	96.5	1.5 ± 1.7[a]	96.8
68	ArtC	$d_7d_{21}d_{35}d_{49}$	15	0/4	3.3 ± 2.5[a]	96.7	1.5 ± 1.3[a]	96.8

WR : worm reduction rate; FWR : female worm reduction
x ± s; [a]p < 0.01 *vs* the control group

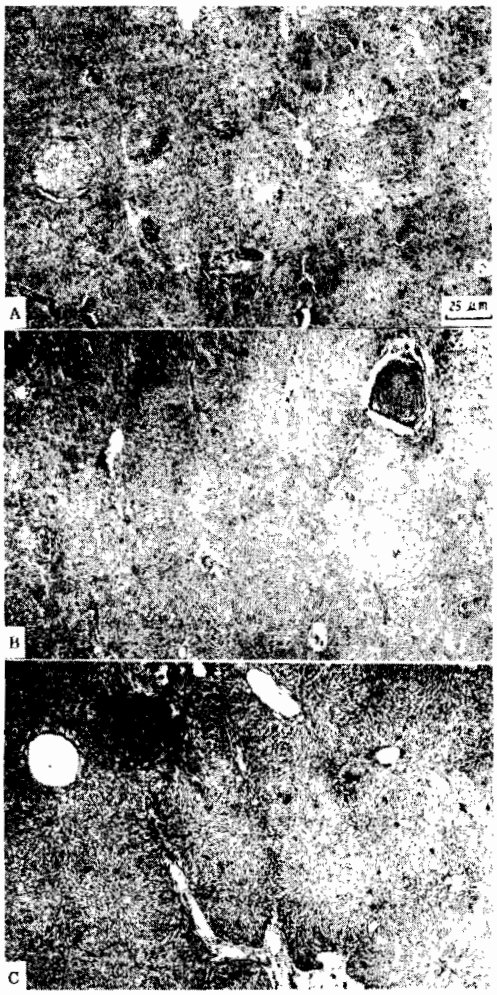

Fig 3–Dogs were initially treated ig with artemether at a single dose of 10 mg kg^{-1} on day 7 after infection with schistosome cercariae, followed by repeated dosing once every 1 week for 2-4 times. The treated animals were killed 4-5 weeks after the last medication (10-11 weeks after infection) for histopathological examination of the liver. ×40, HE. A) Control dog, 10 weeks after infection, showing many schistosome egg granulomas in the liver. B) Group 64 treated with artemether showing formation of fibrous egg granuloma in portal vein area with no apparent change of the liver. C) Group 65 treated with artemether, showing no egg in portal vein area and the normal structure of the hepatic lobule.

In control rabbits (group 59), the livers appeared dark red-grey in color with numerous white-yellow miliary egg tubercles or white fibrous tissue distributed intensively over the whole surface, resulting in hardness of the liver. Histological observation showed many eggs of different developmental stages deposited in the portal vein area, accompanied by formation of extensive inflammatory, fibrous or scarred schistosome egg granulomas. Severe damage of the liver lobules, and many dark grey particles within swollen Kupffer's cells could be seen (Fig 4A). When the rabbits were treated ig with Artemether (group 60 and group 61) at an early stage after infection, their livers showed red, or slight red color with no or few miliary egg tubercles scattered on the surface. The structure of the hepatic lobules and the arrangement of the liver bundles were normal except for a few of fibrous egg granulomas in the portal vein area of individual rabbits. The liver changes induced by schistosome eggs were similar to groups treated with the drug once every 1 or 2 weeks (Fig 4B, C). In rabbits treated *ig* with Artemether 15 mg kg (group 62) on day 49 after the first infection, their livers appeared dark red with more miliary egg tubercles on the liver surface. The inflammatory, fibrous or scarred schistosome egg granulomas found in the liver tissue were less than in the controls and part of the hepatic lobules remaind normal (Fig 4D).

Schistosome egg granulomas

In dog and rabbit control groups, the average numbers of the egg granuloma in the livers were 11 ± 6/0.5 cm^2 (Table 9) and 34 ± 4/0.5 cm^2, respectively (Table 10). When infected dogs and rabbits were treated ig with artemether or ArtC in the early stage of infection, the average number of egg granulomas in the liver was significantly less than the control (Tables 9, 10). In rabbits treated ig with artemether at a single dose of 15 mg kg^{-1} on day 49 after infection (group 62), the number of egg granulomas was less than that in the control group but significantly higher than that in the groups treated ig with the drug in the early stage after infection (Table 10).

In the control dogs, 2 types of granulomas, *ie* inflammatory and fibrous schistosome egg granulomas, were seen in liver sections. In the control rabbits, besides the above-mentioned 2 types of granulomas, another type of scarred schistosome egg granuloma was also detected. After early treatment of dogs with artemether or ArtC, the numbers of inflammatory egg granuloma were reduced markedly (Table 9). When the rabbits were treated *ig* with artemether the numbers of inflammatory and fibrous

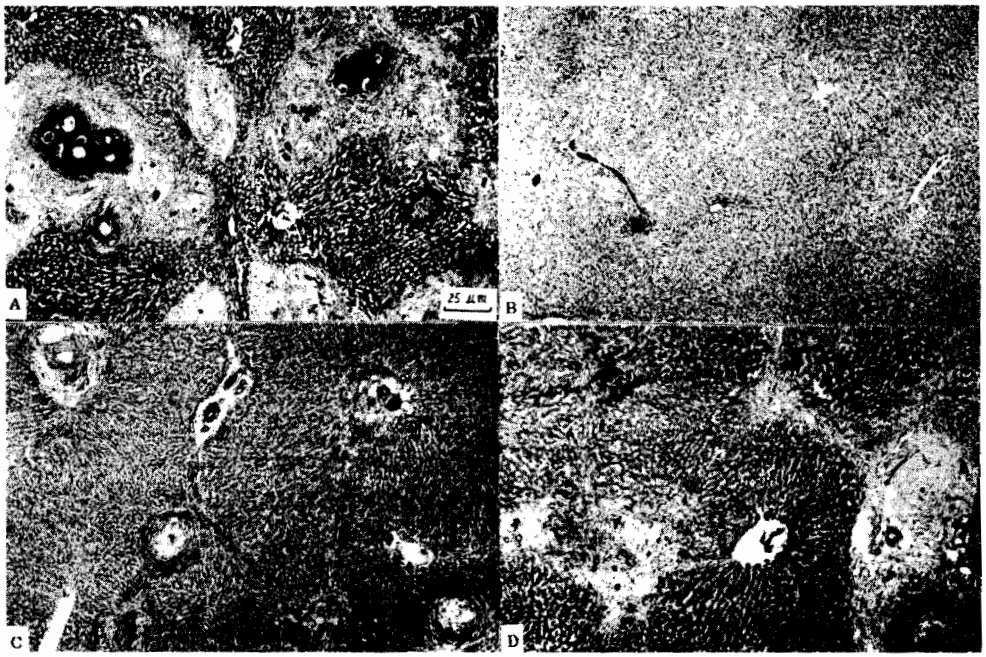

Fig 4–Rabbits were initially treated *ig* with Artemether at a single dose of 15 mg kg^{-1} on day 7 after infection with schistosome cercariae, followed by repeated dosing once every 1 or 2 weeks for 3-4 times. The treated animals were killed 4-5 weeks after the last medication (10-11 after infection) for histopathological examination of the liver. × 40, HE. A) control rabbit (Group 59), 10 weeks after infection, showing deposition of many eggs in the portal vein area; formation of inflammatory, fibrous and scarred egg granuloma, and destruction of the hepatic lobule structure. B) Group 60 treated with artemether, showing normal structure of the liver. C) Group 61 treated with artemether, showing occasional inflammatory or fibrous egg granuloma in the portal vein area with normal structure of the hepatic lobule. D) Group 62 treated with artemether on day 49, showing formation of inflammatory, fibrous and scarred egg granuloma in the portal vein area, but most of the hepatic lobules exhibited normal structure.

Table 9

Quality of schistosome egg granuloma in the liver tissues (section with 0.5 cm^2) of dogs treated *ig* with artemether (Art) and Art C in the early stage after infection with *Schistosoma japonicum*.

Group	Drug	Day of medication	Dose (mg kg^{-1})	Dogs without granuloma	Total No. of granuloma	Granuloma quality		Egg quality	
						Inflammatory	Fibrous	Fresh	degenerated
63	Control	-	-	0/4	11 ± 5	9.9 ± 2.2	1.1 ± 1.5	2.2 ± 1.5	1.2 ± 1.3
64	Art	$d_7d_{14}d_{21}$	10	0/3	2.6 ± 2.0^c	2.4 ± 2.1^c	0.2 ± 0.5^b	0.2 ± 0.4^c	0.1 ± 0.2^c
65	Art	$d_7d_{14}d_{21}d_{28}d_{35}$	10	1/3	0.3 ± 0.6^c	0.3 ± 0.6^c	0^c	0.2 ± 0.9^c	0.1 ± 0.2^c
67	ArtC	$d_7d_{14}d_{21}d_{28}d_{35}$	15	0/4	3.2 ± 1.8^c	2.5 ± 1.4^c	0.5 ± 0.9^a	0.7 ± 1.0^c	0.6 ± 1.1^a
68	ArtC	$d_7d_{21}d_{49}$	15	0/4	2.6 ± 2.2^c	1.2 ± 1.0^c	1.4 ± 1.6^a	0.5 ± 0.9^c	1.1 ± 1.7^a

$x \pm s$; $n = 25$; $^ap > 0.05$; $^bp < 0.05$; $^cp < 0.01$; *vs* the control

Table 10

Quality of schistosome egg granuloma in the liver tissues (section with 0.5 cm²) of rabbits treated *ig* with artemether (Art) in the early stage after infection with *Schistosoma japonicum*.

Group	Drug	Day of medication	Dose (mg kg⁻¹)	Rabbits without granuloma	Total No. of granuloma	Granuloma quality			Egg quality		
						Inflammatory	Fibrous	Scarred	Fresh	degenerated	Calcified
59	Control	-	-	0/3	34 ± 4	15 ± 5	13 ± 2	6 ± 4	41 ± 11	18 ± 14	0.6 ± 0.9
60	Art	$d_7d_{14}d_{21}d_{28}d_{35}$	15	2/3	0.9 ± 1.5[b]	0.4 ± 0.7[b]	0.4 ± 0.7[b]	0[b]	0[b]	0[b]	0[b]
61	Art	$d_7d_{21}d_{35}d_{49}$	15	0/3	8 ± 7[b]	3 ± 3[b]	4 ± 3[b]	1 ± 2[b]	5 ± 5[b]	4 ± 4[b]	0[b]
62	Art	d_{49}	15	0/3	17 ± 4[b]	5 ± 3[b]	8 ± 5[b]	5 ± 4[a]	12 ± 10[b]	29 ± 21[b]	0.6 ± 0.7[a]

x ± s; n = 25; [a]p > 0.05; [b]p < 0.01; *vs* the control

egg granulomas were also reduced markedly as compared with the control (Table 10). In the group treated ig with artemether at a single dose on day 49 after infection (group 62), only reduction of the number of inflammatory egg granuloma was seen (Table 10).

In control dogs, few eggs were seen in the liver. The average numbers of fresh and degenerated eggs were 2.2 ± 1.5 and 1.2 ± 1.3/0.5 cm², respectively (Table 9), No calcified eggs were seen. In control rabbits, the respective numbers of fresh, degenerated and calcified eggs were 41 ± 11, 18 ± 14 and 0.6 ± 0.9/0.5 cm² (Table 10). After treatment of dogs or rabbits with artemether or ArtC the average numbers of fresh eggs were reduced markedly apart from individual group (Tables 9, 10). In rabbits treated ig with a single dose of artemether at day 49 after infection, a lesser number of fresh eggs was seen, but this was higher than that in groups treated with artemether at an early stage after infection (Table 10).

DISCUSSION

In order to understand what time is appropriate for early treatment with artemether, a series of experiments was carried out in mice. The results indicated that day 7 after infection with schistosome cercariae was an appropriate time for early treatment. Thus, the appropriate regimen for early treatment with for artemether is to be given on day 7 after infection, followed by repeated dosing once every week for 4 times, which results in killing most female worms before oviposition. On the other hand, since no apparent difference was seen between dosages and efficacies, it seemed that the mouse was not a good host to use for evaluating efficacy of early treatment with artemether.

When the above-mentioned appropriate regimen of artemether or ArtC was given to rabbits and dogs infected once with cercariae, significant protection of the host from damage induced by schistosome eggs occurred, with apparent reduction in numbers of total and female worms. Since the dose of artemether used in the early treatment of mice was 300 mg kg⁻¹, higher doses of artemether were tested initially in rabbits. Unexpectedly, the effect of artemether 30-60 mg kg⁻¹ on 7-day-old schistosomules in rabbits was rather high even at a single dose, indicating that the effect of artemether on 7-day-old schistosomules in rabbits was more promising. Further study demonstrated that the minimal effective dose of artemether used in early treatment of rabbits was as low as 10 mg kg⁻¹, and administration followed the first dose on day 7 after infection was once every week for at least 2 times. Promising efficacy was also seen in dogs, when lower dose of artemether was given at an early stage after infection. Considering the oviposition of residual 7-day-old female worms treated with artemether was inhibited, administration of the drug once every 2 weeks following the first dose was tested in rabbits and dogs. The protective effect of artemether used at an early stage was also demonstrated by negative results in determination of parameters relevant to acute schistosomiasis, and by low levels of specific antigen and antibody in serum.

Whether artemether could be used in the field for controlling acute schistosomiasis or reducing the

infection rate and intensity of infection depends on the effects of the regimen on reinfection with cercariae. Our experimental results indicated that the above-mentioned appropriate regimens of Artemether were also effective in early treatment of rabbits infected with cercariae once every week for 6 times or every other day for 5 times. However, a higher dose of 15 mg kg^{-1} is necessary when artemether is given once every 2 weeks following the first dose.

For evaluation of efficacy of early treatment, pathological changes in the liver were one of the important parameters, in addition to the total numbers of worms, especially the female worm reduction rate. Our study showed that when artemether or ArtC was given initially to dogs or rabbits on day 7 after infection followed by repeated dosing once every 1 or 2 weeks for 2-4 times, protection of the host liver from egg-induced damage was seen. On the contrary, when artemether was given once to rabbits on day 49 after infection, the total and female worm reduction rates were low and serious damage to the liver could be seen. Since the protective effect of artemether given every week on the liver was similar to that of the drug given every 2 weeks, it is reasonable to suggest that in field trials, the recommended intervals for artemether medication should be every 2 weeks. On the other hand, because 5-14-day-old schistotomules exhibited similar susceptibility to artemether, the first dose could be given between day 5-day 14 after infection.

On the basis of these results, as well as extensive experience from clinical use of artemether in the treatment of malaria, the application of this drug for controlling acute schistosomiasis and reducing the infection rate and intensity of schistosomal infection in a well-designed field trial is deemed to be feasible and practicable.

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