STUDIES ON CHEMOTHERAPY OF PARASITIC HELMINTHS: EFFICACY OF ARTEMETHER ON JAPANESE STRAIN OF *SCHISTOSOMA JAPONICUM* IN MICE

Motohito Sano[1], Cetin Volkan Akyol[2], Anchalee Tungtrongchitr[3], Makoto Ito[4] and Akira Ishih[1]

[1]Department of Parasitology, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan; [2]Department of Parasitology, Faculty of Veterinary Medicine, Uludag University, Bursa, Turkey; [3]Bangkok School of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [4]Department of Parasitology, Medical School, Nagoya City University, Nagoya 467, Japan.

Abstract. Effects of artemether were examined on *Schistosoma japonicum* in mice. When the drug was given at a daily dosage of 200 mg/kg for 4 successive days from 46 days post-infection, a significant reduction in worm recovery was observed. A significant reduction in size of worms from the medicated mice was also seen compared with that from non-medicated controls.

INTRODUCTION

Schistosomiasis japonica is an important endemic parasitic diseases, widely distributed in many countries in Asia. Infection with this trematode results in harmful effects to humans, causing pathological changes in the intestine and liver, and difficulty in treating schistosomiasis is a major factor contributing to the disease as a world health problem. The most effective drugs in current use are praziquantel and oxamniquine, but recently, it was suggested that drug resistance against these drugs might be developing (Coles *et al.* 1987). If so, new drugs may be required. Artemether, a derivative of the antimalarial agent, qinghaosu, has been reported to be therapeutically active against *in vitro* and *in vivo Schistosoma mansoni* infection (Xiao and Catto, 1989). This drug has also been shown to affect *S. japonicum* infections in dogs and mice in China (Le *et al.* 1982). Thus artemether appears to have antischistosoma as well as antimalarial chemotherapeutic potential. In these experiments, the efficacy of this drug against the Japanese strain of *S. japonicum* in mice was assessed.

Correspondence to : Prof Dr Motohito Sano, Department of Parasitology, Hamamatsu University, School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan.

MATERIALS AND METHODS

Compound tested

Artemether was kindly supplied by Dr Xiao Shuhua, of the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai. The drug was suspended in 25% glycerol and 1% cremophor EL solution for administration to experimental animals.

Animal treatment

Five-week-old female ddY mice, weighing 24-25g, were obtained from Japan SLC, Inc. The parasite used in this experiment was a strain of *S. japonicum* from Yamanashi Prefecture in Japan which had been maintained in the laboratory for many years. The cercariae used were obtained from experimentally infected snails, *Onchomelania nosophora* (Yamanashi strain). Mice were exposed to infections with 20 cercariae by intradermal injection, and divided into two groups, medicated and non-medicated controls, consisting of 9 mice each. Forty-six days post-infection (pi), when all mice were found positive with eggs in their feces, in the medicated group the mice were administered orally with artemether at a daily dosage of 200 mg/kg body weight for 4 days successively, while the controls received vehicle only.

Parasitological findings

Changes in body weight of infected mice were regarded as an index of the severity of the disease, and hence recorded at one week intervals, starting from the day of infection to autopsy. Two weeks after end of treatment surviving animals were sacrificed and examined for schistosomes. The worms were recovered by the isotonic saline perfusion technique (Radke *et al,* 1961), and were processed by a standard acid carmine staining method for observing the morphology of tegument and reproductive organs in both sexes. To examine the differences in development of worms recovered, the worm body length was estimated from photographs using a computerized image analyzer (Videopolan, Kontron Co, Munich, FRG).

Statistical analysis

Differences in mortality between medicated and non-medicated groups were analyzed by chi-square test. The mean values of worms recovered and of worm body length were compared by the Mann-Whitney U-test and the Student's *t*-test, respectively. P values less than 0.05 were considered to be statistically significant.

RESULTS

Changes in surviving numbers and body weights of mice in medicated and non-medicated groups are shown in Fig 1. A gradual loss of mean body weight was observed in both groups from 5 weeks pi. Three and 2 mice died in medicated and non-medicated groups, respectively, from 7 weeks pi. After treatment, however, a small increase of mean body weight was observed in medicated group. The results of *in vivo* efficacy of chemotherapy against *S. japonicum* at autopsy are summarized in Table 1. Statistical analysis using x^2-test revealed no significant difference in mortality between two groups. The mean worm recovery from medicated group was significantly lower than that from non-medicated controls. Moreover, it must be stressed that no female worm was recovered from medicated animals, while paired and single males were recovered from non-medicated mice. It was of interest to note that there was a significant difference in size between males medicated and those from non-medicated mice (Table 2). The mean body length of male worms recovered

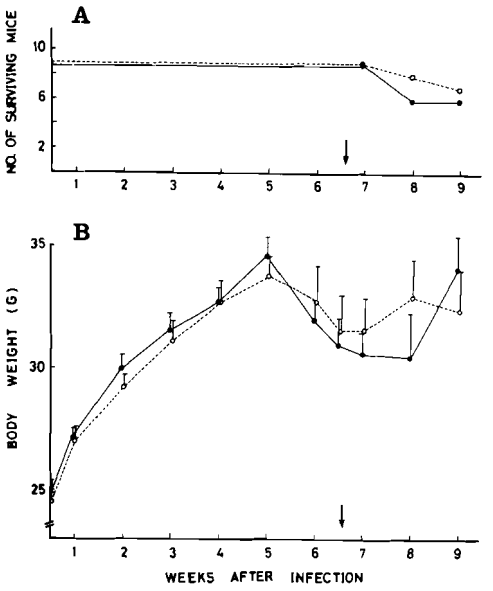

Fig 1—Effects of artemether on surviving number (A) and body weight (B) of mice infected with 20 cercariae of *Schistosoma japonicum*. Each group has 9 mice. Four successive daily doses of 200 mg/kg were administered from 46 days post-infection (arrow) in the medicated group (●—●). Non-medicated control mice (○—○) received vehicle (25% glycerol and 1% cremophor EL solution) only. Each value represents the mean with SE shown as a vertical bar in (B).

from medicated mice was significantly shorter than that of male worms from non-medicated controls. Organ specific damage of worms was assessed by light microscopic observations of carmine-stained worms. Comparing with the morphology of worms from non-medicated control mice, no decrease of the number and no atrophy of testes occurred in males from medicated animals. No tegumental damage of male worms was observed.

DISCUSSION

It has been reported that artemether is effective against adult schistosomes. The effects of this drug on adult worms are characterized by a shift of the worms to the liver after drug administration,

Table 1

Chemotherapeutic effects of artemether on worm recovery in mice infected with *Schistosoma japonicum* (mean ± SD).

Dosage of artemether (mg/kg)	No. of mice used/ No. autopsied	Body weight (g)	No. of worms recovered		
			Female	Male	Total
200 × 4*	9/6	34.1 ± 3.3	0.0 ± 0.0#	4.7 ± 2.5	4.7 ± 2.5#
None	9/7	33.8 ± 3.0	2.9 ± 2.0	7.9 ± 3.2	10.7 ± 4.3

Each mouse was exposed to 20 cercariae by intradermal injection, and sacrificed 14 days after end of treatment.
* The drug was given 4 days successively from 46 days post-infection.
# Significantly lower than the non-medicated control group ($p<0.05$, Mann-Whitney U-test).

Table 2

Body length measurements of adult male and female *Schistosoma japonicum* recovered from medicated and non-medicated mice (mean ± SD).

Dosage of artemether	Sex	No. of worms examined	Body length (mm)
200 × 4*	male	25	5.81 ± 0.71#
	female†	-	-
None	male	31	7.06 ± 1.92
	female	10	10.02 ± 0.96

Each mouse was exposed to 20 cercariae by intradermal injection, and sacrificed 14 days after end of treatment.
* The drug was given 4 days successively from 46 days post-infection.
† No female worm recovered from portal and mesenteric veins in medicated mice.
# Significantly lower than the non-medicated control group ($p<0.01$, Student's *t*-test).

reduction in size of both male and female worms, and degeneration of reproductive organs (Xiao and Catto, 1989). 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 (Le *et al*, 1982). In the present study of mice infected with the Japanese strain of *S. japonicum* and given at daily dosage of 200 mg/kg for 4 successive days from 46 days pi, the worm reduction rate was 56.1%. Moreover, no female worms were recovered from the portal and mesenteric veins in medicated animals. In 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 23.8 to 39.1% (Xiao and Catto, 1989). Thus, the therapeutic effect of the drug against adult *S. japonicum* is apparently greater than that against adult *S. mansoni*. Adult *S. mansoni* worms are generally more susceptible to antischistosomal drugs than adult *S. japonicum* (Davis, 1986), and therefore the present results as well as those by Le *et al* (1982) are particularly interesting. Xiao and Catto (1989) stated that these two species of schistosomes appeared to be affected differently by the drug.

Besides a hepatic shift of worms induced by drug administration in *S. mansoni* infection, a reduction in size of worms recovered is also known to occur. However, in *S. japonicum* infections in the present study an apparent reduction in size of worms from medicated mice was observed compared with that in non-medicated controls.

A gradual loss of body weight was observed in both groups from 5 weeks pi. After being given the drug for 4 successive days from 46 days pi, a small increase of body weight in medicated animals

was noticed. In mice infected with schistosomes, eggs produced by females are a pathogenic factor in the intestine and liver, with resultant body weight loss. Increase of body weight in the medicated group, therefore thus is probably due to the worm shift to the liver and/or the cessation of egg production, but these possibilities are unclear. Xiao and Catto (1989) have reported that, in the treatment of *S. mansoni,* 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, but by 28 days after treatment the size of testes and ovaries returned to normal. However, whether adult worms which survive treatment regain functional integrity and resume egg laying is not known.

Artemether is a chemical derivative of qinghaosu, a new antimalarial drug derived from a traditional Chinese herbal remedy, qinghao or *Artemisia annua* L. (Klayman, 1985), and also is effective in the treatment of experimental schistosomiasis (Le *et al,* 1982; 1983). Further studies are necessary on identification and evaluation of new antischistosomal agents derived from this traditional Chinese herbal remedy.

REFERENCES

Coles GC, Bruce JI, Kinoti GK, *et al.* The potential for drug resistance in schistosomiasis. *Parasitol Today* 1987; 3 : 349-50.

Davis A. Recent advances in schistosomiasis. *Q J Med* 1986; 58 : 95-110.

Klayman DL. Qinghasou (artemisinin): an antimalarial drug from China. *Science* 1985; 228 : 1049-55.

Le WJ, You JQ, Mei JY. Chemotherapeutic effect of artesunate in experimental schistosomiasis. *Acta Pharm Sin* 1983; 18 : 619-21.

Le WJ, You JQ, Yang YQ, *et al.* Studies on the efficacy of artemether in experimental schistosomiasis. *Acta Pharm Sin* 1982; 17 : 187-93.

Radke MG, Berrios-Duran LA, Moran K. A perfusion procedure (Perf-O-Suction) for recovery of schistosome worms. *J Parasitol* 1961; 47 : 366-68.

Xiao SH, Catto BA. ***In vitro*** and ***in vivo*** studies of the effect of artemether on *Schistosoma mansoni. Antimicrob Agents Chemother* 1989; 33 : 1557-62.