REVIEW

ARTEMETHER, AN EFFECTIVE NEW AGENT FOR CHEMOPROPHYLAXIS AGAINST SHISTOSOMIASIS IN CHINA: ITS *IN VIVO* EFFECT ON THE BIOCHEMICAL METABOLISM OF THE ASIAN SCHISTOSOME

Xiao Shuhua[1], Peter J Hotez[2] and Marcel Tanner[3]

[1]Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, WHO Collaborating Center for Malaria, Schistosomiasis and Filariasis, 207, Rui Jin Er Lu, Shanghai 200025, China; [2]Department of Epidemiology and Public Health and Pediatrics, Yale University School of Medicine, 60 College Street, New Haven, CT 06520, USA; [3]Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland

Abstract. Conventional drug chemotherapy against human schistosomiasis currently relies on treatment with praziquantel to eliminate adult schistosome worm pairs. The use of praziquantel for control purposes is limited, however, by high rates of post-treatment re-infection with subsequent parasite egg deposition and host end-organ damage. Artemether, a methyl ether derivative of the anti-malarial drug quinghaosu, was discovered recently to also have anti-schistosomal properties. Because artemether selectively targets the larval migratory stages of the parasite, known as schistosomulae, it blocks the development of ovipositing adult schistosome worm pairs in the vasculature. On this basis, we have since shown in clinical trials conducted in China that artemether has proven benefit as an agent for chemoprophylaxis. *In vivo* studies using laboratory animals suggest that artemether causes damage to the tegument and musculature of schistosomulae. Artemether may exert its helminthotoxic effect through synergy with hemin or related heme-containing compounds. Schistosomes recovered from artemether treated laboratory animals have increased glycogen phosphorylase activity, but decreased glucose uptake. These findings may account for their decreased glycogen content, relative to schistosomes recovered from untreated laboratory animals. The artemether-damaged schistosomes also have decreased activities of a number of enzymes and enzyme systems, including glycolysis.This might suggest common pathways by which artemether may target human parasites that live in the bloodstream.

INTRODUCTION

Because the control of schistosomiasis japonica was a cornerstone of Chairman Mao's patriotic campaigns in the 1950s and 1960s, this disease has had a significant impact on the modern history of China (Hotez *et al*, 1997). As a result of aggressive public health control measures, such as water drainage, snail eradication, and anthelminthic drugs for infected individuals, the number of cases of *Schistosoma japonicum* infection has fallen steadily from 10 million in 1955, to 1.5 million in 1989 (Yu, 1996). However despite these successes, *S. japonicum* infection remains a serious public health threat in China. The population density in schistosome endemic areas is very high and many of these individuals have a daily life which places them in frequent contact with schistosome-infested water (Yu, 1996). Over 40 million Chinese are still at risk for acquiring infections caused by the Asian or oriental schistosome, *S. japonicum*. Currently an estimated 880,000 Chinese harbor the parasite and morbidity associated with hepatosplenic disease has been increasing (Chen, 1995).

Schistosomes are water-borne parasitic platyhelminthes belonging to the order Trematoda - human infection occurs in bodies of fresh water where suitable snail vectors serve as intermediate hosts. These conditions are met in an estimated 121 Chinese counties that comprise the lake regions of Hubei, Hunan, Jiangxi, Anhui and Jiangsu Provinces along the Yangtze River (Yu, 1996). Pockets of infection also occur in the mountainous regions of Sichuan and Yunnan Provinces.

Correspondence: Xiao Shuhua
Tel: 86-21-6-437-6308; Fax: 86-21-6-433-2670; E-mail: shxiao@yahoo.com

The major clinical features of schistosomiasis japonica occur when adult male and female worm pairs living in the mesenteric vasculature of their definitive human host deposit eggs. Many of the eggs make their way to the intestinal lumen where they enter the fecal stream and escape from the host. This process facilitates the continuation of the parasite life cycle through a fresh-water snail intermediate host. Frequently, however, the eggs remain trapped in the intestine or liver where they cause microhemorrhages and elicit granulomas and fibrosis. Liver fibrosis from moderate and heavy *S. japonicum* infections results in portal hypertension and hepatosplenomegaly. Chronically infected children acquire deficits in their physical growth and cognition. Of all the human schistosomes, *S. japonicum* causes the greatest amount of human pathology because of the greater number of eggs released by the parasite.

In order for humans become re-infected with schistosomes, eggs which exit from the body in feces must hatch in fresh water to release a pluripotent ciliated organism called a miracidium. Miracidia have the capacity to infect new snails - asexual reproduction of the parasite occurs outside the definitive mammalian host in fresh-water snails. This process gives rise to thousands of free-swimming larval schistosomes known as cercariae. The cercariae attach to and penetrate through human skin. During percutaneous host entry the cercariae lose their characteristic tails and transform into schistosomulae. The schistosomulae acquire an altered surface biochemistry that allows them to resist the action of host complement and other immunologically important molecules. This feature allows the schistosomulae to travel in the bloodstream so that they migrate through the vasculature until they arrive in the liver. Once in the liver they differentiate into paired male and female schistosomes. The worm pairs enter the portal vasculature and travel to the mesenteric venules where they release eggs.

CHEMOPROPHYLAXIS FOR CONTROL

Both major control measures currently used in China rely on either of two types of chemical agents. First, molluscicides are applied extensively in endemic areas in order to target the Oncomelania snail vectors. However, widespread application of molluscicidal agents toxic to snail intermediate hosts has been limited by the environmental hazards of these agents, as well as the costs associated with extensive manpower requirements to apply them. Second, attempts have been made to actively treat all suspected cases with anthelminthic drugs. Since 1978, praziquantel, the drug of choice for the treatment of human schistosomiasis, has been manufactured in China. Anthelminthic treatment with praziquantel can temporarily reduce the number of adult schistosome worm pairs in a given human host. However, it does not prevent re-infection so that in areas of high transmission (Hu *et al*, 1989; Li *et al*, 1990; Zhang *et al*, 1993; Ministry of Public Health, 1992), praziquantel treatment has failed to control schistosomiasis japonica. There are also concerns about emerging praziquantel anthelminthic drug resistance (Davis, 1986).

These problems have led us to search for alternative methods of schistosomiasis control. A long-term approach being undertaken in China is to genetically engineer candidate schistosome antigens in order to develop a recombinant vaccine. Such a vaccine might one day be suitable for immunoprophylaxis either in humans in order to reduce worm burdens or for use in domestic animal reservoirs in order to interrupt zoonotic transmission (Liu *et al*, 1998).

A more near-term approach has been to develop agents for chemoprophylaxis. The principle behind chemoprophylaxis is to target the cercaria and schistosomula larval stages in order to eradicate the parasites before they become ovipositing adult worms. One approach to chemoprophylaxis has been to apply the compound niclosamide in a topical ointment in order to block cercarial entry during periods of water immersion. Niclosamide has also been applied directly to clothing. In both cases, this approach has not worked well because of the inconvenience and very limited local protection.

ARTEMETHER AS CHEMOPROPHYLAXIS AGAINST SCHISTOSOMIASIS

A more successful approach to chemoprophylaxis has been to exploit the drug artemether (methyldihydroartemisinin), a derivative of qinghaosu (artemisinin), for its schistosomicidal properties. Artemisinin is a naturally occurring sesquinterpene lactone obtained from the leaf of qinghao, or sweet wormwood *Artemesia annua* (Klayman, 1985; Hien and White, 1993; White, 1996). Artemether was first synthesized and used as an antimalarial in China (Klayman, 1985; Hien and White, 1993;

White, 1996). The compound is believed to be the most rapidly acting of all antimalarial drugs, and is both orally and parenterally effective in doses ranging from 2 mg/kg to 4 mg/kg. Over the last twenty years between 2 million and 5 million doses of the drug have been prescribed in China, partly accounting for the precipitous decline in endemic malaria (DeStefano, 1993). Under the trade name Paluther, the methyl ether of dihydroartemisinin (artemether) is manufactured through a joint venture with Rhone-Poulenc Rorer (Vitry-sur-Seine, France). Artemether was shown recently to be as effective as quinine in the treatment of cerebral malaria in children (Boele van Hensbroek *et al,* 1996). The drug is well tolerated in humans and, although neurotoxic in laboratory animals at high doses (>25 mg/kg daily for 6-14 days), there is not yet evidence artemether-associated neurologic damage in humans (Brewer *et al,* 1994).

In early 1980s we found that artemether is also effective against *S. japonicum* (Le *et al,* 1982). Most of the qinghaosu-associated studies on schistosomes have been conducted in China. Unlike praziquantel, artemether was noted to have less effect on adult schistosomes *in vivo*. Instead, the drug was observed to be more toxic for the migrating schistosomula stage (Xiao *et al,* 1985; 1987; 1989). As noted above, upon entry into the host, the invading cercariae lose their tail and transform into schistosomulae. The schistosomulae migrate for up to one month before entering the liver and developing into adult schistosome worm pairs. The drug targets the developing schistosomula during a window period of 5-21 days after cercarial entry into the mammalian host (Xiao *et al,* 1994a,b,c; 1995). Therefore artemether functions as a chemoprophylactic agent by destroying the parasites prior to developing into ovipositing female worms, marked reductions in adult schistosome worm burdens in the vasculature were observed, as were reductions in numbers of eggs reaching the liver. When administered before schistosomula developed into adult worms, the livers of treated animals resembled uninfected controls with respect of the microanatomy of hepatic lobules and absence or decrease of inflammatory granulomata and fibrosis (Xiao *et al,* 1994a,b,c; 1995).

The observation that artemether is helminthotoxic to the schistosomulae and prevents their development *in vivo* to an egg-laying adult worm pairs, suggested to us that this agent might have an exciting and new use for humans exposed to the infective stages of the parasite. Because it ultimately blocked worm development and resulted in reductions in liver pathology in the treated laboratory animals, artemether offered promise as a potential agent for chemotherapy. This hypothesis led to the design of clinical field trials with artemether. Over a two year period from 1994 to 1996 a total of six field trials were conducted in endemic areas of Anhui, Hunan, Jiangxi and Yunnan Provinces, artemether was proven to be effective at reducing both worm burdens among susceptible individuals as well as eliminating symptomatic hepatosplenic schistosomiasis (Xiao *et al,* 1994a,b; Xu *et al,* 1997; Tian *et al,* 1997; Wang *et al,* 1997; Song *et al,* 1998). These studies, which provided proof of concept for schistosomiasis chemoprophylaxis, were reviewed recently (Xiao *et al,* 2000a).

MODE OF ACTION

Over the last few years we have undertaken studies to determine the mechanisms by which artemether is helminthotoxic for schistosomes. The ultrastructural damage caused by artemether differed from praziquantel-induced damage in that the former produced more tegumental swelling and the damage was greatest in the migrating larval stages of the parasite, rather than the adult worms. The major changes in the tegument involved disruptions, loss of definition, vacuolation and lysis. By scanning electron microscopy marked swelling of the tegument was noted. The tegumental swelling resulted in a loss of definition of the characteristic surface ridges as well as the spinous processes which otherwise stud the surface of the parasite (Xiao *et al,* 1996c,d).

Our model for studying the helminthotoxic mechanisms of artemether employed lots of the drug which were made available from the Kunming Pharmaceutical Corporation (Yunnan Province, China), and a laboratory strain of *S. japonicum* that was originally derived from Anhui Province and since maintained in *Oncomelania* snails at our Institute by continuous passage. Outbred (Kunming strain) mice were infected with 60-80 *S. japonicum* cercariae, by percutaneous infection through their shaved abdominal skin. Artemether was then administered intragastrically by gavage feeding at 4-5 weeks post-infection. The drug was administered in a volume of 10 ml/kg either at a subcurative dose of 100 mg/kg or at a curative dose of 300 mg/kg. The mice were sacrificed at either 24 hours or 72 hours after anthelminthic drug administration, and the adult male and female schistosome

were recovered by intrahepatic and intravascular perfusion with ice-cold Hanks balanced salt solution (HBSS). The schistosomes were washed three times with HBSS, prior to biochemical measurements.

Glycogen content: One of the most striking biochemical changes in the schistosomes recovered from the artemether treated mice was the reduction in parasite glycogen content (You *et al,* 1994; Xiao *et al,* 1997). When infected mice were treated intragastrically with artemether at a curative dose of 300 mg/kg for 24 or 72 hours, the glycogen contents of male or female worms were decreased to 50% and 73%, respectively. The basis for glycogen reduction was partly accounted for on the basis of reduced parasite glucose uptake. Male and female worms recovered from the artemether-infected mice and recovered at 24 hours and 48 hours post-treatment were also incubated with [U-^{14}C]glucose for 1, 4 and 24 hours. Within 1-24 hours the ^{14}C contents of the female worms were lower than female worms recovered from untreated mice. The results were less dramatic in male worms. Only 3 of the 6 groups of male worms from artemether-treated mice exhibited decreased glucose incorporation compared with the male worms from untreated mice (Table 1).

The reduced glucose uptake was not accompanied by decreased incorporation of glucose into glycogen. However, there were substantial effects noted in the schistosomes recovered from artemether-treated mice when glycogen phosphorylase was

Table 1
Cabohydrate metabolism of schistosomes, previously harbored in mice 24-48 hours after treated intrastrically with artemether (Art) 300 mg/kg, in medium with 0.5% glucose and [U-^{14}C]glucose 11.1 MBq/l for 1-24 hours.

Group	Time after Art (hr)	Sex of worms	Culture of time (hr)	Number of sample	Glycogen content (μg/worm)	[U-^{14}C]glucose uptake (Bq/worm)	Incorporation of [U-^{14}C]glucose into worm glycogen (%)
Control	24	♂	1	19	9.8 ± 1.4	2.0 ± 0.5	23 ± 16
		♀		20	2.4 ± 0.5	1.6 ± 0.5	17 ± 5
Art	24	♂	1	19	6.2 ± 1.0^{c}	1.8 ± 0.5^{a}	22 ± 7^{a}
		♀		15	1.4 ± 0.4^{c}	1.2 ± 0.5^{b}	20 ± 6^{a}
Control	24	♂	4	18	9.8 ± 1.7	2.9 ± 0.5	36 ± 7
		♀		20	2.3 ± 0.7	2.2 ± 0.5	28 ± 7
Art	24	♂	4	19	7.1 ± 2.1^{c}	3.1 ± 0.6^{a}	38 ± 6^{a}
		♀		18	1.4 ± 0.5^{c}	1.8 ± 0.4^{c}	27 ± 8^{a}
Control	24	♂	24	18	9.5 ± 1.9	5.4 ± 0.6	38 ± 18
		♀		20	2.4 ± 0.5	3.0 ± 1.0	26 ± 9
Art	24	♂	24	20	5.7 ± 1.3^{c}	3.5 ± 0.8^{c}	33 ± 10^{a}
		♀		18	0.8 ± 0.3^{c}	1.9 ± 0.9^{c}	26 ± 9^{a}
Control	48	♂	1	20	10.0 ± 1.8	2.2 ± 0.6	27 ± 8
		♀		18	2.2 ± 0.4	2.0 ± 0.6	26 ± 11
Art	48	♂	1	20	3.9 ± 0.8^{c}	1.7 ± 0.5^{b}	21 ± 5^{b}
		♀		16	0.8 ± 0.3^{c}	1.5 ± 0.5^{b}	21 ± 9^{a}
Control	48	♂	4	20	10.1 ± 1.7	3.2 ± 0.6	37 ± 9
		♀		20	2.1 ± 0.4	2.0 ± 0.6	28 ± 10
Art	48	♂	4	20	3.9 ± 0.7^{c}	2.5 ± 0.5^{c}	28 ± 9^{c}
		♀		15	0.7 ± 0.4^{c}	1.3 ± 0.6^{b}	23 ± 8^{a}
Control	48	♂	24	20	7.2 ± 2.3	4.6 ± 1.6	25 ± 7
		♀		20	2.7 ± 0.4	4.0 ± 1.4	18 ± 6
Art	48	♂	24	20	3.7 ± 0.6^{c}	4.5 ± 1.3^{a}	25 ± 8^{a}
		♀		19	0.6 ± 0.2^{c}	2.5 ± 0.9^{c}	19 ± 15^{a}

X ± S; $^{a}p > 0.05$, $^{b}p < 0.05$, $^{c}p < 0.01$ *vs* control. Each sample consisted of 10 ♂ or 10 ♀ worms.

analyzed. Glycogen metabolism is regulated by controlling the addition or removal of phosphate groups to the glycogen phosphorylase enzyme. There is some evidence to suggest that the reduction in parasite glycogen measured in schistosomes recovered in artemether treated mice occurs as a consequence of increases in schistosome glycogen phosphorylase activities. This was obtained by measuring the enzyme activities of total phosphorylase (PP) and phosphorylase in its active (PPa) and inactive (PPb) conformations (Xiao *et al*, 1999). In schistosomes recovered from mice treated at a curative doses of 300 mg/kg of artemether, the activities of total PP and PPa increased significantly in both male and female worms (Table 2). The increase in PPb was more modest and was not apparent at all in schistosomes obtained 48 hours after a curative dose of artemether. Therefore the reduction in schistosome glycogen may occur both as a result of drug induced increases in the activated form of phosphorylase, as well as decreased glucose uptake.

Glycolytic and related enzyme activities: The enzyme activities of the major glycolytic enzyme tested, hexokinase (HK), glucose phosphate isomerase (GPI), phosphofruktokinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (GPDH), phosphoglycerate kinase (PGK), pyruvate kinase (PK), as well as glucose-6-phosphate dehydrogenase (G6PDH) and the enzymes alkaline phosphatase (AKP), acid phosphatase (ACP) and adenosine triphosphatase (ATPase) were reduced in schistosomes recovered from artemether-treated mice relative to untreated control mice (You *et al*, 1994; Xiao *et al*, 1998a,b). Of these enzyme activities of PFK, PGK and PK (Table 3). were reduced the most. The regulatory role of PFK may be particularly relevant. We are investigating whether the apparent decrease in rates of glycolysis affects parasite glucose consumption and therefore accounts for diminished parasite glycogen content. There was also a corresponding decrease in parasite lactate content. The reduction in lactate may result from either overall inhibition in glucose metabolism, but we have not ruled out that the enzyme activity of lactate dehydrogenase is also reduced. The enzyme reductions associated with glycolysis were usually less evident in worms recovered 24 hours after mice were treated with a subcurative dose of artemether (100 mg/kg), but were more pronounced in schistosomes recovered from mice treated with a curative dose of 300 mg/kg. The reductions were even greater in worms recovered 48 hours after treatment compared to worms recovered after 24 hours treatment. The reductions more pronounced in female worms compared to male worms. For HK and GPI, the reduction in enzyme activities was significant only in schistosomes recovered from mice treated with a therapeutic dose of artemether (300 mg/kg). For PFK and G-6-PDH, the enzyme activity in schistosomes was reduced significantly even in mice

Table 2
Phorphorylase (PP) of schistosomes in mice treated intragastrically with artemether (Art). The enzymed activity was expressed as formation of NADPH 1 μmol/min/worm.

Art (mg/kg)	Time after Art (hr)	Sex of worm	Total PP	Increase (%)	PPa	Increase (%)	PPb	Increase (%)
0	0	♂	0.65 ± 0.10	-	0.43 ± 0.09	-	0.20 ± 0.10	-
	0	♀	0.38 ± 0.07	-	0.30 ± 0.09	-	0.09 ± 0.04	-
100	24	♂	0.71 ± 0.16[a]	9	0.47 ± 0.17[a]	9	0.24 ± 0.17[a]	20
	24	♀	0.57 ± 0.08[c]	50	0.45 ± 0.08[c]	50	0.12 ± 0.03[b]	33
0	0	♂	0.67 ± 0.15	-	0.36 ± 0.11	-	0.32 ± 0.14	-
	0	♀	0.40 ± 0.05	-	0.27 ± 0.05	-	0.14 ± 0.03	-
300	24	♂	0.79 ± 0.13[b]	18	0.43 ± 0.08[b]	19	0.37 ± 0.12[a]	16
	24	♀	0.61 ± 0.11[c]	53	0.42 ± 0.10[c]	56	0.19 ± 0.08[b]	36
0	0	♂	0.59 ± 0.17	-	0.49 ± 0.04	-	0.11 ± 0.03	-
	0	♀	0.32 ± 0.06	-	0.29 ± 0.09	-	0.05 ± 0.03	-
300	48	♂	0.74 ± 0.24[b]	25	0.66 ± 0.23[c]	35	0.10 ± 0.04[a]	-
	48	♀	0.49 ± 0.10[c]	53	0.43 ± 0.09[c]	48	0.05 ± 0.03[b]	-

N = 20 (each sample containing 4 ♂ or 4 ♀ worms); X ± S; [a]$p > 0.05$, [b]$p < 0.05$, [c]$p < 0.01$ *vs* control.

Table 3
Phosphofructokinase (PFK), phosphoglycerate kinase (PGK) and pyruvate kinase (PK) of schistosomes in mice treated intragastrically with artemether (Art). Parenthesis were the number of samples (each sample consisted of 20 ♂ or 20 ♀ worms).

Art (mg/kg)	Time after Art (h)	Sex of worm	PFK activity		PGK activity		PK activity	
			Formation of NADPH 1μ mol/min/ per worm	Inhibition (%)	Consumption of NADH 1μ mol/min/ per worm	Inhibition (%)	Consumption of NADH 1μ mol/min/ per worm	Inhibition (%)
0	0	♂	0.58 ± 0.08 (20)	-	ND	-	ND	-
		♀	0.37 ± 0.04 (20)	-	ND	-	ND	-
100	24	♂	0.52 ± 0.01^{c}(20)	10.3	ND	-	ND	-
		♀	0.17 ± 0.05^{c}(20)	54.0	ND	-	ND	-
0	0	♂	0.61 ± 0.1 (20)	-	0.58 ± 0.17 (20)	-	2.0 ± 0.7 (19)	-
		♀	0.37 ± 0.11 (20)	-	0.21 ± 0.08 (20)	-	1.11 ± 0.24 (19)	-
300	24	♂	0.51 ± 0.06^{b}(20)	16.3	0.23 ± 0.10^{c}(20)	60	1.43 ± 0.57^{c} (20)	29
		♀	0.13 ± 0.05^{c}820)	64.9	0.11 ± 0.03^{c}(18)	48	0.70 ± 0.39^{c} (20)	37
0	0	♂	0.96 ± 0.29 (20)	-	0.79 ± 0.16 (20)	-	2.2 ± 0.6 (20)	-
		♀	0.31 ± 0.14 (20)	-	0.26 ± 0.12 (19)	-	1.27 ± 0.12 (20)	-
300	48	♂	0.44 ± 0.04^{c}(20)	54.2	0.20 ± 0.07^{c}(19)	75	1.33 ± 0.28^{c}(20)	40
		♀	0.09 ± 0.04^{c}(20)	71.0	0.10 ± 0.04^{c}(15)	62	0.5 ± 0.16^{c}(20)	61

ND: not determined.
X ± S; $^{a}p > 0.05$, $^{b}p < 0.05$, $^{c}p < 0.01$ *vs* the corresponding control.

treated with a subtherapeutic dose of 100 mg/kg. For GPDH, the enzyme activities were only marginally reduced, and the enzyme activities of PGK, PK and LDH were significantly reduced in schistosomes recovered from mice treated with a therapeutic dose of 300 mg/kg. The activities of AKP and ACP were significantly reduced (greater than 60%) in female worms, with less impressive findings noted among male worms. Reductions in the three ATPase activities, (Ca^{2+})-ATPase, (Mg^{2+})-ATPase, and (Na-K)-ATPase were reduced significantly in both male and female worms at 48 hours after treatment. It is not possible to conclude from these experiments whether the decrease in these enzyme activities account for the mechanism of action for artemether, or whether they reflect non-specific changes which may occur during drug-induced parasite damage.

Glutathione S-transferase (GST): GST is an important detoxifyng enzyme for schistosomes which are exposed to high amounts of otherwise toxic organic molecules originating from the intestinal tract and entering the portal system. Because this enzyme has been shown to be a potential vaccine target (Liu *et al,* 1998), as well as a possible drug target for praziquantel (McTigue *et al,* 1995), we investigated the enzyme activity of GST in schistosomes obtained from artemether-treated mice. When infected mice were treated intragastrically with artemether 300 mg/kg for 24, 48 and 72 hours, the inhibition rates of GST in female worms were 26.2, 40.0 and 55.1%, respectively. Similar inhibition rates of GST were also seen in male worms (Table 4). The inhibitory effect of artemether on GST of schistosomes could be reversed by the addition of dithiothreitol (DTT) or with cysteine at concentrations of 1-10 mM (Xiao *et al,* 2000b).

THE MALARIA PARADIGM

The findings that artemether reduces parasite glycogen, possibly by increasing glycogen phosphorylase activity, and adversely affects the activities of glycolytic enzymes, as well as GST suggest possible approaches to pinpoint the precise mechanisms of action for this agent. Undoubtedly some of these biochemical changes are simply reflective of drug induced parasite damage. This may account for the overall reductions noted in parasite nucleic acids and protein. However, the finding that glycogen phosphorylase enzyme activity is increased suggests a possible bona fide target site of action.

Table 4
Glutathione S-transferase of schistosomes in mice treated intragastrically with artemether (Art) 300 mg/kg. Parentheses were the number of sample (each sample consisted of 10 ♂ or 10 ♀ worms).

Art (mg/kg)	Time after Art (hr)	Sex of worm	GST activity, conjugation of 1 μmol CDNB with glutathione/min/worm	Inhibition (%)
Control	0	♂	15 ± 5.2 (30)	-
		♀	4.1 ± 1.2 (30)	-
Art	24	♂	12.3 ± 3.0[c](30)	25.5
		♀	3.1 ± 0.7[c](30)	43.2
Control	0	♂	13.6 ± 2.8 (20)	-
		♀	3.0 ± 0.6 (20)	-
Art	48	♂	10.0 ± 1.5[c](20)	26.5
		♀	1.8 ± 0.5[c](20)	40.0
Control	0	♂	18.3 ± 4.2 (30)	-
		♀	4.9 ± 1.6 (30)	-
Art	72	♂	8.7 ± 2.7[c](30)	52.4
		♀	2.2 ± 0.8[c](30)	55.1

CDNB: 1-chloro-2,4-dinitrobenzene.
X ± S; [c]$p < 0.01$ *vs* control.

It is also instructive to examine comparable studies which look at the effects of arteminisin on malaria parasites. Both the parent artemisinin compound and its two more active derivatives [artesunate (a water-soluble hemisuccinate) and artemether (an oil-soluble ether)] are metabolized to a biologically active dihydroartemesinin metabolite (Klayman, 1985; Hien and White, 1993; White, 1996). The endoperoxide bridge within all three molecules is essential for its antimalarial properties. We do not as yet know if there is a similar requirement for helminthotoxcitiy. *In vitro*, artemisinin inhibits both protein and nucleic acid synthesis of malaria parasites (Gu *et al*, 1983; Li *et al*, 1983). However, some evidence also suggests that artemisinin may interact with high concentrations of heme acquired by the parasite through extensive hemoglobin digestion to generate iron-dependent free radicals which is toxic for the parasite (Krungkrai and Yuthavong, 1987; Meschnick *et al*, 1993; 1996). It has also been proposed that artemisinin may form covalent complexes with malaria-specific proteins or specific receptors (Meshnick, 1996).

Schistosomes, like malaria parasites, must ingest host hemoglobin for their nutrition. Schistosomes even contain cathepsins which are similar to malaria cathepsins, which function in the breakdown of host hemoglobin. Therefore studies are underway to determine whether artemether also interacts with hemoglobin degradation products in order to exert its helminthotoxic effects. Our preliminary data indicates that hemin has a synergistic helminthotoxic effect against schistosomulae and adult schistosomes when incubated *in vitro* with artemether. Schistosomes treated in this manner exhibit vesiculation in their tegument, in addition to dilation of their intestine. Interestingly, these worms become discolored and acquire a reddish yellow appearance (Xiao *et al*, 2000c). These data suggest that hemin might also interact with artemether to order to cleave its endoperoxide bridge and generate helminthotoxic free radicals.

ACKNOWLEDGEMENTS

This work is supported by the National Natural Science Foundation of China, Ninth Five-Year Key Research Program of China, Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Swiss Tropical Institute and the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR). Dr Peter Hotez is Chairman of Department Microbiology and Tropical Medicine, the George Washington University and supported by the grants from the NIH (AI-32726) and the Hookworm Vaccine Ini-

tiative of Albert B Sabin Vaccine Institute and the bill and Melinda Gates Foundation. Marcel Tanner is the Director of Swiss Tropical Institute.

REFERENCES

Boele van Hensbroek M, Onyiorah E, Jaffar S, *et al*. A trial of artemether or quinine in children with cerebral malaria. *N Engl J Med* 1996; 335: 69-75.

Brewer TG, Peggins JO, Grate SJ, *et al*. Neurotoxicity in animals due to arteether and artemether. *Trans R Soc Trop Med Hyg* 1994; 88: (suppl1): S33-S36.

Chen MG. Control and research on schistosomiasis in China. *Chin Med J* 1995; 108: 643-6.

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

DeStefano FR. East meets West in laboratory to treat malaria. *Infect Dis News* September 1993; 11-2.

Gu HM, Warhurst DC, Peters W. Rapid action of qinghaosu and related drugs on incorporation of [^{3}H]isoleucine by *Plasmodium falciparum in vivo*. *Biochem Pharmacol* 1983; 32: 2462-6.

Hien TT, White NJ. Qinghaosu. *Lancet* 1993; 341: 603-8.

Hotez PJ, Feng Z, Xu LQ, *et al*. Burden of emerging and reemerging helminthiases on the public health of China. *Emerg Infect Dis* 1997; 3: 303-10.

Hu LS, Xie ZW, Qiu YX, *et al*. Research on surveillance of the control strategy of schistosomiasis in different areas of Poyang Lake region. *Chin J Schisto Control* 1989; 1: 9-10.

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

Krungkrai SR, Yuthavong Y. The antimalarial action on *Plasmodium falciparum* of qinghaosu and artesunate in combination with agents which modulate oxidant stress. *Trans R Soc Trop Med Hyg* 1987; 81: 710-4.

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

Li TS, Wang TP, Qin XY, *et al*. Study on the fluctuation of epidemiological pattern of schistosomiasis after preliminary control in lake areas. *Chin J Schisto Control* 1990; 2: 24-8.

Li ZI, Gu HM, Warhurst DC, Peters W. Effects of qiinghaosu and related compounds on incorporation of [G-^{3}H] hypoxanthine by *Plasmodium falciparum in vitro*. *Trans R Soc Trop Med Hyg* 1983; 77: 522-3.

Liu SX, Song GC, Xu YX, *et al*. Progress in the development of a vaccine against schistosomiasis in China. *Int J Infect Dis* 1998; 2: 176-80.

McTigue MA, Williams DR, Tainer JA. Crystal structures of a schistosomal drug and vaccines target: glutathione S-transferase from *Schistosoma japonicum* and its complex with the leading antischistosomal drug praziquantel. *J Mol Biol* 1995; 246: 21-7.

Meshnick SR, Yang YZ, Lima V. Iron-dependent free radical generation from the antimalarial agent artemisinin (aqinghaosu). *Antimicrob Agents Chemother* 1993; 37: 1108-4.

Meshnick SR. Is haemozoin a target for antimlarial drugs? *Ann Trop Med Parasitol* 1996; 90: 367-72.

Ministry of Public Health. A brief introduction of a nationwide sampling survey on schistosomiasis [Abstract]. International Symposium on Schistosomiasis, Beijing, November 24-26, 1992.

Song Y, Xiao SH, Wu W, *et al*. Preventive effect of artemether on schistosome infection. *Chin Med J* 1998; 111: 123-7.

Tian ZY, Xiao SH, Xiao JW, *et al*. Reduction of *Schistosoma japonicum* infection in an endemic area in islet with embankment after prophylaxis with oral artemether throughout the transmission season. *Chin J Parasitol Parasitic Dis* 1977; 15: 208-11.

Wang JL, Xiao SH, Yang Z, *et al*. Effect of oral artemetehr in controlling schistosomiasis in Yunnan mountainous endemic area. *Chin J Parasitol Parasitic Dis* 1997; 15: 138-42.

White NJ. The treatment of malaria. *N Engl J Med* 1996; 335: 800-6.

Xiao SH, Catto BA, Webster LT Jr. Effects of praziquantel on different developmental stages of *Schistosoma mansoni in vitro* and *in vivo*. *J Infect Dis* 1985; 151: 1130-7.

Xiao SH, Yue WJ, Yang YQ, *et al*. Susceptibility of *Schistosoma japonicum* of different developmental stages to praziquantel. *Chin Med J* 1987; 100: 759-68.

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

Xiao SH, You JQ, Jiao PY, *et al*. Effect of early treatment of artemether against schistosomiasis in mice. *Chin J Parasitol Parasitic Dis* 1994a; 12: 7-12.

Xiao SH, You JQ, Mei JY, *et al*. Early treatment of schistosomal infection with artemether and praziquantel in rabbits. *Acta Pharmacol Sin* 1994b; 15: 447-52.

Xiao SH, You JQ, Mei JY, *et al*. Early treatment with artemether and praziquantel in rabbits repeatedly infected with *Schistosoma japonicum* cercariae. *Chin J Parasitol Parasitic Dis* 1994c; 12: 252-6.

Xiao SH, You JQ, Yang YQ, *et al*. Experimental studies on early treatment of schistosomal infection with artemether. *Southeast Asian J Trop Public Health* 1995; 26: 306-18.

Xiao SH, Shen BG, Catto BA. Effect of artemether on ultrastructure of *Schistosoma japonicum*. *Chin J Parasitol Parasitic Dis* 1996a; 14: 181-7.

Xiao SH, Shen BG, Horner J, *et al*. Tegument changes of *Schistosoma japonicum* and *Schistosoma mansoni* in mice treated with artemether. *Acta Pharmacol Sin* 1996b; 17: 535-7.

Xiao SH, Shi ZG, Zhuo SJ, *et al*. Field studies on the preventive effect of oral artemether against schistosomal infection. *Chin Med J* 1996c; 109: 272-5.

Xiao SH Wang JL, Wang CZ, *et al*. Protection of the residents from schistosome infection using oral artemether in the mountanous area. *Chin J Parasitol Parasitic Dis* 1996d; 14: 11-4.

Xiao SH, You JQ, Mei JY, *et al*. Effect of artemether on glucose uptake and glycogen content in *Schistosoma japonicum*. *Acta Pharmacol Sin* 1997; 18: 363-7.

Xiao SH, You JQ, Guo HF, *et al*. Effect of artemether on hexokinase, glucose phosphate isomerase and phosphofructokinase of *Schistosoma japonicum* habored in mice. *Chin J Parasitol Parasitic Dis* 1998a; 16: 25-8.

Xiao SH, You JQ, Guo HF, *et al*. Effect of artemether on glyceraldehyde-3-phosphate dehydrogenase, phosphglycerate kinase, and pyruvate kinase of *Schistosoma japonicum* harbored in mice. *Acta Pharmacol Sin* 1998b; 19: 279-81.

Xiao, SH, Yu JQ, Guo HF, *et al*. Effect of artemether on phosphorylase, lactate dehydrogenase, and glucosephosphate dehydrogenase of *Schistosoma japonicum* harbored in mice. *Acta Pharmacol Sin* 1999; 20: 750-4.

Xiao SH, Booth M, Tanner M. The prophylactic effect of artemether against *Schistosoma japonicum* infection. *Parasitol Today* 2000a; 16: 122-6.

Xiao SH, You JQ, Guo HF, *et al*. Effect of artemether on glutathione S transferase and superoxide dismuatse of *Schistowsoma japonicum* harbored in mice. 2000b (In preparation).

Xiao SH, Chollet J, Matile H, Mei JY, Tanner N. Artemether administered together with hemin damages schistosomes *in vitro*. *Trans R Soc Trop Med Hyg* 2000c (In preparation).

Xu MS, XiaoSH, Song Q, *et al*. Observation on the effect of artemether on controlling schistosomiasis japonica in an endemic area of marshland. *Chin J Parasitol Parasitic Dis* 1997; 15: 212-5.

You JQ, Guo HF, Mei JY, *et al*. Effect of artemether on glycogen, protein, alkaline phosphatase and acid phosphatase of *Schistosoma japonicum*. *Chin J Parasitol Parasitic Dis* 1994; 12: 275-8.

Yu SH. Control of parasitic diseases in China, current status and prospects. *Chin Med J* 1996; 109: 259-65.

Zhang SJ, Liu ZD, Zhong JH, *et al*. Further study of high transmission areas of schistosomiasis in Poyang Lake region. *Chin J Schisto Control* 1993; 5: 78-81.