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Effect of artemether on phosphorylase, lactate dehydrogenase, adenosine triphosphatase, and glucosephosphate dehydrogenase of *Schistosoma japonicum* harbored in mice[1]

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KEY WORDS *Schistosoma japonicum*; artemether; phosphorylase a, lactate dehydrogenase, glucose-phosphate dehydrogenase; adenosinetriphosphatase; NADH, NADPH oxidoreductases

ABSTRACT

AIM: To study the effect of artemether (Art) on phosphorylase (PP), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), and adenosine triphosphatase (ATPase) of *S japonicum*. **METHODS**: Mice infected with *S japonicum* cercariae for 32 - 38 d were treated ig with Art 100 - 300 mg·kg^{-1} and killed 24 - 72 h after treatment for collection of schistosomes. The activities of PP, LDH, and G-6-PDH were measured by the formation of NADH or NADPH. The activity of ATPase was measured by the rate of release of inorganic phosphate (P_i) from ATP at 37 ℃. **RESULTS**: After infected mice were treated ig with Art 300 mg·kg^{-1} for 24 - 48 h, the activities of total PP and PPa (active form) increased markedly in both male and female worms, while PPb (inactive form) showed no or only a slight increase. At 24 - 72 h after the above-mentioned mice were treated ig with Art 100 - 300 mg·kg^{-1}, the inhibitory rates of LDH and G-6-PDH were 9 % - 59 % (male) and 41 % - 75 % (female) as well as 22 % - 42 % (male) and 74 % - 89 % (female), respectively. When Art 300 mg·kg^{-1} was given to infected mice for 24 h, only the activity of Mg^{2+}-ATPase showed marked inhibition in both male and female worms. At 48 h, the Ca^{2+}-ATPase, Mg^{2+}-ATPase, and Na^{+}-K^{+}-ATPase were all inhibited, the inhibitory rates of 17 % (male) and 19 % (female), 32 % (male) and 48 % (female) as well as 29 % (male) and 44 % (female), respectively. **CONCLUSION**: In schistosomes, the increase in the activity of AMP-independent PPa induced by Art may enhance the decomposition of glycogen and the inhibition of LDH by Art could reduce the formatin of lactate. Moreover, Art exerts a potent inhibition on the G-6-PDH activity of the female *S japonicum*.

INTRODUCTION

In recent years, Artemether (Art), a derivative of artemisinin, has become recognized as a promising chemoprophylactic agent against schistosome infection. Understanding the mode of action by which schistosomes are attacked and damaged is an important prerequisite for the development of novel drugs against schistosome infection. However, knowledge of the action mechanism of Art is very limited. In our previous studies[1-3], the effect of artemether (Art) on the activities of several enzymes involved in glycolysis of *Schlstosoma japonicum* had been measured. The results suggested that phosphofructose kinase (PFK), phosphoglycerate kinase (PGK), and pyruvate kinase (PK) might be the important targets attacked by artemether. Subsequenatly, Wu *et al*[4] reported that artesunate, another derivative of artemisinin, could inhibit the activities of malic dehydrogenase, 6-phosphate mannosease and acid phosphatase of *S japonicum* schistosomules. Gong *et al*[5] reported that

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Art inhibited the proteinase of *S japonicum*. In this paper we studied the effect of artemether on phosphorylase (PP), lactate dehydrogenase (LDH), adenosine triphosphatase (ATPase), and glucose-6-phosphate dehydrogenase (G-6-PDH) of *S japonicum*.

MATERIALS AND METHODS

Parasite *S japonicum* cercariae released from *Oncomelania hupensis* infected artificially with miracidia (Anhui isolate) was provided by the Department of Vector Biology and the Control of our Institute.

Mice Kunming strain mice ($n = 685$), Grade Ⅱ, weighing 18-22 g were provided by animal facilities of our Institute (Certificate № 02-32-1). Each mouse was infected with 60 – 80 cercariae. Mice were divided into groups 32 – 36 d after infection for treatment with Art 100 or 300 mg·kg^{-1}. Mice were killed 24, 48, or 72 h after medication for collection of worms by perfusion with ice-cold Hanks' balanced salt solution (HBSS) and kept in ice bath, the worms were rinsed with HBSS for 3 times.

Drugs and reagents Art was produced by Kunming Pharmaceutical Corporation (lot № 880701) and suspended in 1 % tragacanth at concentrations of 10 – 30 g·L^{-1}. The volume given intragastrically (ig) was 10 mL·kg^{-1}. Adenosine triphosphate (ATP, disodium salt), adenosine diphosphate (ADP, disodium salt), and adenosine monophosphate, coenzyme NAD with a purity of 90 %, NADH (disodium salt with a purity of >90 %), and NADP with a purity of >90 % were products of Shanghai Dongfeng Biochemical Technique Company. Glucose-6-phosphate (G-6-P), glucose-1,6-diphosphate, glutathione, ouabain, glycogen, glucose-6-phosphate dehydrogenase (G-6-PDH), phosphoglucomutase were purchased from Sigma. Other reagents were all of AR grade.

Worm homogenate Ten to twenty male or female worms were placed in a glass homogenizer containing 1 mL of phosphate buffer (pH 7.4) or Tris-HCl buffer (pH 7.4) in ice bath. After centrifugation (1200 × *g*, 4 ℃, 15 min), the supernatant was stored in ice bath.

PP measurement The tube containing glycogen, NADP, glucose-1,6-diphosphate, with or without adenosine monophosphate, $MgCl_2$, and sodium edetate was warmed at 30 ℃ for 5 min. The worm homogenate, phosphoglucomutase, and G-6-PDH were then added, and the absorbances at 340 nm were measured at 10 s and 30 min by the formation of NADPH[6].

LDH measurement The enzyme reaction system containing sodium lactate and NAD in glycerine buffer 0.1 mol·L^{-1} was preincubated at 37 ℃ for 5 min and the absorbance was measured at 340 nm. The worm homogenates preincubated at 37 ℃ for 20 min were then added to the reaction system and the absorbance at 340 nm was measured again at 5 min by the formation of NADH[6].

ATPase measurement ATPases of *S japonicum* including Mg^{2+}-ATPase, Ca^{2+}-ATPase, and Na^+-K^+-ATPase were measured[7,8].

G-6-PDH measurement The tube containing $MgCl_2$, NADP, and G-6-P was warmed at 30 ℃ for 5 min. The worm homogenate was then added into the tube and the absorbances at 340 nm were then measured at 20 s and 5 min by the formation of NADPH[6].

RESULTS

PP In *S japonicum*-infected mice treated ig with Art at a subcurative dose of 100 mg·kg^{-1}, the activities of total PP, PPa, and PPb increased markedly with the increase rates of 50 %, 50 %, and 33 %, respectively, in female worms as compared with the control. No marked increase in the activities of PP, PPa, and PPb was seen in male worms (Tab 1).

When the infected mice were treated ig with Art at a curative dose of 300 mg·kg^{-1} for 24 and 48 h the activities of total PP and PPa increased markedly in both male and female worms. While for PPb, at 24 h after treatment, the increase was only seen in female worms but not in male ones (Tab 1).

LDH When infected mice were treated ig with Art at a curative dose of 300 mg·kg^{-1} for 24 h, no apparent impact on LDH of the male worms was seen, while that of the female worms was showed an inhibitory rate of 41 %. At 72 h after treatment the LDH activity of both male and female worms was inhibited by 59 % and 75 %, respectively (Tab 2).

G-6-PDH In infected mice treated ig with artemether at a subcurative dose of 100 mg·kg^{-1} for 24 h, the G-6-PDH of male and female worms was

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Tab 1. Phosphorylase (PP) of schistosomes in mice treated ig with artemether (Art). The enzyme activity was expressed as formatin of NADPH 1 μmol·min^{-1} per worm. $n = 20$ (each sample containing 4 ♀ or 4 ♂ worms). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* the corresponding control.

Art/ mg·kg^{-1}	Time after Art/h	Worm	Total PP Activity	Total PP Increase/%	PPa Activity	PPa Increase/%	PPb Activity	PPb Increase/%
0	0	♂	0.65 ± 0.10	–	0.43 ± 0.90	–	0.20 ± 0.10	–
0	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
100	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	♀	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
300	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 ± 0.06	–
0	0	♀	0.32 ± 0.06	–	0.29 ± 0.09	–	0.05 ± 0.03	–
300	48	♂	0.74 ± 0.24^b	–	0.66 ± 0.23^c	35	0.10 ± 0.04^a	–
300	48	♀	0.49 ± 0.10^c	53	0.43 ± 0.09^c	48	0.05 ± 0.03^a	–

inhibited by 22 % and 77 %, respectively. Similar inhibitory effects of artemether given at a dose of 300 mg·kg^{-1} on G-6-PDH of both male and female worms were seen 24 h after treatment, but higher inhibitory rates of 42 % and 89 % were detected in male and female worms, respectively at 48 h after treatment (Tab 2).

ATPase The activities of Ca^{2+}-ATPase and Mg^{2+}-ATPase in both male and female worms were higher than that of Na^+-K^+-ATPase. After infected mice were treated ig with Art at a curative dose of 300 mg·kg^{-1} for 24 h, inhibition of Mg^{2+}-ATPase was seen in both male and female worms with inhibitory rates of 19 % and 40 %, respectively. Inhibition of

Tab 2. Lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) of schistosomes in mice treated ig with artemether (Art). LDH activity was expressed as formation of NADH 1 μmol·min^{-1} per worm; G-6-PDH activity was expressed as formation of NADPH 1 μmol·min^{-1} per worm. Parentheses were the number of samples (each sample containing 4 ♀ or 4 ♂ worms). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* the corresponding control.

Art/ mg·kg^{-1}	Time after Art/h	Worm	LDH activity/ μmol·min^{-1} per worm	Inhibition/%	G-6-PDH activity/ μmol·min^{-1} per worm	Inhibition/%
0	0	♂	ND	–	1.61 ± 0.18 (20)	–
0	0	♀	ND	–	0.52 ± 0.11 (20)	–
100	24	♂	ND	–	1.25 ± 0.22 (20)^c	22
100	24	♀	ND	–	0.12 ± 0.03 (20)^c	77
0	0	♂	0.94 ± 0.48 (72)	–	0.97 ± 0.19 (20)	–
0	0	♀	1.16 ± 0.46 (65)	–	0.42 ± 0.17 (20)	–
300	24	♂	0.86 ± 0.54 (66)^a	9	0.68 ± 0.13 (20)^c	30
300	24	♀	0.69 ± 0.42 (71)^c	41	0.11 ± 0.05 (20)^c	74
0	0	♂	ND	–	1.28 ± 0.11 (20)	–
0	0	♀	ND	–	0.78 ± 0.12 (20)	–
300	24	♂	ND	–	0.74 ± 0.11 (20)^c	42
300	24	♀	ND	–	0.09 ± 0.04 (20)^c	89
0	0	♂	1.17 ± 0.71 (45)	–	ND	–
0	0	♀	1.45 ± 0.84 (54)	–	ND	–
300	72	♂	0.48 ± 0.44 (51)^c	59	ND	–
300	72	♀	0.36 ± 0.22 (35)^c	75	ND	–

Ca^{2+}-ATPase was only seen in male worms, while Na^+-K^+-ATPase of the worms was not significantly affected although the inhibitory rate of 28 % was seen in female worms. At 48 h after treatment, the activities of the above-mentioned 3 ATPases were inhibited markedly in both male and female worms, and the inhibitory rates were higher in Mg^{2+}-ATPase and Na^+-K^+-ATPase but less in Ca^{2+}-ATPase (Tab 3).

DISCUSSION

In previous papers we indicated that the glycogen content of schistosomes decreased significantly after treatment with Art and such decrease might be related to an inhibition of the glycolysis induced by Art rather than an interference with the glucose uptake[11]. Our earlier study also demonstrated that Art strongly inhibited the three key enzymes involved in glycolysis, *ie* PFK, PGK and PK of *S japonicum* which resulted in reduction of energy supply and consumption of more glucose for generation of the energy. As *S mansoni*[8] the glycogen regulated by PPa (active type) and PPb (inactive type) is the major energy reserve in *S japonicum*. Therefore, in Art-treated worms, the glycogen PP remained sustainedly active, resulting in depletion of glycogen. This might account for the significant reduction of the glycogen content after treatment with Art, being similar to the effect of niridazole on schistosomes and the effect of amoscanate on *Hymenolopis diminuta*[9,10]. In addition, the activity of LDH was also inhibited by Art significantly, this might explain why the generation of lactate, the end product of glycolysis decreased significantly during Art treatment. As to ATPases, Mg^{2+}-ATPase of schistosomes was more sensitive to Art than the other two ATPases, although Na^+-K^+-ATPase was also inhibited and emerged late after treatment. Since the extent to which the ATPase was inhibited was lower than 50 %, it seemed that the ATPase of the worm might not be the major site attacked by Art[11]. Another effect of Art detected biochemically was the marked inhibition of schistosomal G-6-PDH. It is known that both *S japonicum* and *S mansoni* possess G-6-PDH linking to the pentose phosphate pathway and the main role of this pathway is to provide NADPH and pentose[12]. The activity of G-6-PDH was present in both the male and female worms and inhibited by Art, and the inhibitory action of Art on the female worms was 2.5 times greater than that on the male worms, indicating that Art interfered with extensive biochemical metabolism of female schistosomes.

Tab 3. Adenosine triphosphatase (ATPase) of schistosomes in mice treated ig with artemether (Art). The enzyme activity was expressed as formation of P_i 1 μmol · h^{-1} per worm. Each sample containing 4 ♀ or 4 ♂ worms. $n = 20$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* the corresponding control.

Art/ mg·kg^{-1}	Time after Art/h	Worm	Ca^{2+}-ATPase activity	Inhibition/ %	Mg^{2+}-ATPase activity	Inhibition/ %	K^+-Na^+-ATPase activity	Inhibition/ %
0	0	♂	127 ± 19	–	196 ± 47	–	33 ± 15	–
0	0	♀	143 ± 21	–	169 ± 39	–	29 ± 19	–
300	24	♂	109 ± 18^c	14	159 ± 30^b	19	32 ± 11^a	3
300	24	♀	143 ± 13^a	–	102 ± 13^c	40	21 ± 7^a	28
0	0	♂	84 ± 16	–	131 ± 43	–	41 ± 11	–
0	0	♀	106 ± 25	–	114 ± 28	–	39 ± 12	–
300	48	♂	70 ± 15^b	17	89 ± 20^c	32	29 ± 9^a	2
300	48	♀	86 ± 11^c	19	59 ± 24^c	48	22 ± 6^c	44

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蒿甲醚对小鼠体内日本血吸虫磷酸化酶、乳酸脱氢酶、三磷酸腺苷酶和磷酸葡萄糖脱氢酶的影响[1]

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关键词 日本血吸虫；蒿甲醚；磷酸化酶a；乳酸脱氢酶；磷酸葡萄糖脱氢酶；三磷酸腺苷酶；NADH、NADPH氧化还原酶类

目的：观察蒿甲醚（Art）对日本血吸虫磷酸化酶（PP）、乳酸脱氢酶（LDH）、6-磷酸葡萄糖脱氢酶（G-6-PDH）和三磷酸腺苷酶（ATPase）的影响。**方法**：感染32-38天的小鼠于灌服Art 100-300 mg·kg^{-1}后24-72 h剖杀，收集雌（♀）、雄虫（♂），按NADH和NADPH的形成和无机磷的释放量测定虫的上述4种酶。**结果**：感染小鼠用Art 300 mg·kg^{-1}治疗后24-48 h，♀、♂虫的总PP和PPa（激活型）活力明显增加，而PPb则无或仅有轻度增加。上述小鼠用Art 100-300 mg·kg^{-1}治疗后24-72 h，LDH和G-6-PDH的抑制率分别为9 %-59 %（♂）和41 %-75 %（♀）及22 %-42 %（♂）和74 %-89 %（♀）。用300 mg·kg^{-1}治疗后24 h，仅虫的Mg^2-ATPase明显受抑制，48 h后，Ca^{2+}-ATPase、Mg^{2+}-ATPase和Na^+-K^+-ATPase分别抑制17 %（♂）和19 %（♀），32 %（♂）和48 %（♀），及29 %（♂）和44 %（♀）。**结论**：Art引起血吸虫PPa活力的增加，使虫的糖原分解，并抑制LDH使虫糖酵解的终产物乳酸明显减少。此外，对血吸虫♀虫的G-6-PDH有明显的抑制作用。

（责任编辑 朱倩蓉）