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Effect of artemether on glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and pyruvate kinase of *Schistosoma japonicum* harbored in mice[1]

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KEY WORD *Schistosoma japonicum*; artemether; glyceraldehyde-3-phosphate; NADH, NADPH oxidoreductases; phosphoglycerate kinase; pyruvate kinases; lactates

AIM: To study the effect of artemether (Art) on glyceraldehyde-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), and pyruvate kinase (PK) of *S japonicum*. **METHODS**: Mice infected with schistosome cercariae for 32 - 38 d were treated ig with Art 100 - 300 mg·kg^{-1} and killed 24 - 72 h after medication for collection of schistosomes. The activities of GAPDH, PGK, and PK of the worms were determined by measuring the formation of NADH or consumption of NAD. The lactate content of the worms was also measured. **RESULTS**: After the infected mice were treated ig with Art 300 mg·kg^{-1} for 24 h, the inhibition rates of GAPDH were 13 % (♂) and 21 % (♀), and 48 h later the inhibition rates of the enzyme were 6 % (♂) and 28 % (♀). When Art 300 mg·kg^{-1} was given to infected mice for 24 h and 48 h, the inhibition rates of PGK were 60 % (♂) and 48 % (♀) as well as 75 % (♂) and 62 % (♀), respectively. Similar results were seen in PK activity. At 72 h after treatment the reduction rate of lactate content in ♀ worm was 72 %, while that of ♂ was 48 %. **CONCLUSION**: In the glycolytic pathway of both ♂ and ♀ schistosomes, PGK and PK activities were inhibited by Art. The GAPDH activity of ♀ worms was also susceptible to Art, while that of ♂ worms showed only temporary inhibition after treatment with Art. The Art reduced lactate content more in ♀ than in ♂ worms.

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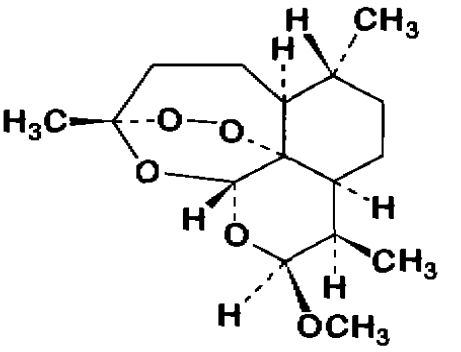
Artemether (Art), a derivative of artemisinin, was first synthesized by Shanghai Institute of Materia Medica, Chinese Academy of Sciences[1] and found to be effective against schistosomes[2].



Artemether

In *S japonicum*, we found the inhibitory effect of Art on phosphofructokinase which might be one of the targets attacked by Art[3]. The present communication was specifically directed at the effect of Art on glyceraldehyde-3-phosphate degydrogenase (GAPDH), phosphoglycerate kinase (PGK), and pyruvate kinase (PK) of the glycolytic pathway of *S japonicum*.

MATERIALS AND METHODS

Parasite An Anhui isolate of *S japonicum* cercariae released from artificially infected *Oncomelania hupensis* snails was provided by the Department of Vector Biology of this Institute.

Mice Kunming strain mice (n = 480, ♀ and ♂), weighing 20 - 24 g were obtained from the Animnal Facilities of this Institute (Certificate № 02-32-1). Mice were each infected with 60 - 80 cercariae and divided into groups 32 - 38 d after infection for treatment with Art 100 or 300 mg·kg^{-1}. Mice were killed 24 or 48 h after medication for collection of worms by perfusion with ice-cold Hanks' balance salt solution (HBSS) and kept in ice bath. The worms were rinsed with HBSS for 3 times.

Worm homogenate Ten to twenty ♀ or ♂ worms were placed in a glass homogenizer containing 1 mL of distilled water, HBSS or Tris-HCl buffer (pH 7.4) in ice bath. After centrifugation (1200 × g, 4 ℃, 20 min), the supernatant was stored in ice bath.

Drugs and reagents Art was the product from Kunming Pharmaceutical Corp (lot No 880701). Art was suspended 10 or 30 $g \cdot L^{-1}$ in 1 % tragacanth and given to mice by intragastric gavage (ig) 10 $mL \cdot kg^{-1}$.

Glyceraldehyde-3-phosphate (GAP), phosphoenolpyruvate (PEP), and adenosine triphosphate (ATP, disodium salt) were the products of Sigma. Coenzyme NADH (disodium salt with a purity of > 70 %), coenzyme NAD with a purity of > 90 % and adenosine diphosphate (disodium, ADP) were the products of Shanghai Dong Feng Biochemical Technique Co. Other reagents were all of AR grade.

GAPDH measurement The homogenate containing sodium pyrophosphate, tertiary sodium phosphate, 1,4-dithiothreitol (DTT), and NAD was preincubated at 25 ℃ for 5 min. The GAP was then added and the absorbance at 340 nm was measured at 10 s and 3 min for the formation of NADH[4].

PGK measurement The tube containing ATP, edetic acid-Na_2, NADH, and GAP was warmed to 37 ℃. The worm homogenate was added and the absorbance at 340 nm was measured at 10 s and 5 min for the consumption of NAD[4].

PK measurement The tube containing ADP, NADH, and PEP was warmed to 30 ℃. The worm homogenate was added and the absorbance at 340 nm was measured at 10 s and 4 min for the formation of NADH[4].

Lactate measurement The infected mice treated ig with Art 300 $mg \cdot kg^{-1}$ were killed at 24 or 72 h and the worms were collected by perfusion with ice-cold HBSS. The lactate content in the worm homogenate containing 20 ♀ or ♂ worms were measured[5].

RESULTS

GAPDH In infected mice treated ig with Art 100 $mg \cdot kg^{-1}$ for 24 h, the GAPDH activities of ♀ and ♂ worms were decreased 18 % and 26 %, respectively. When Art was given 300 $mg \cdot kg^{-1}$, no further inhibition of GAPDH activity was seen. The same was true as the GAPDH activity of ♂ was measured 48 h later, whereas that of ♀ worm was inhibited with an inhibitory rate of 28 % (Tab 1).

PGK When infected mice were treated ig with Art 300 $mg \cdot kg^{-1}$, the PGK activities of ♀ and ♂ worms were inhibited 60 % and 48 %, respectively. Further inhibition of PGK activity was seen at 48 h after medication (Tab 1).

PK After infected mice were treated ig

Tab 1. Glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and pyruvate kinase of schistosomes in mice treated ig with artemether (Art). Parentheses were the number of samples (each sample contained 20 ♀ or ♂ worms). $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Art/ $mg \cdot kg^{-1}$	Time after Art/h	Worms	GAPDH activity Formation of NADH 1 $\mu mol \cdot min^{-1}$ per worm	Inhibition /%	PGK activity Consumption of NADH 1 $\mu mol \cdot min^{-1}$ per worm	Inhibition /%	PK activity Consumption of NADH 1 $\mu mol \cdot min^{-1}$ per worm	Inhibition /%
0	0	♂	6.6 ± 0.1 (20)	-	-	-	-	-
	0	♀	4.7 ± 0.6 (20)	-	-	-	-	-
100	24	♂	5.4 ± 0.8^b (20)	18	-	-	-	-
	24	♀	3.5 ± 0.7^c (20)	26	-	-	-	-
0	0	♂	6.1 ± 0.9 (20)	-	0.58 ± 0.17 (20)	-	2.0 ± 0.7 (19)	-
	0	♀	4.8 ± 0.5 (20)	-	0.21 ± 0.08 (20)	-	1.11 ± 0.24 (19)	-
300	24	♂	5.3 ± 0.1^b (20)	13	0.23 ± 0.10^c (20)	60	1.43 ± 0.57^c (20)	27
	24	♀	3.8 ± 0.6^c (20)	21	0.11 ± 0.03^c (18)	48	0.70 ± 0.39^c (20)	37
0	0	♂	6.3 ± 0.4 (20)	-	0.79 ± 0.16 (20)	-	2.2 ± 0.6 (20)	-
	0	♀	4.7 ± 0.7 (20)	-	0.26 ± 0.12 (19)	-	1.27 ± 0.12 (20)	-
300	48	♂	5.9 ± 0.4 (20)	6	0.20 ± 0.07^c (19)	75	1.33 ± 1.28^c (20)	40
	48	♀	3.4 ± 0.7 (20)	28	0.10 ± 0.04^c (15)	62	0.5 ± 0.16^c (20)	61

with Art 300 mg·kg^{-1}, the PK activities of ♀ and ♂ worms were inhibited 37 % and 27 %, respectively. At 48 h later, the PK activities of the ♀ and ♂ worms were further decreased to 61 % and 40 %, respectively (Tab 1).

Lactate When infected mice were received Art at 300 mg·kg^{-1} for 24 h, the lactate content of ♂ worms was similar to that of the control. At 72 h, the lactate content of ♂ worms was decreased to 49 %. In ♀ worms the lactate contents were decreased to 49 % and 72 % at 24 h or 72 h, respectively (Tab 2).

Tab 2. Effect of artemether (Art) on lactate content of schistosomes harbored in mice. Each sample contained 20 ♀ or ♂ worms. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

Group	Time after Art/h	Worm	Lactate Samples	Lactate μg/worm	Reduction rate/%
Control	24	♂	18	0.52 ± 0.10	–
		♀	18	0.36 ± 0.08	–
Art		♂	18	0.46 ± 0.16^a	12
		♀	18	0.18 ± 0.07^c	50
Control	72	♂	20	0.68 ± 0.16	–
		♀	20	0.36 ± 0.07	–
Art		♂	19	0.35 ± 0.10^c	49
		♀	20	0.10 ± 0.06^c	72

DISCUSSION

Although the GAPDH activities of both ♀ and ♂ worms were inhibited significantly by Art given to the schistosome-infected mice, the inhibition ranges were low and no further inhibition was seen either in increasing the dosage of Art or extending the observation period. Thus, the less inhibition of the GAPDH might not severely interference with the glycolysis of the worms. However, in both ♀ and ♂ worms exposed to Art in infected mice at a curative dose, the PGK and PK activities were inhibited significantly 24 h after medication and further inhibition sustained relatively for as long as 48 h, suggesting that Art exerted its action selectively on both PGK and PK, providing further support that glycolytic pathway of *S japonicum* is a vulnerable target for selective inhibition by Art.

Previously we have demonstrated that the lactate dehydrogenase of schistosomes harbored in mice treated with Art showed significant inhibition[6], the end product of glycolysis, ie, lactate was measured. It was found that 24 h after a curative dose of Art was given to the infected mice, the lactate content of ♂ worms decreased significantly 48 h later, while in ♀ worms the lactate contents were significantly lower than those of the control at a reduction rate of over 70 %, being in accordance with the difference in susceptibility of PK and PFK of glycolysis to Art between ♀ and ♂ worms[3].

REFERENCES

1 Li Y, Yu PL, Chen YX, Li LQ, Gai YZ, Wang DS, *et al*. Synthesis of some derivatives of artemisinine. Kexue Tongbao 1979; 24: 667 – 9.

2 Le WJ, You JQ, Yang YQ, Mei JY, Guo HF, Yang HZ, *et al*. Studies on the efficacy of artemether in experimental schistosomiasis. Acta Pharm Sin 1982; 17: 187 – 93.

3 Xiao SH, You JQ, Guo HF, Jiao PY, Mei JY, Yao MY, *et al*. Effect of artemether on hexokinase, glucose phosphate isomerase and phosphoructokinase of *Schistosoma japonicum* harbored in mice. Chin J Parasitol Parasitic Dis 1998; in press.

4 McManus DP, Symth JD. Intermediary carbohydrate metabolism in protoscoleces of *Echinococcus granulosus* (horse and sheep strains) and *E multilocularis*. Parasitology 1982; 84: 351 – 66.

5 Huang TY, Chu CH. *In vitro* studies on glycolysis of *Schistosoma japonicum*. Acta Biochem Sin 1959; 2: 102 – 10.

6 You JQ, Guo HF, Mei JY, Jiao PY, Feng JJ, Yao MY, *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.

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蒿甲醚对小鼠体内日本血吸虫 3-磷酸甘油醛脱氢酶、磷酸甘油酸激酶和丙酮酸激酶的影响[1]

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关键词 日本血吸虫; 蒿甲醚; 甘油醛 3-磷酸; NADH, NADPH 氧化还原酶类; 磷酸甘油酸激酶类; 丙酮酸激酶类; 乳酸盐类

目的: 研究蒿甲醚(Art)对日本血吸虫 3-磷酸甘油醛脱氢酶(GAPDH)、磷酸甘油酸激酶(PGK)和丙酮酸激酶(PK)的影响. **方法**: 小鼠感染血吸虫尾蚴 32 – 38 d 后 ig Art 100 – 300 mg·kg^{-1}, 24 – 72 h 后取虫测定上述 3 种酶和乳酸含量. **结果**: 小鼠 ig Art 300 mg·kg^{-1}后 24 – 48 h, 血吸虫♀、♂虫的 PGK 和 PK 活力被抑制 27 % – 48 %; ♀虫的 GAPDH 对 Art 亦较敏感, ♂虫则否. 给药后 72 h, ♀虫乳酸含量降低 72 %, ♂虫的降低 49 %. **结论**: Art 对血吸虫的 PGK 和 PK 活力有明显抑制作用, ♀虫的 GAPDH 对 Art 亦较敏感, ♀虫乳酸含量降低较♂虫明显.