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Effect of artmether, hemin and Fe^{3+} on recombinant lactate dehydrogenase from *Schistosoma japonicum*

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ABSTRACT

Objective: To explore antischitosome effects of artemether, hemin and Fe^{3+} on *Sj*LDH. **Methods:** Enzyme activity of r*Sj*LDH was assayed in the standard reaction system by adding different concentration of reagents (0.00–0.10 mM artemether, 0.00–0.02 mM hemin, 0.00–0.50 mM Fe^{3+}). Same solvents of the each reagent were used as control. **Results:** There was no enzyme activity inhibition observed at 0.10 mM artemther; obivious inhibition for lactate oxidation reaction and pyruvate reduction reaction were detected at 0.002 mM and 0.004 mM of hemin, respectively; comparing with that of the control ($P<0.05$). The relative enzymatic activity inhibitions for pyruvate reduction reaction and lactate oxidation reaction at 0.02 mM hemin were 93.48% and 100.00%, respectively, comparing with that of the control ($P<0.01$); both pyruvate reduction and lactate oxidation reaction were inhibited completely at 0.50 mM Fe^{3+}, comparing with that of the control ($P<0.01$). **Conclusions:** The results implied that *Sj*LDH was not the direct molecular target of artemether. Hemin and Fe^{3+} are inhibitors of *Sj*LDH.

1. Introduction

Schistosomiasis remains as a major public health problem in the world. More than 600 million people in 74 countries in the tropical areas are under the risk of infection. Approximately 200 million people are infected currently, and 120 million are symptomatic[1]. Potential vaccine strategies against schistosomiasis continue to be investigated and public health measures can be important for reducing incidence of the disease[2]. The treatment of schistosomiasis remains dependent on chemotherapeutic intervention, particularly with praziquantel (PZQ), the key drug for chemotherapy since 1980s[3]. But the major weakness of PZQ, lack in efficacy against juvenile schistosoma can cause rapid re-infection after treatment[4]. Some direct epidemic evidences on resistance or low sensitivity to PZQ had been reported[5-7]. These reports made scientists to find out new drugs to prevent and cure schistosomiasis.

In 1980′s, antischistosomal properties of the artemisinin and its derivatives (artemether, artesunate, *etc*), key drugs of antimalaria especially to multidrug resistant malaria, was discovered by Chinese scientists[8]. Since then, notable progress has been made in antischistosoma studies with artemether and artesunate[9-13]. Because artimisinins were efficacy to the juvenile stages of the schistosomes, which overcome the weakness of PQZ, that makes artemther and artesunate to be the chemoprophylactic drugs against schistosomiasis.

The antischistosome mechanism of artemisinins has not yet been elucidated. The most striking biochemical changes measured in the schistosomes collected from infected mice treated with artemether were the reductions in their glycogen content and almost all glycolytic enzymes were inhibited different extent[14,15]. Lactate dehydrogenase in schistosomes was also reduced substantially after artemether administration[15]. It implied that glycolysis pathway or glycolytic enzymes might be the target(s) of artemisinins. Other works proved that hemin or Fe plays an important role in the action of the artimisinins *in vitro*[16-18].

In our previous work, lactate dehydrogenase from

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Schistosome japonicum (*Sj*LDH) was cloned and expressed in *Escherichia coli (E. coli)*, the enzymatic characterization of the recombinant *Sj*LDH (r*Sj*LDH) was described[19]. In this study, we focus on the effect of artemether, hemin and Fe^{3+} on the r*Sj*LDH.

2. Materials and methods

2.1. Preparation of the stock solution

Artemether (Kunming Pharma. Corp., China) was dissolved in DMSO, then used Hank's solution to prepare 5 mM stock solution. The stock solution was serially diluted with the Hank's solution to make various concentrations of 0.02, 0.04, 0.06, 0.08 and 0.10 mM. Same concentration of DMSO in Hank's solution used as a control (DMSO in reaction system was not exceeded 0.1%).

Hemin (Guangzhou Whiga Technology Co., Ltd, China) was dissolved in 5 mM NaOH solution to prepare 5 mM stock solution. Hemin was examined at concentration of 0.002, 0.004, 0.006, 0.008, 0.010, 0.015 and 0.020 mM. Five mM NaOH was used as control.

Fe^{3+} ($FeCl_3$, Guangzhou Chemical Reagent Factory, China) was dissolved in distilled water to prepare 5 mM stock solution and examined at concentration of 0.10, 0.20, 0.30, 0.40 and 0.50 mM. Distilled water was used as control.

2.2. Preparation of rSjLDH and enzymatic assay for the effect of reagents on rSjLDH

r*Sj*LDH preparation and enzymatic assay for the effect of the reagents on r*Sj*LDH were performed by the methods described before[19]. Various concentrations of reagents were added in the standard system. The reaction was started by addition of enzyme and incubated at 37 ℃ by following changes in optical density at 340 nm, $\varepsilon = 6.22\ mM^{-1}cm^{-1}$. Enzymatic activity was measured with a DU530 DNA/Protein Analyzer (Beckman, USA) for 1 min after starting the reaction. Each reaction was done for 3 times.

2.3. Statistical analyses

Arithmetic mean and standard error (mean±SE) of the changes in optical density at 340 nm (△OD) of each reaction and the significance of drug influence (student *t* test) on LDH activity were computed using the SPSS13.0 statistical software. Relative enzymatic activity (%) of drug or control group was computed used that of the blank group as 100%, figures were formed by OriginPro 7.5SR1 software.

3. Results

3.1. Effect of artemether on rSjLDH

The effect of artemether on reversible reaction catalyzed by r*Sj*LDH was shown in Table 1 and Figure 1. Compared with that of the control, at concentration 0.02–0.10 mM of artemether, inhibitions of pyruvate reduction and lactate oxidation reaction were not observed.

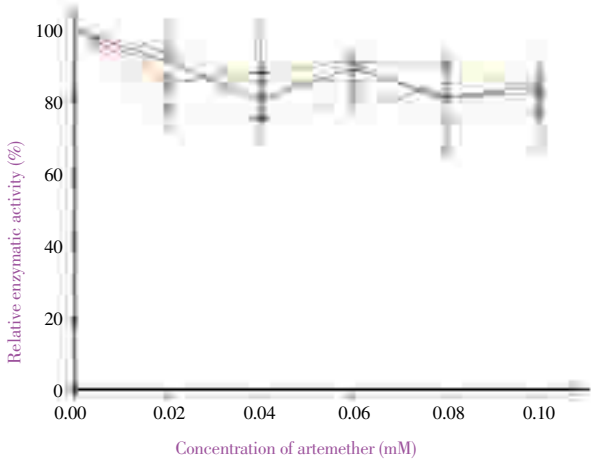

Figure 1. Effect of artemether on pyruvate reduction and lactate oxidation reaction catalyzed by r*Sj*LDH.
△: Reduction Reaction; ○: Oxidation Reaction; —: PZQ; ---: Control.

3.2. Effect of hemin on rSjLDH

The effect of hemin on reversible reaction catalyzed by r*Sj*LDH was shown in Table 2 and Figure 2. Obivious inhibition for lactate oxidation reaction and pyruvate reduction reaction were detected at 0.002 mM and 0.004 mM of hemin respectively, compared with that of the control ($P<0.05$). The relative enzymatic activity inhibitions for pyruvate reduction reaction and lactate oxidation reaction at 0.02 mM hemin were 93.48% and 100.00%, respectively, compared with that of control ($P<0.01$).

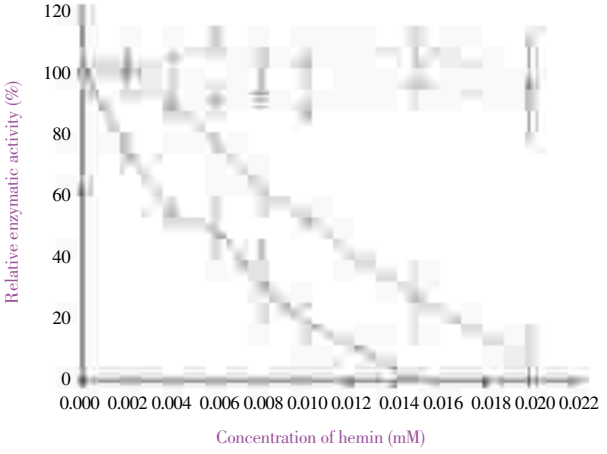

Figure 2. Effect of hemin on pyruvate reduction and lactate oxidation reaction catalyzed by r*Sj*LDH.
△: Reduction Reaction; ○: Oxidation Reaction; —: PZQ; ---: Control.

3.3. Effect of Fe^{3+} on rSjLDH

The effect of Fe^{3+} on r*Sj*LDH was shown in Figure 3 and

Table 3. Obvious inhibition for oxidation reaction and reduction reaction were observed at 0.1mM and 0.2mM, respectively, compared that of the control (P<0.05). The enzymatic activities for both reaction were completely inhibited by 0.5 mM Fe^{3+}, compared with that of the control (P<0.01).

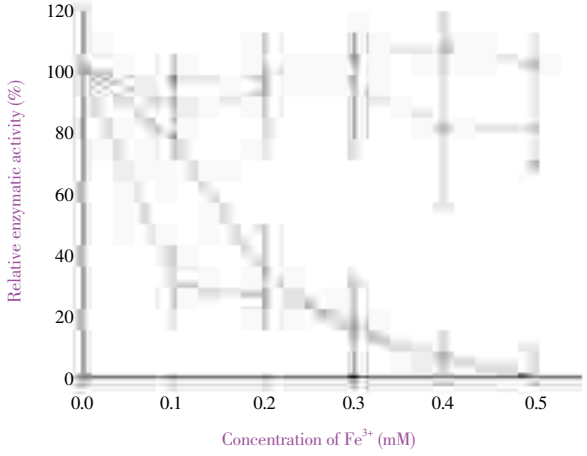

Figure 3. Effect of Fe^{3+} on pyruvate reduction and lactate oxidation reaction catalyzed by r*Sj*LDH.
△: Reduction Reaction; ○: Oxidation Reaction; —: PZQ; ---: Control.

4. Discussion

It is known that parasitic stages of schistosoma largely depend on anaerobic energy metabolism[20]. Glycolysis is essential for the survival of the parasite and glycolytic enzymes appear to be potential targets for chemotherapeutic attacks. LDH is a terminal glycolytic enzyme, which catalyzes the interconversion reaction of pyruvate and lactate in the presence of the nicotinamide adenine dinucleotide coenzyme. Studies on LDH from plasmodium (*p*LDH) and *Toxoplasma gondii* (LDH_1, LDH_2) had shown that they have distinct properties from that of the hosts and appear to be an attractive target for therapy[21,22]. Works on artemether were reported that it can inhibit the activity of the most glycolytic enzymes include LDH in schistosomes collected from artemether-treated mice[23]. These implied that these enzymes may serve as the targets of artemther.

About the mechanism of the artemether on schistosomes, scientists presumed that it might be similar to that of for malaria[8]. Artemisinins are activated within the parasites by intraparasitic heme-iron or other iron system, which results in the cleavage of the endoperoxide bridges and generation of free radicals. These free radicals then may form covalent bounds with specific proteins or receptors and kill the parasites[24].

Table 1
Effect of artemether on r*Sj*LDH.

Reaction group		△OD/min of the enzymatic reaction at each drug concentration					
		0 mM	0.02 mM	0.04 mM	0.06 mM	0.08 mM	0.1 mM
R	Art	0.111±0.007	0.101±0.006	0.089±0.006	0.099±0.004	0.090±0.014	0.094±0.008
	Ctr		0.104±0.008	0.095±0.003	0.095±0.004	0.084±0.011	0.085±0.010
O	Art	0.108±0.003	0.092±0.009	0.095±0.014	0.098±0.003	0.097±0.001	0.089±0.005
	Ctr		0.084±0.006	0.081±0.007	0.087±0.004	0.089±0.003	0.088±0.006

R: Reduction reaction; O: Oxidation reaction; Art: artemether; Ctr: control (DMSO+Hank's solution). Data presented in the table are means of three determinations±SE.

Table 2
Effect of hemin on r*Sj*LDH.

Reaction	group	△OD/min of the enzymatic reaction at each drug concentration							
		0 mM	0.002 mM	0.004 mM	0.06 mM	0.08 mM	0.01 mM	0.015 mM	0.02 mM
R	Hem	0.098±0.003	0.102±0.004	0.087±0.006☆	0.075±0.003★	0.060±0.006★	0.051±0.004★	0.006±0.010★	0.026±0.010★
	Ctr		0.097±0.008	0.102±0.006	0.106±0.005	0.101±0.008	0.101±0.007	0.092±0.018	0.106±0.005
O	Hem	0.121±0.006	0.090±0.006☆	0.065±0.008★	0.059±0.010★	0.038±0.017★	0.023±0.008★	0	0
	Ctr		0.118±0.007	0.108±0.005	0.109±0.006	0.113±0.006	0.106±0.004	0.116±0.006	0.109±0.007

R: Reduction reaction; O: Oxidation reaction; Hem: hemin; Ctr: control (5 mM NaOH). Data presented in the table are means of three determinations±SE; ☆:P<0.05; ★: P<0.01.

Table 3
Effect of Fe^{3+} on r*Sj*LDH.

Reaction group		△OD/min of the enzymatic reaction at each drug concentration					
		0 mM	0.1 mM	0.2 mM	0.3 mM	0.4 mM	0.5 mM
R	Fe^{3+}	0.067±0.009	0.052±0.004	0.023±0.008☆	0.010±0.009★	0.005±0.004★	0.000±0.006★
	Ctr		0.059±0.006	0.062±0.013	0.063±0.014	0.055±0.017	0.054±0.008
O	Fe^{3+}	0.085±0.007	0.026±0.010☆	0.023±0.009★	0.016±0.012★	0.001±0.006★	0.000±0.009★
	Ctr		0.082±0.007	0.084±0.100	0.089±0.006	0.092±0.007	0.087±0.005

Fe^{3+}: $FeCl_3$; Ctr: control (Distilled water); R: Reduction reaction; O: Oxidation reaction; △OD/min is following change in optical density 1min after starting the reaction; data are the means of three determinations±SE. ☆:P<0.05; ★: P<0.01.

The data obtained *in vitro* and *in vivo* studies with schistiosomes described before showed that no effect on the worms was observed when schistosomes were exposured to media containing only artemether or hemin; cultivation of the parasites in the artemther plus hemin containing media obvious effect on the worms[25]; the activity of lactate dehydrogenase in schistosomes collected from artemether-treated mice was inhibited substantially[15]. In the present work, no inhibition was observed on r*Sj*LDH at 0.02-0.10 mM of artemether, but significant inhibition was presented at 0.004-0.020 mM of hemin and 0.2-0.5mM of Fe^{3+}. We can conclude that artemether or artemether plus hemin had no direct effect on *Sj*LDH, the inhibition of the *Sj*LDH by artemether observed *in vivo* might simply reflect non-specific changes occurring during drug-induced parasite damage.

Interestingly, we observed that hemin and Fe^{3+} was reacted by *Sj*LDH and also was inhibited their activities, especially hemin. Hemin is one of hemoglobin degrading product from schistosoma ingested red blood cell. Hemin is a strong oxidant and has cytotoxicity on cells of parasites or host. Ferrous Hb undergoes spontaneous oxidation to ferric iron-containing methemoglobin, which releases its heme groups more readily than ferrous Hb. Heme is broken down to biliverdin, Fe^{2+}, and CO catalyzed by heme oxygenases (HO). And biliverdin is reduced to atoxic bilirubin by the biliverdin reductase then[26]. A complete degrading enzyme system of hemin was found in schistosoma. It is deduced that HO may play a key role in antischistosome mechanism of artemisinins.

Conflict of interest statement

We declare that we have no conflict of interest.

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