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 PZQ, that makes artemether 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 artemisinins in vitro[16–18].

In our previous work, lactate dehydrogenase from...
Schistosome japonicum (SjLDH) was cloned and expressed in Escherichia coli (E. coli), the enzymatic characterization of the recombinant SjLDH (rSjLDH) was described[19]. In this study, we focus on the effect of artemether, hemin and Fe^{3+} on the rSjLDH.

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

rSjLDH preparation and enzymatic assay for the effect of the reagents on rSjLDH 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 °C by following changes in optical density at 340 nm, \( \varepsilon = 6.22 \text{mM}^{-1}\text{cm}^{-1} \). 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 (\( \Delta \text{OD} \)) 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 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 rSjLDH 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.

3.2. Effect of hemin on rSjLDH

The effect of hemin on reversible reaction catalyzed by rSjLDH was shown in Table 2 and Figure 2. Obvious 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 (\( P<0.01 \)).

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

The effect of Fe^{3+} on rSjLDH 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 ($P<0.01$).

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 (pLDH) and Toxoplasma gondii (LDH1, LDH2) 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 artesunate.

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].
The data obtained in vitro and in vivo studies with schistosomes described before showed that no effect on the worms was observed when schistosomes were exposed to media containing only artemether or hemin; cultivation of the parasites in the artemether plus hemin containing media obvious effect on the worms\[26\]; 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 $SjLDH$ at 0.02–0.10 mM of artemether, but significant inhibition was presented at 0.004–0.020 mM of hemin and 0.2–0.5 mM of Fe\(^{3+}\). We can conclude that artemether or artemether plus hemin had no direct effect on $SjLDH$, the inhibition of the $SjLDH$ by artemether observed in vitro might simply reflect non-specific changes occurring during drug–induced parasite damage.

Interestingly, we observed that hemin and Fe\(^{3+}\) was reacted by $SjLDH$ 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.

**References**


