BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 May; 18 (3): 198 - 201

Original Research

Inhibitory effect of artemether on proteinase of *Schistosoma japonicum*[1]

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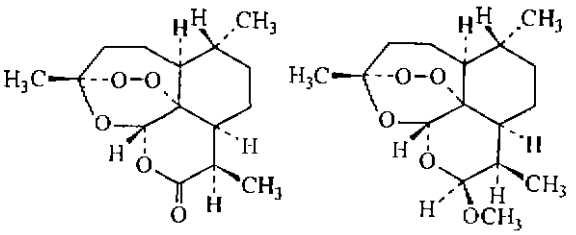
KEY WORDS *Schistosoma japonicum*; artemether; hemoglobins; peptide peptidohydrolases; proteinase inhibitors; polyacrylamide gel electrophoresis

AIM: To study the effect of artemether (Art) on the thio proteinase ("hemoglobinase", Hem) of *Schistosoma japonicum*. **METHODS:** Hem was extracted from *S japonicum* adults. The inhibitory effect of Art on the activity of Hem to degrade human hemoglobin (Hgb) was examined with UV-photometer at 280 nm, SDS-PAGE and scanning at 600 nm on a chromoscanner. **RESULTS:** Human Hgb was degraded at pH 4.0 by the Hem. The activities of Hem preincubated at 37 ℃ with Art 0.14, 1.4, and 14 mmol·L^{-1}, were inhibited by 30.2 %, 39.8 %, and 45.0 %, respectively. **CONCLUSION:** Art possesses an inhibitory effect to Hem of *S japonicum*.

Praziquantel (Pra) is effective for schistosomiasis. However, resistance to Pra can be induced under drug pressue in mice[1] and concern exists for a possible development of resistance in endemic areas. Accordingly, alternative drugs should be developed.

Artemether (Art) is a methyl derivative of the antimalarial artemisinin which was fristly developed by Chinese scientists[2]. It was active against *Schistosoma japonicum*[3,4] and *S mansoni*[5] *in vitro* and *in vivo*. Experiments *in vivo* showed both of size and body weight of *S japonicum* and *S mansoni* were reduction compared with that in non-medicated controls[3,4], the glycogen and protein contents in ♀ and ♂ worms were significantly reduced[6], these phenomena seemed to be involved in malnutrition. Host's hemoglobin (Hgb) is an important source of nutrients for schistosome[7]. It has been demonstrated that schistosome "hemoglobinase", a thio proteinase capable of degrading of host's Hgb, was located in the gut of the worms and found in homogenates of the worms[8]. Radioactivity from erythrocytes labeled with tritiated leucin was incorporated into schistosome proteins[9]. In this paper, the effect of Art on hemoglobinolytic activity from *S japonicum* was investigated.



Artemisinin **Artemether**

MATERIALS AND METHODS

Extract of Hem Adult worms of *S japonicum* were collected by perfusion from the rabbits 45 d after infection with 1000 - 1500 cercariae. Dulbecco's modified Eagles medium containing 5 % calf serum was used asperfusion buffer. The collected worms were washed several times with isotonic saline to remove host tissue and blood. Hem of worms was extracted as the thio proteinase[9] with slight modifications. Briefly, the fresh worms were homogenized in citrate buffer 0.2 mol·L^{-1} at pH 4.0 in a glass homogenizer on ice. The homogenate was spun at 10 000 × *g* for 10 min at 4 ℃. The supernatant was stored at −30 ℃ until use. The protein content of the supernatant was determined with BCA-reagent (Pierce, Rockford IL, USA).

Assay of proteolytic activity of Hem in extract Human Hgb (Yi Hua Medical Science Company, Shanghai) was dissolved in citrate buffer 0.2 mol·L^{-1}, pH 4.0, to a concentration of 8 g·L^{-1}. Then 25 μL of extracts and 20 μL

[1]Project supported by the Ministry of Health, China, № 94-1-130.
Received 1995-10-19 Accepted 1997-01-08

of Hgb solution were incubated in citrate buffer 205 μL at 37 ℃ for 2 h The reaction was terminated by addition of trichloroacetic acid (TCA) (final concentration of 7 %) at 0, 30, 60, 90, and 120 min followed by centrifugation at 10 000 × *g* for 10 min, and then 250 μL of each supernatant was analyzed by UV-photometer (Hitach UV-2000) at 280 nm. Assays with Hgb solution or the extract containing Hem served as controls. For reduction of the errors from experiments, three samples of the same sort were tested and the mean value was used.

Inhibitory effect of Art on activity of Hem Art (Guilin Pharmaceutical Factory, China) 14 mg were dissolved in Me_2SO 1 mL. Art 20 μL was incubated with extracts from worms 25 μL at 37 ℃ for 10 min and then incubated with 20 μL Hgb (8 $g \cdot L^{-1}$) in citrate buffer 205 μL at 37 ℃ for 2 h. Solution 25 μL were taken for SDS-polyacrylamid gel electrophoresis (SDS-PAGE) followed by scanning. The rest was terminated by addition of TCA and spun at 10 000 · *g* for 10 min. The absorbance of the supernatants were measured at 280 nm. The effect of Me_2SO on the absorbing UV light at 280 nm were tested in the system. Three samples of the same sort were tested and the mean value was used.

SDS-PAGE was made on 10 % – 14 % acrylamide gels[10]. After the electrophoresis at 4 ℃ for about 4 h, the gel was stained with Coomassie brilliant blue R-250 (Sigma, Deisenhofen, Germany) and scanned at 600 nm on a chromoscanner (Schimadzu Cs-930).

RESULTS

Proteolytic activity of Hem The Hgb degradation was increased with the concentration of extract (Tab 1).

Tab 1. Hgb degradation by the Hem extracted from *S japonicum*. *n* = 3 samples (homogenates of 3 adult worms from 1 rabbit), $\bar{x} \pm s$.

Hgb/$g \cdot L^{-1}$	Hem/$g \cdot L^{-t}$	Absorbance at 280 nm
0	1.0	0.034 ± 0.004
8	0	0.055 ± 0.024
8	0.25	0.361 ± 0.034
8	0.50	0.576 ± 0.041

At the same concentration of extract, Hem activity to degrade Hgb was increased with the time of incubation and reached to the peak at 90 min (Fig 1).

Inhibitory effect of Art on Hem When extract containing Hem was preincubated with Art,

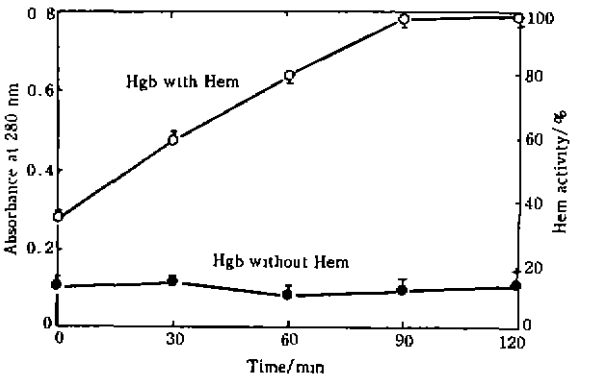


Fig 1. Hemoglobinase activity to degrade Hgb. *n* = 3 samples (homogenates of 3 adult worms from 1 rabbit), $\bar{x} \pm s$.

the proteolytic activity of Hem was inhibited (Tab 2). The absorbance of Me_2SO at 280 nm was 0.011 ± 0.003, no interferences were found at 280 nm when Me_2SO with other samples (Hgb, Hem or Art) in the system ($P > 0.05$).

Tab 2. Effect of Art on activity of Hem. *n* = 3 samples (homogenates of 3 adult worms from 1 rabbit), $\bar{x} \pm s$.

Art/$mmol \cdot L^{-1}$	Inhibitive rate/%	
	Hem 0.25 $g \cdot L^{-1}$	Hem 0.5 $g \cdot L^{-1}$
0.14	39.3 ± 1.8	30.2 ± 1.1
1.40	49.0 ± 1.2	39.8 ± 1.7
14.0	58.2 ± 1.5	45.0 ± 1.4

Hgb showed 2 main bands after SDS-PAGE and the scanning spectra of the electrophoresis indicated that the peaks of the Hgb bands were enlarged when the Hem was treated with Art (Fig 2).

DISCUSSION

Host's Hgb is an important source of nutrients for schistosome[7]. An enzyme capable of degrading Hgb was found in homogenates of adult schistosomes. The enzyme was active at acid pH and exhibited particularly high activity against Hgb[8]. This enzyme activity can be inhibited by some compounds such as leupeptin, tosyl-lysine-chloromethyketone or stimulators (sulphydryl compounds)[9]. This report demonstrates that

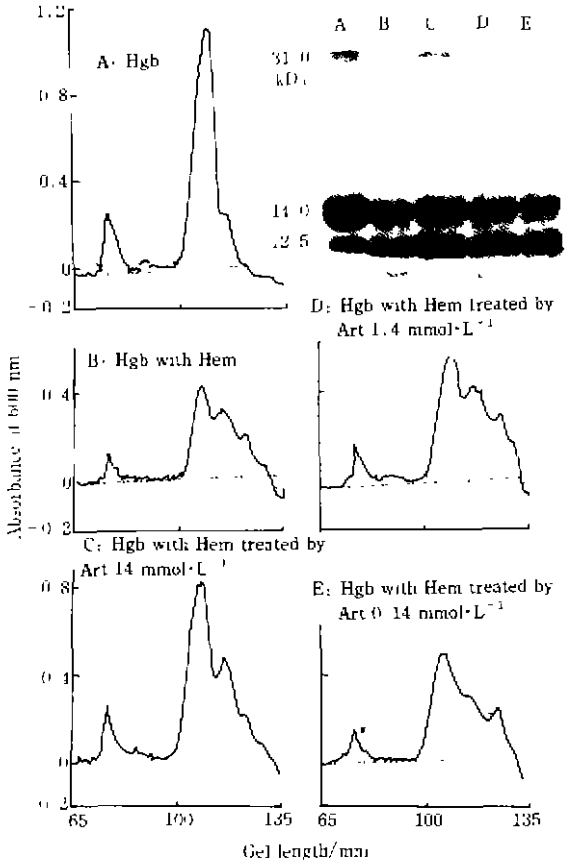


Fig 2. Patterns on Hgb by SDS-PAGE and scanning.

human Hgb is degraded at pH 4.0 by Hem from *S japonicum* and Art can inhibit the proteolytic activity of Hem from *S japonicum*.

Art was evidenced to be effective in treating schistosomiasis japonica[2,3,12] and schistosomiasis mansoni[5]. Our results suggested that interference with proteolytic activity of Hem is one of the mechanisms of antischistosome of Art. Reduction of size and body weight of the worms[3,4], lower of the glycogen and protein contents in *S japonicum* with respect to worms grown in untreated controls[6] seemed to be involved in inhibitory effect of Art on Hem in the parasite.

It should be noted that Hem was extracted from worms which contained host's Hgb and the proteolytic activity of the Hem had been partly consumed before extraction, therefore, the proteolytic activity of Hem in this paper was the surplus activity.

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蒿甲醚对日本血吸虫血红蛋白酶的抑制作用

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关键词 日本血吸虫；蒿甲醚；血红蛋白类；肽水解酶类；蛋白酶抑制剂；聚丙烯酰氨凝胶电泳

目的：研究蒿甲醚(Art)对日本血吸虫血红蛋白酶(Hem)活性的影响. 方法：从日本血吸虫成虫提取 Hem，将 Hem 与人血红蛋白(Hgb)于37 ℃共同孵育 2 h 后，用紫外分光光度计(280 nm)，SDS-聚丙烯酰氨凝胶电泳(SDS-PAGE)和色谱扫描仪(600 nm)分析 Hem 对人 Hgb 的降解作用及在有 Art 存在的条件下，Art 对日本血吸虫 Hem 活性的影响. 结果：在酸性 pH 条件下，血吸虫的 Hem 能降解人的 Hgb，而 Art 抑制 Hem 对人 Hgb 的降解，当含有 Hem 的提取物(0.5 $g \cdot L^{-1}$)分别与 0.14，1.4 和 14 $mmol \cdot L^{-1}$的 Art 在 37 ℃预先孵育 10 min 后，Hem 的活性被抑制 30.2 %，39.8 % 和 45.0 %. 结论：Art 对日本血吸虫的 Hem 活性具有抑制作用.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 May; 18 (3): 201 - 203

Protection of ebselen against anoxic damage of cultured neurons of cerebral cortex

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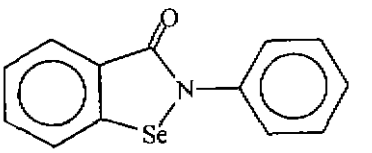
KEY WORDS ebselen; glutathione peroxidase; lactate dehydrogenase; cultured cells; thiobarbituric acid reactive substances; cerebral cortex; neurons; anoxia

AIM: To study the protective effect of ebselen on anoxic damage of brain cells. **METHODS**: On d 10 after plating of the cortical neurons from 1-d-old rat, cultures were placed under 95 % N_2 + 5 % CO_2 for 2 - 6 h. Lactate dehydrogenase (LDH) in supernatant, thiobarbituric acid reactive substance (TBARS) and glutathione peroxidase (GSH-Px) activity of neurons were determined. **RESULTS**: Under anoxia, efflux of LDH and TBARS from cultured neurons increased while GSH-Px activity decreased. Ebselen reduced the efflux of LDH and TBARS in a dose-related manner and increased the total GSH-Px activity. **CONCLUSION**: Ebselen can protect neurons from anoxic damage.

Ischemia/reperfusion made superoxide dismutase (SOD) activity decrease[1]. It is little known whether activity of glutathione peroxidase (GSH-Px, also an endogenous antioxidant enzyme) decrease when neurons suffer from anoxia.

Received 1996-03-25 Accepted 1996-12-23

Ebselen (Ebs) is a seleno-organic compound with antioxidant activity[2]. Protection of ebselen against ischemia and/or reperfusion injury of liver[3] and myocardium[4] has been reported. In the present study, we examined the antioxidant effect of ebselen on newborn rat cortical neurons in culture.



Ebselen (2-phenyl-1,2-benzisoselenazolin-3-one)

MATERIALS AND METHODS

Reagents Ebselen (M_r 274.18, mp 183 ℃ - 186 ℃) was synthesized by Department of Medicinal Chemistry, College of Pharmacy. It was dissolved in Me_2SO as 100 $mmol \cdot L^{-1}$ and diluted with Dulbecco's modified Eagle medium (DMEM) just before use. Cultures were pretreated with medium containing ebselen 1 h before anoxia. SOD was purchased from Sigma.

Cell culture[5] Cortex isolated from 1-d-old Sprague-Dawley rats was incubated in 0.125 % trypsin for 30 min. The tissue was dissociated by passing through a flame-polished