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.

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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.

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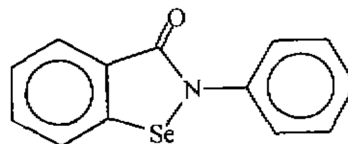
Ebselen (Ebs) is a seleno-organic compound with antioxidant activity[2]. Protection of ebselen against ischemia and/or reperfusion injury of liver[3] and myocardium[4] has been reported. In the present study, we examined the antioxidant effect of ebselen on newborn rat cortical neurons in culture.



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

Pasteur pipette The dissociated cells were plated at a density of 0.9×10^9 cells·L^{-1} in 35-mm poly-L-lysine-coated dishes (Falcon). Cells were maintained in growth medium consisting of 80 % DMEM, 10 % fetal bovine serum, and 10 % horse serum. On d 5, cytosine arabinoside (Hualian Pharmaceutical Co Ltd, Shanghai, 941024) 2.5 mg·L^{-1} was added to inhibit further growth of nonneurons. Culture medium was replaced 50 % with fresh medium twice a week.

Anoxia On d 12, culture medium was replaced with serum-free medium for 1 d. Oxygen was deprived with 95 % N_2 + 5 % CO_2 for 2, 4, 5, and 6 h, and then returned to normoxic, humidified incubator for 24 h prior to biochemical assay.

Biochemical analysis LDH activity[6] in culture supernatant and TBARS content[7] were assayed spectrophotometrically. GSH-Px activity was determined with kit made by Nanjing Jian-Cheng Institute of Biological Engineering.

RESULTS

Anoxia resulted in a time-dependent increase of LDH efflux (Fig 1).

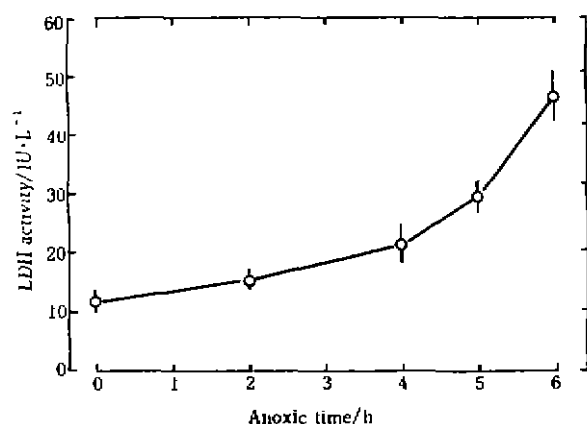


Fig 1. LDH activity in supernatant of cortical neuron culture deprived of O_2. n = 8 dishes (pooled from 3 mice and assayed in triplicate), $\bar{x} \pm s$.

TBARS content of neurons increased but GSH-Px activity decreased time-dependently when deprived of oxygen (Fig 2).

SOD decreased LDH efflux and TBARS content of neurons deprived of oxygen for 6 h ($P<0.01$). Ebselen 5, 10, 25, and 50 μmol·L^{-1} negated LDH efflux induced by anoxia in a dose-dependent manner. All four concentrations of ebselen decreased content of TBARS of the 6 h-

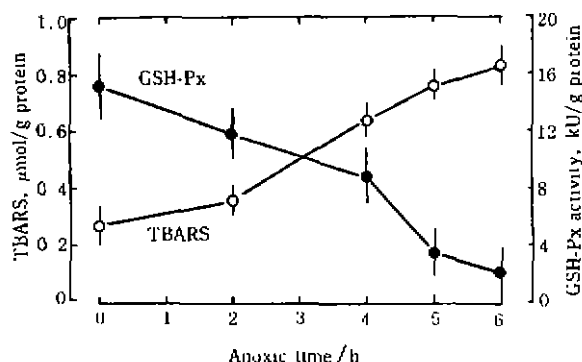


Fig 2. Influence of anoxia on TBARS content and GSH-Px activity of cortical neuron culture. n = 8 dishes (pooled from 3 mice and assayed in triplicate), $\bar{x} \pm s$.

anoxic neurons in a concentration-related manner ($P<0.01$). Total GSH-Px activity of the neurons treated with ebselen increased (Tab 1).

Tab 1. Effects of ebselen on LDH activity, TBARS content and GSH-Px activity from cultured neurons deprived of O_2 for 6 h. n = 8 dishes (pooled from 3 mice and assayed in triplicate), $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ vs control.

Treatment	LDH IU·L^{-1}	TBARS nmol/g protein	GSH-Px kU/g protein
–	45.6±1.6	852±27	2.5±1.4
SOD 1000 kU·L^{-1}	19.7±0.9^c	414±18^c	5.8±1.4^c
Ebselen 5 μmol·L^{-1}	44.5±1.4^b	736±31^c	3.0±1.1
10	39.2±1.1^c	546±27^c	4.8±1.2
25	32.8±1.0^c	423±23^c	8.0±1.2^c
50	29.0±1.1^c	382±36^c	10.4±1.3^c

DISCUSSION

The present results showed that anoxia made cultured cortical neurons release LDH and TBARS content increase. But on the contray, GSH-Px activity decreased when deprived of oxygen, all time-dependently. These results implies that decrease of GSH-Px activity may be partly responsible for anoxia-induced lipid peroxidation damage. Ebselen, a GSH-Px-mimic compound, lowered the damage and lipid peroxidation of cortical neurons caused by 6 h anoxia. Furthermore, paralleling protective effect of ebselen, total GSH-Px activity of neurons treated with ebselen went up

dose-dependently. All above results indicate that anoxia-caused neurons damage is at least partly related to decrease of GSH-Px activity. GSH-Px, one of three endogenous antioxidant enzymes (the other two is SOD and catalase), degrades hydrogen peroxide and organic hydroperoxides such as lipid hydroperoxides. As there is rarely catalase in CNS, decrease of GSH-Px activity is deemed to exagerate the damage caused by reactive oxygen.

Ebselen has been shown to reduce damage of endothelial cell induced by 15-hydroperoxyeicosatetraenoic acid[8], protect against ischemia-reperfusion injury of myocardial infarction model[4]. In our experiment, ebselen dose-dependently lowered the damage of cortical neurons caused by 6 h anoxia.

This effect of ebselen against anoxic damage may mainly derive from its antioxidant property because ebselen ameliorated lipid peroxidation as shown in Tab 1. Antioxidant activity of ebselen derives from its GSH-Px activity[2,8,9]. But some other mechanisms may also be invovled in, among which are modification of IP_3-induced Ca^{2+} release[10], inhibition of nitric oxide synthase[11].

Ebselen mimics as a GSH-Px, enabling it function of limiting peroxidation. But different from GSH-Px, ebselen is a relatively small, liphilic compound. So it can access across the blood-brain barrier and reach intracellular space with ease. From this point of view, ebselen may have a prospective future.

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依伯硒啉对培养大鼠皮层神经元缺氧损伤的拮抗作用

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关键词 依伯硒啉；谷胱甘肽过氧化物酶；乳酸脱氢酶；培养的细胞；硫代巴比妥酸反应活性物质；大脑皮层；神经元；缺氧症 脑缺氧

目的：研究依伯硒啉对神经细胞缺氧损伤的拮抗作用. **方法**：培养新生大鼠皮层神经元置于95 % N_2 + 5 % CO_2. 测定了神经元乳酸脱氢酶(LDH)释放，硫代巴比妥酸反应活性物质(TBARS)含量和谷胱甘肽过氧化物酶(GSH-Px)活性. **结果**：缺氧后，LDH 释放和 TBARS 含量增加；同时，GSH-Px 随缺氧时间延长活性下降；依伯硒啉减少缺氧时 LDH 释放和 TBARS 的增加，使缺氧神经元的总 GSH-Px 活性上升. **结论**：依伯硒啉对神经元的缺氧损伤具拮抗作用.