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Salvianolic acid B protects brain against injuries caused by ischemia-reperfusion in rats

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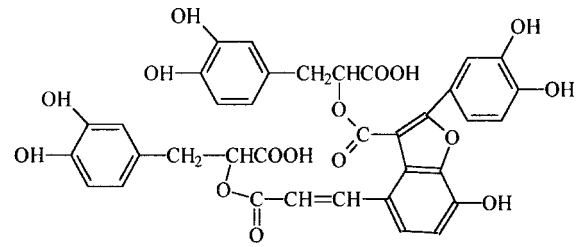
KEY WORDS salvianolic acid B；superoxide dismutase；glutathione；malondialdehyde；lactic acid；transient cerebral ischemia

ABSTRACT

AIM：To study the protective effects of salvianolic acid B (Sal B) against the ischemia-reperfusion induced rat brain injury. **METHODS**：Focal cerebral ischemia-reperfusion model in rats was employed to study the protective effects of Sal B. The behavioural tests were used to evaluate the damage to the central nervous system. Spectrophotometric assay methods were used to measure the activity of superoxide dismutase (SOD)，contents of reduced glutathione (GSH)，malondialdehyde (MDA)，adenosine 5-triphosphorate (ATP)，and lactate acid (LA) in experimental rats' brain homogenate. **RESULTS**：Focal cerebral ischemia-reperfusion resulted in abnormal behavior which could be alleviated by Sal B 10 mg·kg^{-1} iv，and nimodipine (Nim) 4 mg·kg^{-1} ip. At the same time，Sal B 10 mg·kg^{-1} and Nim 4 mg·kg^{-1} could inhibit the decrease in SOD，GSH，and ATP levels and the increase in MDA and LA levels caused by ischemia-reperfusion in brain. **CONCLUSION**：Sal B showed a protective action against the ischemia-reperfusion induced injury in rat brain by reducing lipid peroxides，scavenging free radicals and improving the energy metabolism.

INTRODUCTION

Sal B is a new chemical compound isolated from traditional Chinese drug — *Salvia miltiorrhiza*. Previous experiments have proved that it has potent antioxidative effects *in vivo* and *in vitro*[1]. It has been reported that ischemia-reperfusion induced brain injury is related to the excessive production of free radicals and the loss of antioxidative ability of the brain. Free radicals attack membrane polyunsaturated fatty acids，damaged the mitochondrial membrane，and affect the energy metabolism，and aggravate the injury caused by ischemia-reperfusion[2]. In present experiments，we studied the protective effects of Sal B against the brain injuries in focal cerebral ischemia-reperfusion model induced by middle cerebral artery (MCA) occlusion. The effects of Sal B on free radicals and energy metabolism in ischemia-repefusion rat brain were also observed.



Salvianolic acid B

MATERIAL AND METHODS

Rats Male wistar rats (Grade Ⅱ，Certificate № 01-3008) were purchased from the Center of Experimental Animal，Chinese Academy of Medical Sciences.

Drugs and reagents Sal B (purity > 99 %) was provided by the Department of Phytochemistry in our institute. Nimodipine (Nim) was purchased from Shandong Xin Hua Pharmaceutical Factory. ATP，glucose-6-P dehydrogenase，hexokinase，and bovine serum albumin (BSA) were from Sigma Chemical Co. Other reagents and chemicals were of AR and from standard commercial sources.

Preparation of focal cerebral ischemia-reperfusion model This model was performed following the method of Nagasawa with slight modification[3]. Adult male Wistar rats weighing 250－300 g were anesthetized with 12 % chloral hydrate in 0.9 % NaCl (350 mg·kg^{-1}，ip). The right common carotid artery (CCA)，

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external, and internal carotid artery (ECA, ICA) were carefully dissected. A nylon suture (5 cm in length and 0.24 cm in diameter) was introduced to the ICA from ECA to occlude the origin of MCA. By withdrawing the nylon suture, the reperfusion was performed 1 h after the onset of ischemia.

Experimental groups Rats were divided into 5 groups. 1) Sham group was operated but without ischemia-reperfusion. 2) Control group. 3 – 5) Treatment groups as Nim group receiving Nim 4 mg·kg^{-1} ip, Sal B groups receiving Sal B 3 mg·kg^{-1} iv, and Sal B 10 mg·kg^{-1} iv 10 min before the onset of ischemia.

Tests of behavior The stroke index of each rat was marked carefully 24 h after reperfusion following the method of Bederson *et al*[4]. The damage was graded on a scale of 0 – 3. Grade 0: no neurological deficits, marked 0; Grade 1: contralateral forelimb flexed to the injured hemisphere when held up by the tail, marked 1; Grade 2: reduced resistance when shoulder was pushed towards the paretic side, marked 3; Grade 3: rat circled towards the paretic side, marked 6.

Slopping board experiment[5] was performed at 24 h after reperfusion according to the procedure followed by NIU Xin-Yi with slight modification. The rat was placed in the center of a rough rubber pad (with a board) which was set at an 85° angle. The time for which each rat stayed on the board was recorded. The cut-off time was 180 s.

Free radical and energy metabolism assays Rats were decapitated at 24 h after reperfusion. The ischemic area in the right hemisphere of the forebrain was rapidly separated and homogenized in 10 volumes of ice-cold normal saline. The content of MDA was determined by the method of thiobarbituric acid (TBA) colorimetric analysis[6]. The contents of SOD, GSH, and LA were measured by spectrophotometric assay methods[7]. The content of ATP was measured by the enzyme-fluorometric method[8]. The protein determination was done following the method of Lowry *et al*[9].

Statistical analysis All data were expressed as $\bar{x} \pm s$. Statistical analysis was performed using unpaired t test.

RESULTS

Effects of Sal B on the behavior of rat cerebral ischemia-reperfusion model The score of stroke index in sham group was 0, and in the control group it was 7.1. Sal B 10 mg·kg^{-1} iv and Nim 4 mg·kg^{-1} ip significantly decreased the stroke index. The period of staying on the slop board in focal cerebral ischemia-reperfusion rat was much shorter than that in the sham group. Sal B 10 mg·kg^{-1} iv and Nim 4 mg·kg^{-1} ip prolonged the rat's staying time on the slop board. It shows that Sal B 10 mg·kg^{-1} iv and Nim 4 mg·kg^{-1} ip can significantly relieve the behavioural disorder caused by ischemia-reperfusion injury in the rat brain (Tab 1).

Tab 1. Effects of Sal B on the stroke index and staying time on slope board experiment in the rats with focal cerebral ischemia-reperfusion. n **= number of rats.** $\bar{x} \pm s$. $^{a}P > 0.05$, $^{c}P < 0.01$ *vs* **model group.** $^{f}P < 0.01$ *vs* **sham group.**

Group	n	Stroke index (score)	Slope board experiment/s
Sham	6	0^{c}	180^{c}
Model	7	7.1 ± 1.2^{f}	10 ± 7^{f}
Nim 4 mg·kg^{-1}	6	4.7 ± 1.1^{cf}	43 ± 12^{cf}
Sal B 3 mg·kg^{-1}	5	6.8 ± 0.7^{af}	13 ± 7^{af}
Sal B 10 mg·kg^{-1}	6	4.3 ± 1.2^{cf}	50 ± 18^{cf}

Effects of Sal B on the SOD activity and contents of GSH and MDA The SOD activity and GSH content in the control group were very low than those in sham group. Nim and Sal B significantly increased both of them. At the same time, Nim and Sal B inhibited the increase in MDA levels in focal cerebral ischemia-reperfusion rats. It shows that Nim and Sal B can significantly decrease the free radical generation in brain caused by ischemia and reperfusion injury (Tab 2).

Effects of Sal B on the ATP and LA levels in the brain of ischemia-reperfusion rats Twenty-four hours after reperfusion, the ATP levels in control group were lower than that in the sham group. A significant increase in ATP was observed in Nim 4 mg·kg^{-1} and Sal B 10 mg·kg^{-1} groups. At the same time, Nim and Sal B decreased the LA levels in the brain of focal cerebral ischemia-reperfusion rats. It shows that Nim and Sal can significantly reduce the abnormal energy metabolism in brain caused by ischemia-reperfusion injury (Tab 3).

DISCUSSION

Stroke is a major cause of debilitation and death. Growing evidences support the role of oxidative

Tab 2. Effect of Sal B on the SOD activity and the GSH and MDA levels in the brain of focal cerebral ischemia-reperfusion rats. n = number of rats. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* model group. $^{d}P > 0.05$, $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* sham group.

Group	n	SOD/ ku·g^{-1} protein	GSH/ mg·g^{-1} protein	MDA/ mol·g^{-1} protein
Sham	6	0.92 ± 0.08^{c}	4.31 ± 0.82^{c}	0.270 ± 0.037^{c}
Model	7	0.55 ± 0.11^{f}	1.78 ± 0.62^{f}	0.432 ± 0.041^{f}
Nim 4 mg·kg^{-1}	6	$0.82 \pm 0.06^{c,e}$	$3.82 \pm 0.96^{c,d}$	$0.333 \pm 0.037^{c,e}$
Sal B 3 mg·kg^{-1}	5	$0.76 \pm 0.10^{b,e}$	$3.00 \pm 0.94^{b,e}$	$0.368 \pm 0.058^{c,e}$
Sal B 10 mg·kg^{-1}	6	$0.84 \pm 0.05^{c,d}$	$3.34 \pm 0.67^{c,d}$	$0.327 \pm 0.062^{c,d}$

Tab 3. Effect of Sal B on the ATP and LA levels in the brain of focal cerebral ischemia-reperfusion rats. n = number of rats. $\bar{x} \pm s$. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* model group. $^{d}P > 0.05$, $^{f}P < 0.01$ *vs* sham group.

Group	n	ATP/ μmol·g^{-1} wet	LA/ μmol·g^{-1} wet
Sham	6	2.55 ± 0.64^{c}	3.11 ± 0.28^{c}
Model	7	1.61 ± 0.44^{f}	8.27 ± 0.56^{f}
Nim 4 mg·kg^{-1}	6	$1.90 \pm 0.49^{b,d}$	$5.69 \pm 0.78^{c,f}$
Sal B 3 mg·kg^{-1}	5	$1.09 \pm 0.61^{a,d}$	$7.13 \pm 0.44^{c,f}$
Sal B 10 mg·kg^{-1}	6	$2.20 \pm 0.34^{c,d}$	$6.06 \pm 0.54^{c,f}$

mechanisms in ischemia-reperfusion injury[2]. The brain is very susceptible to energy depriving injuries and is particularly sensitive to oxygen radical mediated injury due to its charactristics low fuel reserves, high aerobic metabolism, and low concentrations of O_2 radical scavenging enzymes, and with high levels of polyunsaturated fatty acids that constitute the neuronal cell membrane[10]. The mitochondrial membrane which is similar to the cell membrane in constitution, can also be attacked by free radicals, aggravating the dysfunction in energy metabolism thereby leading to a vicious cycle.

The present study showed that Sal B improved the above mentioned abnormalities caused by cerebral ischemia-reperfusion injury. Sal B also increased the SOD activity and GSH content and decreased the MDA levels, as well as raised ATP and reduced LA levels in the ischemia-reperfusion forebrain. In conclusion, the protective action of Sal B against the injury caused by ischemia-reperfusion may be due to its antioxidative action, its free radicals scavenging effect and by improving cerebral energy metabolism.

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丹酚酸 B 拮抗大鼠缺血再灌注引起的脑损伤

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关键词　丹酚酸 B；超氧化物歧化酶；谷胱甘肽；丙二醛；乳酸；短暂性脑缺血

目的：研究丹酚酸 B 对缺血再灌注脑损伤的拮抗作用．方法：采用大鼠局灶性脑缺血再灌注模型．以行为学实验评价中枢神经系统的损伤．采用比色法检测脑匀浆液中超氧化物歧化酶（SOD）的活性，以及还原型谷胱苷肽（GSH）、丙二醛（MDA）、三磷酸腺苷（ATP）和乳酸（LA）的含量．结果：局灶性脑缺血再灌注可造成行为学异常，丹酚酸 B 10 $mg\cdot kg^{-1}$ 可减轻此种异常．同时，丹酚酸 B 10 $mg\cdot kg^{-1}$ 可以改善由于脑缺血再灌注所造成的 SOD、GSH、和 ATP 含量降低以及 MDA 和 LA 含量增加．结论：丹酚酸 B 对脑缺血再灌注脑损伤具有保护作用，其作用机制与减轻脂质过氧化、促进自由基的清除以及改善能量代谢有关．

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