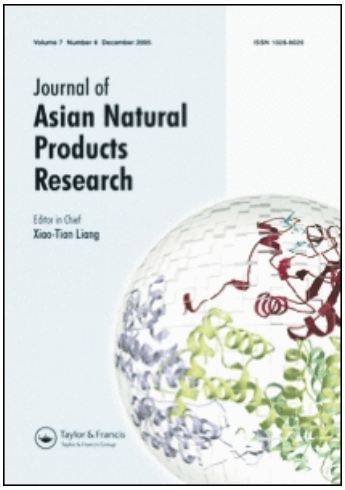

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Salvianolic Acid B Protects the Memory Functions against Transient Cerebral Ischemia in Mice

Guan-Hua Du; Yue Qiu[a]; Jun-Tian Zhang[a]
[a] Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China

To cite this Article Du, Guan-Hua , Qiu, Yue and Zhang, Jun-Tian(2000) 'Salvianolic Acid B Protects the Memory Functions against Transient Cerebral Ischemia in Mice', Journal of Asian Natural Products Research, 2: 2, 145 — 152
To link to this Article: DOI: 10.1080/10286020008039903
URL: http://dx.doi.org/10.1080/10286020008039903

JANPR, Vol. 2, pp. 145–152
Reprints available directly from the publisher
Photocopying permitted by license only

SALVIANOLIC ACID B PROTECTS THE MEMORY FUNCTIONS AGAINST TRANSIENT CEREBRAL ISCHEMIA IN MICE

GUAN-HUA DU*, YUE QIU and JUN-TIAN ZHANG

Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, 1 Xian Nong Tan Street, Beijing 100050, China

(Received 14 July 1999; Revised 21 August 1999; In final form 30 August 1999)

The objective of this work was to study the protective effects of salvianolic acid B (Sal B) on the dysfunctions of learning and memory induced by transient cerebral ischemia in mice. The mechanisms of its actions also were researched both *in vivo* and *in vitro*. The model of dysfunction of learning and memory induced by transient cerebral ischemia in mice was used. One trail passive avoidance tests were used to evaluate the learning and memory functions and experiments *in vitro* were employed to observe the antioxidative effects of Sal B. Cerebral transient ischemia would impair the function of memory in mice. In step down test, the error number and latency were 2.63 and 120.5 in control group and were 1.35 and 234.4 respectively in sham operated group ($p < 0.05$). In Sal B treated groups, the error number was less and latency was longer significantly than those of control group. Meanwhile, 3 and 10 mg kg^{-1} of Sal B iv. reduced the malondialdehyde contents in cortex, hippocampus and striatum of cerebral transient ischemia rat *in vivo*. Sal B 10–100 nmol L^{-1} also inhibited lipid-peroxidation and scavenged free hydroxyl radicals *in vitro*. As conclusion, Sal B ameliorated learning and memory dysfunctions induced by cerebral transient ischemia. Its actions might be related to its antioxidant activity.

Keywords: Salvianolic acid B; Cerebral transient ischemia; Learning and memory; Lipid-peroxidation

INTRODUCTION

Salvianolic acid B (Sal B, Fig. 1) was isolated from *Salvia miltiorrhiza*, which has been used therapeutically in the treatment of cardiovascular

* Corresponding author. Tel.: 0086-10-63165184. Fax: 0086-10-63017757.
E-mail: dugh@imm.ac.an.

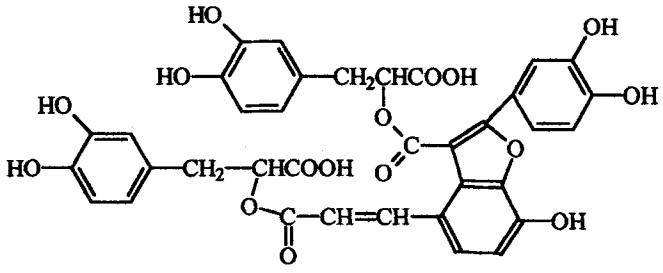

FIGURE 1 Chemical structure of Sal B.

diseases and neuronal disorders since 20 centuries in traditional Chinese medicine [1]. In modern Chinese Pharmacopoeias, *Salvia miltiorrhiza*, is also recommended for treating disorders of cardiovascular system. Some extracts of *Salvia miltiorrhiza*, have been used in clinical practice. Sal B is one of the water-soluble components in extract of *Salvia miltiorrhiza* [2].

It has been demonstrated that transient ischemia, e.g. ischemia reperfusion, in brain could impair the brain tissue and functions of learning and memory. Although the brain damage caused by ischemian reperfusion related with a lot of factors such as disorder of energy metabolism, excited amino acids, the oxygen free radicals generated during ischemia reperfusion play a very important role in this kind of injuries. Oxygen free radicals induce peroxidative reactions with the biochemical components in cells especially the membrane and cause damage of the tissue and then their functions [4–8].

Previous papers reported that Sal B showed antioxidative effects and scavenging oxygen free radical effect *in vitro* [2,3]. In present experiments, the protective effects of Sal B against impairment of learning and memory functions caused by transient cerebral ischemia were investigated in mice. The possible mechanism of its protective actions was also studied by measuring the scavenging hydroxyl radical effect and antioxidant effects of Sal B *in vivo* and *in vitro*.

RESULTS AND DISCUSSION

Improving Effects of Sal B on Impairment of Learning and Memory

In the step down test, the learning trial was performed 24 h after transient ischemia. The results showed that there was no significant difference in learning functions between the groups. But in testing trials (performed in the following day), the latencies in sham operation group and Sal B treated

TABLE I Effects of Sal B on the latency and error number of step down test in ischemia-reperfusion mice

Groups	*n*	*Learning*		*Memory*	
		Reactive time log(s)	*Error number* log(counts)	*Latency* log(s)	*Error number* log(counts)
Sham	11	1.05 ± 0.53	0.46 ± 0.16	2.45 ± 0.07**	0.14 ± 0.19**
Control	12	1.10 ± 0.49	$0.56 + 0.19$	1.74 ± 0.67	0.40 ± 0.20
Sal B 3	8	1.10 ± 0.40	0.45 ± 0.16	2.38 ± 0.27*	0.11 ± 0.15**
Sal B 10	9	1.10 ± 0.43	0.58 ± 0.27	2.43 ± 0.10**	0.07 ± 0.12**

Sal B 3 and Sal B 10 indicated that the mice were treated with Sal B 3 or 10 mg/kg, ip. $x \pm s$, $^*p < 0.05$, $^{**}p < 0.01$ compared with control group.

groups were longer and the number of errors were less than those of the control group (Table I).

In step through test, there was no significant difference in observed indexes between control and treated groups in the first training (learning). But in the testing training (second training), the number of errors and accumulated stimulating time were less and the latencies were longer in Sal B treated groups and sham group than those in control group (Fig. 2). The results obtained from step down tests and step through tests indicated that Sal B could improve the functions of memory in transient ischemic mice.

Effects of Sal B on Elevated Malondialdehyde Induced by Ischemia-Reperfusion in Mice *In Vivo*

After 5 min of ischemia and followed by 10 or 30 min of reperfusion, the content of malondialdehyde (MDA) in the brain cortex was increased by 29.8% ($p < 0.05$) and 15.5% respectively. Sal B (3, 10 mg kg^{-1}, iv.) reduced the content of MDA in the brain cortex dose-dependently. In hippocampus–striatum, the contents of MDA were increased by 43.0% (10 min reperfusion, $p < 0.05$) and 25.5% (30 min reperfusion) respectively. Sal B also inhibited the production of MDA in hippocampus–striatum (Table II).

Inhibitory Effect of Sal B on Lipid Peroxidation in Brain Homogenate

In the Vit C–Fe^{2+} hydroxyl radical generating system, brain homogenate could generate MDA. After reacting for 10 min, Sal B 10–100 nmol L^{-1} decreased the production of MDA. Its IC_{50} was about 2.8 μmol L^{-1}. The 95% confidence interval was 0.55–4.99 μmol L^{-1} (Fig. 3).

It has been demonstrated that transient cerebral ischemia in mice could induce damage in brain tissue [4,5]. The major regions damaged during

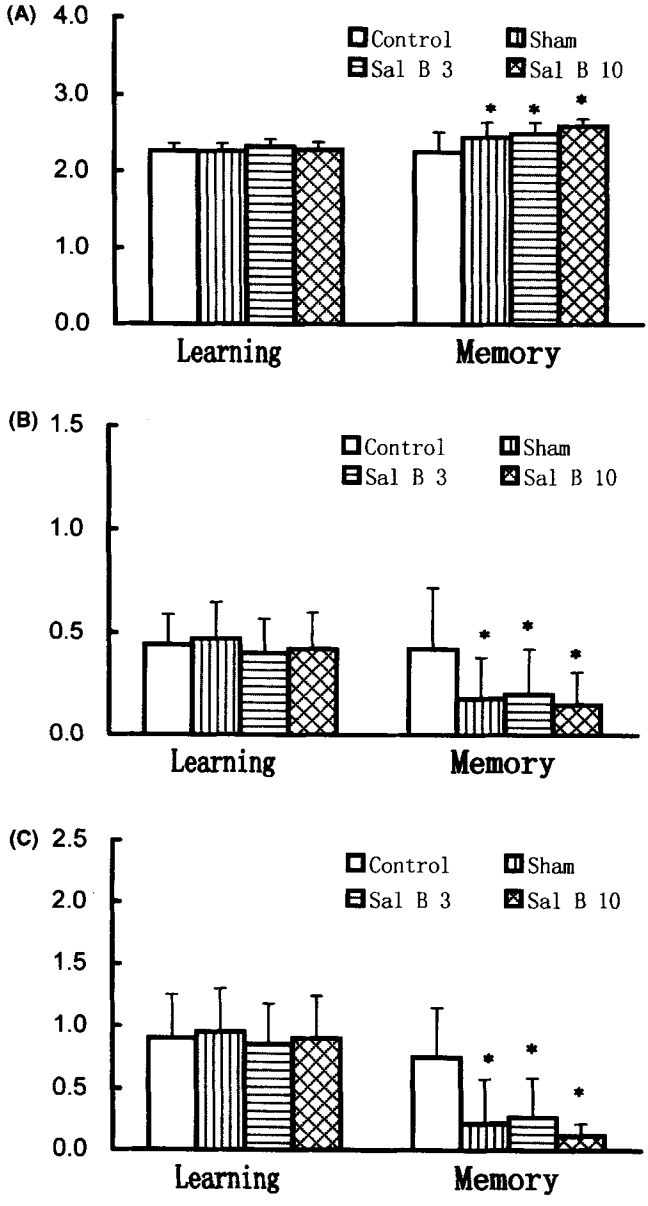

FIGURE 2 Effects of Sal B on the latency (A, logs), error number (B, logcounts) and accumulative stimulating time (C, logs) of step through tests in ischemia-reperfusion mice. ($n = 8-12$, $x \pm s$), $^*p < 0.05$, compared with the control group.

TABLE II Inhibitory effects of Sal B on the production of MDA (nmol g tissue^{-1}) in brain cortex, hippocampus and striatum during cerebral ischemia-reperfusion in mice

Groups	*n*	*Brain cortex*	*Hippocampus–striatum*
Control	4	292.2 ± 32	302 ± 43
Anesthetized	4	288.0 ± 34	350 ± 45
Ischemia	4	335.0 ± 37	303 ± 40
Is-Re 10	5	345.0 ± 11^{a}	420 ± 48^{a}
Is-Re 10 + Sal B 3	5	288.0 ± 28^{b}	340 ± 64
Is-Re 10 + Sal B 10	4	260.0 ± 29^{b}	275 ± 18^{b}
Is-Re 30	5	337.0 ± 40	410 ± 18
Is-Re 30 + Sal B 3	5	294.0 ± 18^{c}	403 ± 34
Is-Re 30 + Sal B 10	4	292.0 ± 12^{c}	403 ± 32

The control, anesthetized and ischemia groups indicate the normal conscious mice, anesthetized mice and cerebral ischemia mice. Is-Re 10 and Is-Re 30 indicates that the mice ischemia for 5 min and followed by 10 or 30 min reperfusion. Sal B 3 and Sal B 10 means the mice were treated with Sal B in 3 or 10 mg/kg iv. $x \pm s$. The a, b, c indicate that $p < 0.05$ compared with anesthetized group, ischemia 5 min and followed by 10 or 30 min reperfusion groups.

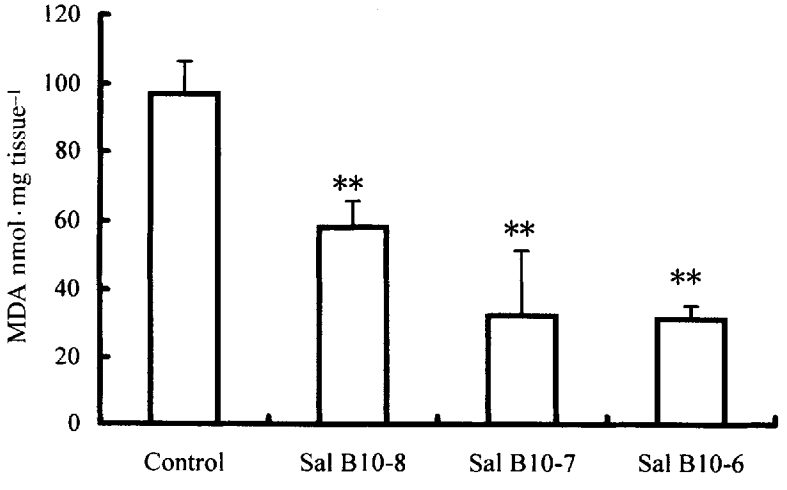

FIGURE 3 Effects of Sal B on the brain-lipid peroxidation induced by Vit C–Fe^{2+} *in vitro*. $x + s$, $n = 4$, $^{**}p < 0.05$ compared with control.

brain transient ischemia are the forebrain, hippocampus–striatum, as well as the hippocampus CA1 region [6]. As a result, the learning and memory functional impairment happened during transient cerebral ischemia [7–9]. It has been well known that the impairment of learning and memory functions induced by transient cerebral ischemia were related to many factors, such as increase of intracellular calcium, release of excitatory amino acids and generation of oxygen free radicals [10].

Our previous experiments demonstrated that Sal B showed potential antioxidant effects [2,3]. In the present experiment it was demonstrated that ischemia-reperfusion significantly increased the content of MDA and caused generation of hydroxyl radicals in the brain cortex and hippocampus–striatum *in vivo*, while Sal B inhibited both MDA and hydroxyl radicals formation. So it is suggested that the facilitation of memory induced by Sal B might be related to inhibiting of brain-lipid peroxidation and to scavenging free radicals.

EXPERIMENTAL SECTION

General Experimental Procedures

The mice transient cerebral ischemia model was prepared as described by Himori *et al.* with some modification [11]. Briefly, the mouse was anesthetized with sodium pentobarbital (60 mg/kg, ip.) and both carotid arteries were carefully exposed. The arteries were wound with ligatures separately and their 4 ends passed through a 5 mm long PE50 tube and were exteriorized through skin at the back of neck. Another silk suture for reperfusion was put through the ligature loops and its ends were out of skin at the cervical part. The skin was closed and the animals housed in 30°C as described above. Forty-eight hour after the operation, 5 min of ischemia was performed by pulling the back exteriorized ligatures and then loosing the artery for reperfusion. Sal B was treated intravenously once a day for 4 days and the first administration was at the moment of 15 min before ischemia. Sal B was administrated 3 or 10 mg kg^{-1}, iv., 15 min before ischemia and once a day until all tests were finished.

Animals

Male mice of Kunming strain, weighing 19.9 ± 0.9 g and Wistar rats, weighing 200–250 g were housed in groups of 6–10 animals with free access of water and standard food. All animals were supplied by the Experimental Animal Center of the Chinese Academy of Medical Sciences.

Chemicals

Sal B was isolated from *Salvia miltiorrhiza* by the Department of Phytochemistry in our institute; mannitol, MDA, cysteine and benzoic acid were

purchased from the Sigma company; ammonium ferrous sulfate and other chemicals were of the highest analytical grade available.

Tests of Learning and Memory Functions [12]

After 24 h transient cerebral ischemia, the learning and memory functions of mouse were tested by the methods of step down test and step through test with some modification. In brief, the mouse was placed in step down apparatus for 3 min to adapt itself to the environment, and then received electric stimulation (38 V, 50 Hz) from the grid of bottom for 5 min as a learning trial. Twenty-four hour later, the mouse was again exposed in the apparatus on a platform (with electric stimulation in the bottom) for 5 min as a testing trial. The latency and number of errors (times of stepping down on the grid of bottom during 5 min) were recorded. In the step through test, the mouse (after operation for 48 h) was placed in the light compartment for 5 min. The latency and error number (counts of the animal moving into the dark compartment and shocked with electric stimulation, 38 V, 50 Hz) were recorded. This process was repeated at the same time the next day (24 h later) to test the animals' memory acquisition.

Measurement of MDA Content in the Brain

Forty mice were randomly divided into 6 groups: Control group: normal conscious mice; Anesthetized group: mice anesthetized with sodium pentobarbital 60 mg/kg; Ischemic group: mice were anesthetized and submitted to cerebral–ischemia by 5 min occlusion of 2 carotid arteries; Ischemia for 5 min and reperfusion for 10 min group (ischemia-re 10) and Sal B treated groups (Sal B 3 or 10 mg kg^{-1} iv. 5 min before occlusion). Mice in this group were anesthetized and their aorta arteries were occluded for 5 min and then loosen for reperfusion; ischemia 5 min–reperfusion 30 min groups (ischemia-re 30) and Sal B treated groups (Sal B 3 or 10 mg kg^{-1} iv. 5 min before occlusion): mice were treated as above except reperfusion for 30 min. Mouse was decapitated after treatment and brain was removed immediately into cold condition. The brain cortex, hippocampus and corpus striatum were separated as samples and were homogenized at 0°C in 100 mmol L^{-1} phosphorus buffer (PBS). Protein contents in samples were measured by the method of Lowry *et al.* [13]. The contents of MDA in brain samples were measured by TBA method with RF-5000 spectrofluorophotometer (Shimadzu, Japan) [14].

Brain Homogenate Lipid-Peroxidation

Wistar rats were decapitated and their brains were removed immediately into ice cold 100 mmol L^{-1} PBS and were homogenated at a concentration of about 10% (g/v). Vit C–$FeSO_4$ peroxidatant system was used to induce brain homogenate lipid peroxidation and MDA formation was measured by the TBA fluorescence method [14–16]. The reaction system contained: Na_2HPO_4 80 mmol L^{-1}, pH 7.4), Vit C 0.5 mmol L^{-1} and proper amount of brain homogenate.

Statistical Analysis

All results were expressed as mean $\pm$ *s* except those of learning and memory, which were expressed in terms of common logarithm. The data were analyzed by Student's *t*-test.

References

[1] C.B. Ai and L.N. Li. *Natural Products* 1988; **5**: 145–149.
[2] Y.S. Huang and J.T. Zhang. *Acta Pharm. Sinica* 1992; **27**(2): 97–100.
[3] Y. Liu and J.T. Zhang. *J. Chin. Pharmaceu. Sci.* 1994; **3**: 43–50.
[4] M.L. Hess and N.H. Manson. *J. Mol. Cell. Cardiol.* 1984; **16**(10): 969–985.
[5] H.P. Davis. *Physiol. Behavior* 1986; **37**: 387–392.
[6] W.A. Pulsinelli, J.B. Brierley and F. Plum. *Ann. Neurol.* 1982; **11**: 491–498.
[7] M. Ohno, T. Yamamoto, S. Ueki and S. Watanabe. *Neuroscience Letters* 1992; **37**: 387–392.
[8] J. Itob, M. Ukai and T. Kaveyama. *Eur. J. Pharmacol.* 1993; **234**: 9–15.
[9] B.T. Volpe, J. Holtzman and W. Hirst. *Neurology* 1985; **35**: 1793–1797.
[10] H. Hagberg, A. Lehmann, M. Sandberg, B. Nystrom, I. Jacobson and A. Hamberger. *J. Cereb. Blood Flow Metab.* 1990; **10**: 646–653.
[11] H. Himori, H. Watanabe, N. Akaike, M. Kurasawa, J. Itoh and Y. Tanaka. *J. Pharmacol. Meth.* 1990; **23**: 311–317.
[12] J.T. Zhang and H. Saito. *Acta Pharm. Sinica* 1986; **21**(1): 12–19.
[13] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall. *J. Biol. Chem.* 1951; **193**: 265–275.
[14] H. Ohkawa, N. Ohishi and K. Yagi. *Anal. Biochem.* 1979; **95**(2): 351–358.
[15] F. Haber and J. Weiss. *J. Proc. R. Soc. A* 1984; **147**: 332–351
[16] G. Minotti and S.D. Aust. *J. Biol. Chem.* 1987; **262**(3): 1098–1104.