Effects of oxysophoridine on amino acids after cerebral ischemic injury in mice

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Abstract

Background: Our previous studies demonstrated that oxysophoridine (OSR) had neuroprotective effects on mice through antioxidant and anti-apoptotic mechanisms. In this study, we investigated whether OSR could influence the release of amino acids in ischemic mice brains. **Materials and Methods:** Male ICR mice were scheduled to undergo 2 h middle cerebral artery occlusion (MCAO) and 24 h reperfusion. Before MCAO, mice in corresponding groups were intraperitoneally injected with OSR (62.5, 125 and 250 mg/kg) for seven successive days. After reperfusion, neurological scores were estimated, infarct volume and the brain water content were assessed. The levels of glutamate (Glu), aspartate (Asp), γ-aminobutyric acid (GABA) and Glycine (Gly) were measured by amino acid analyzer. **Results:** OSR significantly decreased neurological scores, reduced infarct volume and the brain water content. After treatment with OSR of 250 mg/kg, the contents of Glu, Asp, GABA and Gly in mice brains could maintain at a normal level compared with MCAO group mice. The Glu/GABA ratio was significantly decreased in OSR group mice. **Conclusion:** These findings indicate that OSR has a protective effect on cerebral ischemic injury and helps to maintain the amino acids homeostasis after reperfusion for a long time.

Key Words

Amino acids homeostasis, cerebral ischemic injury, excitatory amino acids, inhibitory amino acids, oxysophoridine

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Introduction

Cerebral ischemic injury is one of the most serious diseases which cause human death and disability in the world.[1] Excessive release of excitatory amino acids (EAA) is a primary pathological change in the process of cerebral ischemia reperfusion.[2] EAA are widely distributed in the neuron synapse, neuron soma and glial cell cytoplasm. Glutamate (Glu) and aspartate (Asp) are two important members of EAA and they are abundant in brain tissue. During the early stage of cerebral ischemia, excessive Glu and Asp may be released from neurons which play an important role in the pathways leading to cell death.[3,4] The view of Glu receptor antagonist can protect the cerebral ischemia injury is proved to be true in many studies.[5-7] Inhibitory amino acids (IAA), like γ-aminobutyric acid (GABA) and Glycine (Gly) can inhibit the excessive release of Glu. It is fully accepted that the imbalance between EAA and IAA causes cerebral ischemic injury.[8]

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Oxysophoridine (OSR), an alkaloid derivative based on sophoridine, is a natural alkaloid extracted from Sophora alopecuroides L. Sophora, its chemical structure is made of two piperidine rings [Figure 1]. From earlier researches, we demonstrated that OSR has protective effects on cerebral ischemic mice induced by middle cerebral artery occlusion through antioxidant and anti-apoptotic mechanisms.[9,10] In this study, we further investigated whether OSR could maintain the balance of EAA and IAA after a long time of reperfusion.

Materials and Methods

Animals and drug preparation

Male ICR mice (n = 85) aged from 4 to 5 weeks and weighed from 20.0 to 25.0 g were supplied by animal center of

Ningxia Medical University. The experiments were performed as approved by the institutional animal care and use committee of Ningxia Medical University and all the efforts were made to minimize the suffering. Mice were randomly assigned to five groups: Sham-operated group (n = 17), vehicle group (n = 17), OSR 62.5, 125 and 250 mg/kg groups (n = 17 each group). Mice in OSR groups were intraperitoneally injected with OSR for seven successive days. OSR was supplied by the Institution of Chemistry and Chemical Engineering, Ningxia Medical University. Sham-operated and vehicle groups were treated with physiological saline under the same conditions.

Mice model of cerebral ischemia

The mice cerebral ischemic injury was induced by the model of middle cerebral artery occlusion as described by Longa *et al*.[11] After pretreatment with OSR or saline for seven days, mice in each group were anesthetized with 3.5% of chloral hydrate. Under sterile condition, the left common carotid artery (CCA) was exposed through a neck incision and the external carotid artery (ECA) was then isolated. The internal carotid artery (ICA) was separated carefully from the adjacent vagus nerve. Next, a nylon monofilament (15 mm in length and 0.15 mm in diameter) was introduced into the left ICA through the ECA stump to block the origin of the left middle cerebral artery (MCA). The monofilament was left in place for 2 h and then removed to restore blood flow for 24 h reperfusion. Mice in the sham-operated group were treated identically, except the MCA occlusion after the neck incision.

Evaluation of neurological deficits

Animals were examined for neurological deficits after 24 h of reperfusion using a five-point neurological function score described previously:[12]

0. No observable neurological deficit;
1. Unable to extend the right paw fully;
2. Circling to the right;
3. Falling to the right;
4. Being unable to walk spontaneously and depression of consciousness.

All of these observations were performed by an investigator blinded to the identity of the groups.

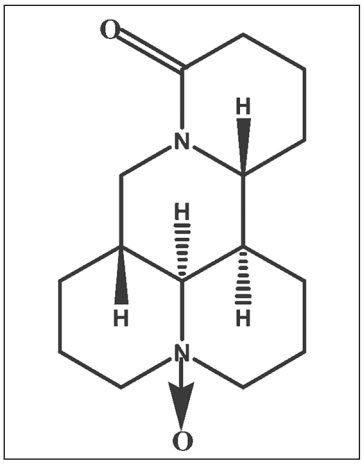

Figure 1: Structure of oxysophoridine (OSR)

Measurement of infarct volume

Following the neurological evaluation, mice (n = 6, for each group) were decapitated to remove brains. Then each brain was cut into 1 mm-thick slices. The brain slices were stained with 2% 2, 3, 5-triphenyltetrazolium chlorides (TTC) (Sigma, St Louis, MO, USA) at 37 °C for 30 min in the dark, and then transferred into 4% of formaldehyde for fixation. The unstained area of the brain slice was defined as infarction, and infarct volumes were calculated with microscope image-analysis software (Image-Pro plus, USA).

Assessment of brain edema

Mice (n = 6, for each group) were sacrificed after 24 h of reperfusion. The brains were divided into two hemispheres: The ischemic hemisphere (left side) and contralateral hemisphere. The ischemic hemisphere was weighed to obtain the wet weight and then dried at 110 °C for 24 h to measure the dry weight. The brain water content in the ischemic hemisphere was calculated as follows: Water content = (wet weight — dry weight)/wet weight × 100%.[13]

Free amino acids analysis

After 2 h of MCAO and 24 h of reperfusion, 5 mice from each group were decapitated. The brain tissues were collected and weighed, then homogenized in 8% 5-sulfosalicylic acid. The homogenate was centrifuged at 10,000 g for 15 minutes and was used to measure amino acids contents. Levels of amino acids were determined by an automatic amino acid analyzer (Model S-433D, sykam, Germany) according to the manufacturer's instructions.

Statistical analysis

Data are presented as mean ± SEM. Comparisons between groups were statistically evaluated by one-way ANOVA with a *post hoc* Fisher's test. Comparisons between two groups were assessed by unpaired t-test. A probability of $P < 0.05$ was considered to be statistically significant by SPSS 13.0 Statistical Software.

Results

Effects of OSR on cerebral ischemic injury in MCAO mice

After 2 h of MCAO and 24 h of reperfusion, the neurological deficits were significantly increased in the vehicle group mice compared to sham-operated group. OSR at the dose of 62.5, 125 and 250 mg/kg groups reduced the neurological deficit scores respectively [Table 1]. In vehicle group, the percentage of infarct volume was significantly increased (44.50 ± 1.10%,

Table 1: Effects of OSR on neurological deficit scores induced by 2 h MCAO followed by 24 h reperfusion

Groups	n	Dose (mg/kg)	No. of mice each grade					Score ($\bar{x}$ ± s)
			0	1	2	3	4	
Sham	12	–	12	0	0	0	0	0.00
Vehicle	12	–	0	1	4	7	0	2.50±0.19##
OSR	12	62.5	0	3	5	4	0	2.10±0.23
	12	125	0	5	6	1	0	1.67±0.19**
	12	250	0	6	6	0	0	1.50±0.15**

Data are expressed as mean ± SEM (n = 12); ##$P < 0.01$ vs. sham-operated group; **$P < 0.01$ vs. vehicle group

$P < 0.01$) compared with sham-operated group. In OSR treatment groups, the infarct volume was reduced to 39.06 ± 1.97% ($P < 0.05$) in OSR 62.5 mg/kg group and 36.32 ± 1.00% ($P < 0.01$) in OSR 125 mg/kg group and 22.44 ± 0.82% ($P < 0.01$) in OSR 250 mg/kg group [Table 2]. The brain water content, as an index of cerebral edema, was noticeably elevated from 79.96 ± 1.72% in sham-operated group to 86.54 ± 1.43% in vehicle group ($P < 0.01$). In OSR 62.5, 125 and 250 mg/kg group, the brain water content was decreased to 83.55 ± 1.02% ($P < 0.01$), 82.85 ± 0.78% ($P < 0.01$) and 80.08 ± 0.83% ($P < 0.01$) [Table 2]. All of these findings indicated that OSR can protect against cerebral ischemic injury in mice and the 250 mg/kg OSR group showed the best neuroprotective effect.

Effects of OSR on amino acids contents

As shown in Figure 2, in sham-operated group, the contents of four amino acids in brain were Glu: 14.33 ± 1.17 μmol/g; Asp: 5.91 ± 0.33 μmol/g; GABA: 11.44 ± 1.09 μmol/g and Gly: 3.30 ± 0.25 μmol/g. After 2 h of ischemia and 24 h of reperfusion, content of Glu was markedly reduced to 10.46 ± 0.76 μmol/g and ASP level significantly decreased to 4.71 ± 0.25 μmol/g ($P < 0.05$) [Figure 2a and b]. The GABA content noticeably decreased to 6.23 ± 1.49 μmol/g ($P < 0.05$) and Gly level was similar to that of the sham-operated group [Figure 2c and d]. Pretreatment with 250 mg/kg OSR could increase all of these four amino acids levels. Glu, Asp, GABA and Gly contents increased to 13.01 ± 0.52 ($P < 0.05$), 5.03 ± 0.29, 10.40 ± 0.55 ($P < .05$) and 4.19 ± 0.18 μmol/g ($P < 0.05$) respectively.

Table 2: Effects of OSR on brain infarct volume and brain water content induced by 2 h MCAO followed by 24 h reperfusion

Groups	*n*	Dose (mg/kg)	Infarct/total Volume (%)	Brain water Content (%)
Sham	6	–	0.00	79.96±1.72
Vehicle	6	–	44.50±1.10##	86.54±1.43##
OSR	6	62.5	39.06±1.97*	83.55±1.02**
	6	125	36.32±1.00**	82.85±0.78**
	6	250	22.44±0.82**	80.08±0.83**

Data are expressed as mean ± SEM ($n = 6$); ##$P < 0.01$ vs. sham-operated group; *$P < 0.05$; **$P < 0.01$ vs. vehicle group

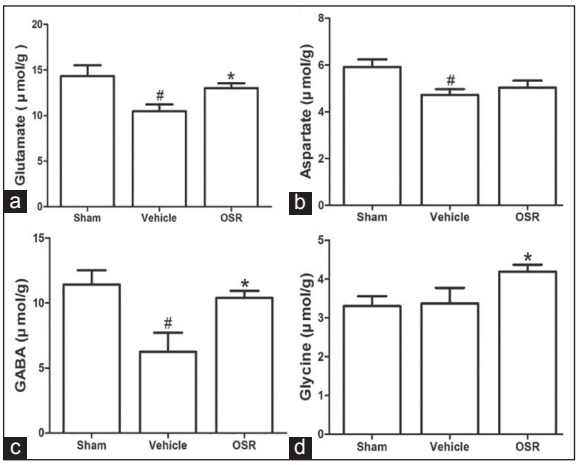

Figure 2: Effects of OSR (250 mg/kg) on the contents of Glu, Asp, GABA and Gly in ischemic brain tissues. (a) Effect of OSR (250 mg/kg) on Glu levels in mice brains at 24 h after reperfusion. (b) Effect of OSR (250 mg/kg) on Asp levels in mice brains at 24 h after reperfusion. (c) Effect of OSR (250 mg/kg) on GABA levels in mice brains at 24 h after reperfusion. (d) Effect of OSR (250 mg/kg) on Gly levels in mice brains at 24 h after reperfusion. Data are expressed as mean ± SEM ($n = 5$). #$P < 0.05$ vs. sham-operated group; *$P < 0.05$ vs. vehicle group

As representative amino acids of EAA and IAA, we further measured the ratio of Glu and GABA. In sham-operated group, Glu/GABA ratio was 1.31 ± 0.21. It was much higher in vehicle group than that of sham group (2.52 ± 0.34, $P < 0.01$). At OSR 250 mg/kg group, the ratio significantly reduced to 1.60 ± 0.19 ($P < 0.05$) [Figure 3].

These findings demonstrated that OSR contributes to keep the amino acids homeostasis and maintain an equitable ratio of EAA/IAA in ischemic brain tissues.

Discussion

In the present study, cerebral ischemic injury in mice was induced by the model of middle cerebral artery occlusion. Our current findings revealed that cerebral ischemic injury could be greatly attenuated by OSR *in vivo*. In OSR 62.5, 125 and 250 mg/kg treatment groups, neurological scores, infarct volume and brain water content were decreased significantly, which of these demonstrated OSR could protect against cerebral ischemic injury.

Amino acids, as important neurotransmitters in the central nervous system, play important roles in the information transmission between neurons. In the process of cerebral ischemia-reperfusion, metabolic disorder between excitatory amino acids (EAA) and inhibitory amino acids (IAA) causes acute neurons damage and intracellular calcium overload, which eventually leads to neuron death.[14] Glutamate (Glu) and aspartate (Asp) are important excitatory amino acids and γ-aminobutyric acid (GABA) and Glycine (Gly) are important inhibitory amino acids in brain. Glu can increase cortical cells activities and mediate excitatory synaptic transmission and

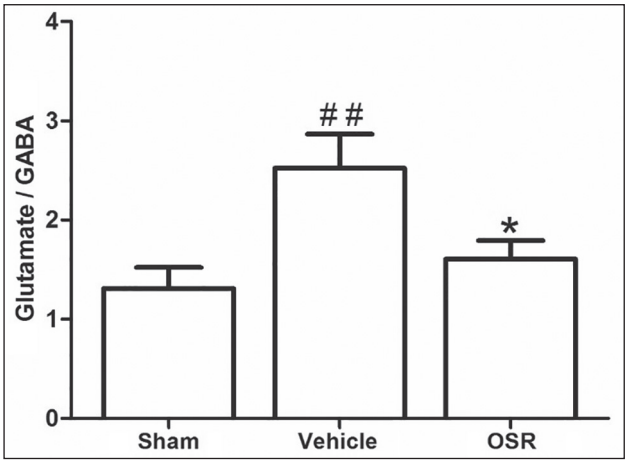

Figure 3: Effects of OSR (250 mg/kg) on the changes of Glu/GABA ratio in ischemic brain tissues. Data are expressed as mean ± SEM ($n = 5$). ##$P < 0.01$ vs. sham-operated group; *$P < 0.05$ vs. vehicle group

excitotoxicity between central neurons. The contents of Glu and Asp will be markedly increased after cerebral ischemia-reperfusion, these changes further cause neurons death and apoptosis and then lead to acute brain injury. GABA can reduce cell injury through postsynaptic inhibition and decrease the release of Glu and eventually attenuate EAA induced toxicity.

During the different time point of cerebral ischemia and reperfusion, the concentration of amino acids in ischemia region changed significantly by some previous studies.[15,16] In this study, we measured the contents of Glu, Asp, GABA and Gly after 24 h of reperfusion, which is a relatively late time point. The results indicated that most of the amino acids levels were reduced at 24 h after reperfusion compared to normal standards. We consider that the excessive release of EAA and IAA at the early stages of cerebral ischemia leads to the low contents of amino acids after 24 h of reperfusion. OSR 250 mg/kg could inhibit the reduction of these four amino acids after cerebral ischemia reperfusion and help to maintain the contents of these four amino acids at a normal level. The imbalance of Glu/GABA ratio, is considered to regard as an important standard to promote the ischemic cerebral damage, was observed obviously in MCAO group mice. In the OSR 250 mg/kg group, the Glu/GABA ratio was significant lower. All of these findings indicated that OSR could maintain the amino acids homeostasis after 24 h of reperfusion.

In this study, we did not measure the contents of these four amino acids at the early time of cerebral ischemia and reperfusion. At the early stages of cerebral ischemia, a large number of free amino acids were released in ischemic brains. According to some previous studies, we make a surmise that OSR could decrease the excessive release of free amino acids at the early time point of cerebral ischemia. The possible mechanisms may be associated with the effects that OSR enhance the free amino acids reuptake of neurons and inhibit the EAA excessive release. Our future study will measure these four amino acids' contents at different time point after cerebral ischemia and reperfusion and wish to obtain a series of more convincing evidences to prove or revise our surmise.

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References

1. Chacon MR, Jensen MB, Sattin JA, Zivin JA. Neuroprotection in cerebral ischemia: Emphasis on the SAINT trial. Curr Cardiol Rep 2008;10:37-42.
2. Farber JL, Chien KR, Mittnacht S Jr. Myocardial ischemia: The pathogenesis of irreversible cell injury in ischemia. Am J Pathol 1981;102:271-81.
3. Kato H, Kogure K. Biochemical and molecular characteristics of the brain with developing cerebral infarction. Cell Mol Neurobiol 1999;19:93-108.
4. Nishizawa Y. Glutamate release and neuronal damage in ischemia. Life Sci 2001;69:369-81.
5. Kawaguchi K, Graham SH. Neuroprotective effects of the glutamate release inhibitor 619C89 in temporary middle cerebral artery occlusion. Brain Res 1997;749:131-4.
6. Minger SL, Geddes JW, Holtz ML, Craddock SD, Whiteheart SW, Siman RG, *et al*. Glutamate receptor antagonists inhibit calpain-mediated cytoskeletal proteolysis in focal cerebral ischemia. Brain Res 1998;810:181-99.
7. Marcoli M, Bonfanti A, Roccatagliata P, Chiaramonte G, Ongini E, Raiteri M, *et al*. Glutamate efflux from human cerebrocortical slices during ischemia: Vesicular-like mode of glutamate release and sensitivity to A(2A) adenosine receptor blockade. Neuropharmacology 2004;47:884-91.
8. Bogaert L, Scheller D, Moonen J, Sarre S, Smolders I, Ebinger G, *et al*. Neurochemical changes and laser Doppler flowmetry in the endothelin-1 rat model for focal cerebral ischemia. Brain Res 2000;887:266-75.
9. Wang TF, Lei Z, Li YX, Wang YS, Wang J, Wang SJ, *et al*. Oxysophoridine protects against focal cerebral ischemic injury by inhibiting oxidative stress and apoptosis in mice. Neurochem Res 2013;38:2408-17.
10. Rui C, Yuxiang L, Ning J, Ningtian M, Qingluan Z, Yinju H, *et al*. Anti-apoptotic and neuroprotective effects of oxysophoridine on cerebral ischemia both *in vivo* and *in vitro*. Planta Med 2013;79:916-23.
11. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 1989;20:84-91.
12. Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H. Rat middle cerebral artery occlusion: Evaluation of the model and development of a neurologic examination. Stroke 1986;17:472-6.
13. Yan H, Zhang Y, Lv SJ, Wang L, Liang GP, Wan QX, *et al*. Effects of glutamine treatment on myocardial damage and cardiac function in rats after severe burn injury. Int J Clin Exp Pathol 2012;5:651-9.
14. Barth A, Barth L, Newell DW. Combination therapy with MK-801 and alpha- phenyl-tert-butyl-nitrone enhances protection against ischemic neuronal damage in organotypic hippocampal slice cultures. Exp Neurol 1996;141:330-6.
15. Shimizu H, Graham SH, Chang LH, Mintorovitch J, James TL, Faden AI, *et al*. Relationship between extracellular neurotransmitter amino acids and energy metabolism during cerebral ischemia in rats monitored by microdialysis and *in vivo* magnetic resonance spectroscopy. Brain Res 1993;605:33-42.
16. Bie X, Chen Y, Han J, Dai H, Wan H, Zhao T. Effects of gastrodin on amino acids after cerebral ischemia-reperfusion injury in rat striatum. Asia Pac J Clin Nutr 2007;16:305-8.

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