The Neuroprotection of Oxymatrine in Cerebral Ischemia/Reperfusion Is Related to Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2)-Mediated Antioxidant Response: Role of Nrf2 and Hemeoxygenase-1 Expression

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Cerebral ischemia-reperfusion (CI/R) injury remains a major medical problem due to the lack of effective therapies. Previous studies have shown that increasing the activity of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and gene targets in cell culture and stroke animal models is highly neuroprotective. Oxymatrine is the major quinolizidine alkaloid extracted from the root of Sophora flavescens Ait., and has been proved to be protective after ischemia in recent studies. The present study was designed to investigate the potential effect of oxymatrine in ischemia-reperfusion injury in rat’s brain and to explore the possible role of oxymatrine in Nrf2 pathway. The results indicated that the ischemic infarct and edema were significantly reduced in rats that received oxymatrine, with a corresponding improvement in neurological function after CI/R. In immunohistochemistry and Western blotting analyses, Nrf2 and hemeoxygenase-1 (HO-1) were up-regulated in ischemic cortex, beginning at 6 h, peaking at 48 h and declining at 72 h after CI/R. Intraperitoneal injection of oxymatrine inhibited the production of lipid peroxidation and increased the activities of Nrf2 and HO-1 in rats brain after CI/R. Taken together, these results suggest that oxymatrine administered systemically protected brain against focal ischemia-reperfusion damage at the early stage of stroke, and that activating Nrf2/HO-1 pathway may contribute to the neuroprotective action of oxymatrine in rat focal brain ischemia-reperfusion model. Thus, treatment of stroke with oxymatrine may prevent severe consequences after brain attack.

Key words cerebral ischemia-reperfusion; hemeoxygenase-1; neuroprotection; nuclear factor erythroid 2-related factor 2; oxymatrine

Cerebrovascular disease, a kind of common and frequently-occurring disease endangering the human health seriously, is one of the leading causes of death and disability worldwide, with the most frequent prevalence of ischemic cerebrovascular disease. Recanalization following ischemia is the most effective method for treatment of acute cerebral infarct and correction of hypoxia, but paradoxically causing severe cerebral ischemia-reperfusion (CI/R) injury. Due to the lack of efficient neuroprotective therapies, the CI/R injury is still a major medical problem urgently needed to be further studied and discussed.

A variety of cascade reactions induced by CI/R involve disequilibrium of calcium homeostasis, cytotoxicity of excitatory amino acid, oxidative stress, mitochondrial dysfunction, etc. which has been accompanied by protease-activation and changing expression of genes, and ultimately resulting in neuron death and apoptosis. Increasing evidences suggest a critical role for oxidative stress in mediating tissue injury and neuron death during CI/R. Brain is the most activated organ with aerobic metabolism, and produces a certain level of reactive oxygen species physiologically by aerobic metabolism, with a dynamic equilibrium of reactive oxygen species generation and elimination. Pathological unbalance occurs as the free radicals are produced excessively or endogenous anti-oxidative systems perform functional disturbance, and then, excessive accumulation of free radicals may induce oxidative reactions in attacked cells and tissues. The ability of free radicals production is greater than that of elimination by endogenous anti-oxidative systems in ischemic tissues especially in tissues after CI/R. Elevated reactive oxygen species produced in cerebral ischemia could directly disrupt the structures of lipids, proteins and DNA, and induce cell death in various ways. Previous studies have well documented that increasing the activities of antioxidant enzymes, or administration of antioxidants, attenuates neuron damage during CI/R injury. Conversely, lowering the antioxidant capacity increases neuron death after CI/R. The nuclear factor erythroid 2-related factor 2 (Nrf2) pathway is a recently elucidated pathway to induce the production of antioxidant enzymes—known as phase II enzymes including hemeoxygenase-1 (HO-1), reduced nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase 1, and γ-glutamyl cysteinyl ligase (γ-GCL)—through antioxidant-response element (ARE). Activating this way enhances the activities of those antioxidant enzymes and provides efficient cytoprotection, partly, by detoxifying reactive oxygen species and xenobiotics, regulating glutathione (GSH) production and use, and maintaining intracellular redox state. Recent experiments have suggested that increasing the activity of Nrf2 and gene targets in cell culture models and stroke animal models that simulate components of cerebral ischemia damage is highly neuroprotective. Phase II enzymes are critical components of endogenous defense against oxidative stress, and crucial for reducing oxidative injury and preventing neuronal death. Among these enzymes HO-1 has attracted special attention because of its significantly therapeutic effects for neuroprotection. Cells from HO-1−/− mice were highly susceptible to oxidative injury than these from wild type mice in vitro. Studies in animal models using gene-knockout and transgenic mice have also demonstrated a biological significance of HO-1 as an endogenous antioxidant. Primary cultured cells of atrocities and neurons from Nrf2−/− mice are also more likely susceptible to oxidative injury, calcium influx and mitochondrial toxicity than that of wild type neuronal cells, whereas, Nrf2 overexpression and small-molecule-inducible Nrf2...
enhanced neuronal resistance to cytotoxicity. Some work had also been presented in our previous study, and proved the availability of drugs which increase the activity and expression of Nrf2/HO-1 for the neuroprotective therapy during cerebral ischemia. Therefore, inducing Nrf2 pathway expression or increasing its activation may be a novel therapeutic target for neuroprotection and anti-oxidative responses.

Oxymatrine is an alkaloid extracted from the root of *Sophora flavescent* Ait (Kushen). We recently found that oxymatrine protected brain against inflammatory damage through nuclear factor-κB (NF-κB) pathway during permanent ischemia. This study was designed to investigate the potential role of oxymatrine in CI/R, and to investigate whether there was an association of oxymatrine and Nrf2/HO-1 pathway during CI/R.

**MATERIALS AND METHODS**

**Transient Cerebral Ischemia Model** Our experimental protocol was approved by the institutional animal care and use committee and the local experimental ethics committee. Healthy adult male Sprague-Dawley (SD) rats, weighing 260 to 280 g at the time of stroke, were purchased from Animal Experiment Center of Hebei Medical University, and housed in a 12/12 h light/dark cycle with food and water ad libitum. Anesthesia was conducted with 10% chloral hydrate (350 mg/kg, intraperitoneally (i.p.)). Body temperature was monitored and maintained at 37 °C. Rat middle cerebral artery occlusion/reperfusion model (MCAO/R) was established with suture method. Briefly, a midline incision was made on the ventral side of the neck and the muscles were pulled aside gently, and the right common carotid artery and the junction of internal and external carotid artery were dissected carefully. The external carotid artery was ligated and catherized. A 2-0 nylon monofilament (diameter 0.234 mm) with its tip rounded by heating near a flame was inserted into the internal carotid artery through a nick of the external carotid stump to block the origin of middle cerebral artery. After 3 h of ischemia, the filament was pulled out for reperfusion. Animals in sham-operated group underwent the same surgery process but for filament insertion.

**Experimental Groups and Drug Administration** Male adult SD rats were randomly divided into three groups: Sham-operated group (Sham), MCAO/R group, and oxymatrine group (OMT). Oxymatrine with a purity of more than 98% was purchased from Huike Botanical Development Company (Shanxi, China). Oxymatrine was prepared at a final concentration of 5 g/100 ml in saline and administered by intraperitoneal injection (i.p., 120 mg/kg/d) at the onset of cerebral ischemia, and then once a day on the following days. In MCAO/R and Sham-operated groups, equal volume of saline was injected via i.p.

**Neurological Function Assessment** An examiner blinded to the experimental groups performed behavior assessments. Neurological deficit was scored based on a previous description with some modifications. Test included the following: 0, no deficits; 1, difficulty in fully extending the contralateral forelimb; 2, unable to extend the contralateral forelimb; 3, mild circling to the contralateral side; 4, severe circling and 5, falling to the contralateral side.

**Infarct Measurement** The extent of infarction was measured with 2% 2,3,5-triphenyl-tetrazolium chloride (TTC) vital staining for 72 h survival times after ischemia. For TTC staining, animals were deeply anesthetized with 10% chloral hydrate, and the brains were removed and washed in normal saline at room temperature and then sliced into 2 mm sections. The sections were immersed in 2% TTC in saline and incubated for 15 min at 37 °C. Then, TTC solution was removed and replaced with 4% paraformaldehyde for fixation overnight at 4 °C. Infarct area was measured using image analysis software (Image-Pro Plus 5.1). To compensate for the effect of brain edema, we normalized the infarct sizes of each section to ipsilateral hemisphere area. The percentage of hemisphere lesion volume (%HLV) was calculated according to the following equations:

\[
\%HLV = \left( \frac{\text{total infarct volume} - \text{right hemisphere volume} - \text{left hemisphere volume}}{\text{left hemisphere volume}} \right) \times 100\%.
\]

**Determination of Oxidative Stress (Lipid Peroxidation Assay)** Malondialdehyde (MDA) is one of the products of lipid peroxidation which is induced by the attack of reactive oxygen species on polyunsaturated fatty acid. MDA levels in brain cortices were determined by thiobarbituric acid method and expressed as nmol MDA/mg protein. Brain tissue homogenates were prepared as follows; 100 mg of tissue sample was homogenized in 0.9 ml of ice-cold normal saline, and then centrifuging 15 min at 2000 rpm. The amount of MDA was measured using spectrophotometer according to the introduction.

**Brain Water Content** Brain water content (BW) was measured using wet–dry method.Brains were removed quickly and placed on a pre-prepared dry tray. The frontal pole about 4-mm-thickness was removed and a 2-mm-thick coronal brain tissue behind the frontal pole was obtained, and the coronal tissue was quickly separated into ischemic and non-ischemic hemispheres. The two hemisphere sections were respectively wrapped with tinfoil and weighted on an electronic balance to get wet weight, and then dried for 24 h at 100 °C to get dry weight. BW = (wet weight–dry weight)/ wet weight) × 100%.

**Immunohistochemistry** After deep anesthesia with 10% chloral hydrate, brains were removed quickly and fixed in 4% paraformaldehyde over 24 h to prepare for paraffin-embedded sections according to the standard histological process. The paraffin-embedded brain tissue was cut into 5 μm thick sections to perform the following procedures including dewaxing, hydration, antigen repairing, and blocking with serum to eliminate endogenous peroxidase. The slices were separately incubated with Nrf2 rabbit polyclonal antibodies (1 : 100, Santa Cruz Biotechnology) and HO-1 rabbit polyclonal antibody (1 : 200, Stressgen Biotechnologies, Victoria, Canada) overnight at 4 °C, then incubated with secondary antibody (goat anti-rabbit), biotinylated conjugates and diaminobenzidine ordinarily (Zhongshan Biology Technology Company, China). Finally, hematoxylin was used for counterstain. An examiner blinded to the experiment groups counted the cells labeled with Nrf2 and HO-1 antibodies of 5 random lesion regions in ischemic hemisphere cortex under a light microscope at 400 ×.

**Western Blotting** Total protein and nuclear protein of ischemic cortex were extracted using Protein Extraction Kits (Apply Gen Technologies Inc., Beijing) following the manufacturer’s protocols at 48 h after MCAO. Protein concentra-
tion was determined using BCA Protein Assay reagent kit (Novagen, Madison, WI, U.S.A.). Equal volume of proteins were separated by polyacrylamide gel electrophoresis, and transferred to polyvinylidene fluoride (PVDF) membrane. Membranes loaded with interest proteins were incubated in blocking buffer (5% non-fat dried milk in phosphate buffered saline (PBS)) at room temperature, and then incubated with the primary antibodies—polyclonal rabbit anti-Nrf2 antibody (1 : 200, Santa Cruz Biotechnology), polyclonal rabbit anti-HO-1 antibody (1 : 500, Stressgen Biotechnologies, Victoria, Canada) and rabbit anti-rabbit β-actin (1 : 500, Zhongshan Biotechnology, Beijing)—at 4 °C overnight. On the second day, membranes were washed with TPBS for three times and incubated with the second antibodies (goat anti-rabbit, 1 : 3000, Rockland) for 1 h at room temperature. The optical density value (OD) of each band was analyzed with an imaging densitometer (LI-COR Bioscience), and normalized by β-actin immunoreactivity to correct samples differences.

Statistical Analysis
SPSS 13.0 software was used to perform the statistical analysis in this study. Except neurological deficit, the data were expressed as the mean ± S.D. Mann–Whitney U test was used to analyze neurological deficit scores. Except neurological function assessment, all other data were analyzed with One-way analysis of variance (ANOVA) comparison test. Differences with p<0.05 were considered statistically significant.

RESULTS

Effect of Oxymatrine on Neurological Deficit Scores after CI/R

Neurological deficits at 3 h, 6 h, 12 h, 24 h, 48 h and 72 h were separately scored on a modified 6-point scaling system (n=6). Comparisons between groups were tested by Mann–Whitney U test. Rats in Sham group didn’t perform any neurological function defect, thus we didn’t consider the operation itself as a contributive factor of brain lesion. All of experimental rats in MCAO/R and OMT groups developed varying degrees of palsy of contralateral forelimbs. The average neurological deficit scores (Avg. Neurol. Score) of each group at different time points were shown in Fig. 1A. The result showed that all animals with cerebral ischemia developed significant neurological function defect with high scores at 3 h after operation. The high scores continued over the next time points, separately at 6 h, 12 h, 24 h, 48 h and 72 h after operation. After treatment with oxymatrine, the neurological deficit score was lower in OMT group than in MCAO/R group. Statistic analysis showed that the acute motor deficits during the stroke (3 h after occlusion) were similar, suggesting that rats in both groups received the same degree of ischemia. However, neurological deficit scores were lower in rats with operation and oxymatrine treatment than in control rats with operation only as examined separately at 12 h, 24 h, 48 h, and 72 h post-ischemia (p<0.05), but not at 6 h (p>0.05).

Effect of Oxymatrine on Brain Water Content after CI/R

Brain water contents of ischemic hemispheres were expressed as a percentage of the weight (n=6). The dynamic changes of the brain water content percentages in groups at different time points were shown in Fig. 1B. Compared with that of normal brain, the water content percentage of ischemic brain tissue (MCAO/R and OMT groups) increased at 6 h, peaked at 48 h, and then declined at 72 h after operation. No obvious changes of the percentages could be found in Sham groups, suggesting that the operation didn’t contribute to the increased brain water content during CI/R (p>0.05). However, the water content percentages in MCAO/R groups were markedly increased after operation (at 12 h, 24 h, 48 h and 72 h after operation) separately compared with those in Sham groups. Oxymatrine treatment can obviously reduce the percentage of brain water content at these time points after operation (p<0.05).

Effect of Oxymatrine on Infarct Size after CI/R

The volume of cerebral infarction was detected by TTC staining (Fig. 2A, n=6). All animals underwent a transient ischemia (3 h) and were sacrificed at 72 h after operation (Fig. 2B). Oxymatrine was injected intraperitoneally immediately after operation, then once daily on the following days. The acute motor deficits during the stroke (3 h) were similar, suggesting that animals in both groups received the same degree of ischemia (Fig. 2C). Motor deficit scores in oxymatrine-treated group were lower than that of MCAO/R group when evaluated at 72 h after operation (Fig. 2D). Figure 2A displayed the image of TTC-stained brain sections of MCAO/R and OMT-treated animals at 72 h after operation. TTC, a fat-soluble complex being sensitive to light, is the proton receptor of enzyme systems with the structure of pyridine-nucleotide in the respiratory chain. Normal tissues appeared red in TTC solution for reacting with dehydrogenase. Ischemic
tissue showed white rather than red color because of the decreased activity of dehydrogenase. The result showed that no infarct area was observed in brain sections of Sham group. However, a large area of infarct was developed in striatum and lateral cortex of ischemic hemispheres of rats in MCAO/R group and OMT group. After treatment with oxymatrine, the infarct size of OMT group was lower than that of MCAO/R group, with significant difference (p<0.05).

**Time Course Expression of Nrf2 and HO-1 after CI/R, and the Effect of Oxymatrine on the Expressions of Nrf2 and HO-1**

Immunohistochemistry was firstly used to observe the expressions of Nrf2 and HO-1 at 48 h after operation (Fig. 3, n=3). The numbers of HO-1 positive cells and Nrf2 nuclear positive cells were counted manually under a microscope (400×). As expected, low levels of constitutive expressions of Nrf2 and HO-1 were observed in normal cortices tissue in Sham rats. Moreover, Nrf2 and HO-1 proteins mostly located in cellular plasm, and the positive cells had been stained dark brown. At 48 h after operation, the immunoreactivities of Nrf2 and HO-1 in ischemic cortices were significantly enhanced, and the positive cells of Nrf2 and HO-1 were markedly increased, with strong positive staining of HO-1 in cytoplasm and strong positive staining of Nrf2 both in cytoplasm and nucleus.

Then we observed the dynamic expression of Nrf2 and HO-1 in the ischemic cortices and countered HO-1 positive cells and Nrf2 nuclear positive cells histologically (Fig. 4, n=3). The result showed that elevated HO-1 and Nrf2 immunoreactivities in cells as well as increased Nrf2 immunoreactivity in nucleus were presented in the injured regions of cortices in rats with cerebral ischemia (MCAO/R and OMT) at 6 h after operation, with a maximum presence at 48 h and then a decline at 72 h. HO-1 positive cells and Nrf2 nuclear positive cells in ischemic cortices were increased more in experimental rats of OMT group separately at 6 h, 12 h, 24 h, 48 h, and 72 h after operation than those of MCAO/R group, with significant differences statistically. Despite increased total protein levels of Nrf2 in ischemic cortices after operation by Western blotting, no sig-
significantly dynamic changes were presented among these time points (Fig. 5A, n = 3). Conversely, HO-1 protein expression measured with Western blotting increased at 6 h after operation, peaked at 48 h, and declined at 72 h, with a consistence with histological result (Fig. 5A, n = 3).

In order to further confirm the neuroprotection of oxymatrine during CI/R, we further examined the changing content of MDA in ischemic cortices at 24 h after operation, and evaluated the changing expression of total HO-1 protein, total Nrf2 protein and nuclear protein of Nrf2 in ischemic cortices by Western blotting at 48 h after operation (Figs. 5B—F), and significantly increased the total protein expression of Nrf2 and HO-1, as well as nuclear Nrf2 protein in ischemic cortices at 48 h after operation, suggesting that oxymatrine had a better action on enhancing the activity of Nrf2/HO-1, and implying the potential role of this neuroprotection in CI/R.

**DISCUSSION**

Cellular antioxidants are important for reducing oxidative stress and preventing neuronal death. Nrf2 and its gene targets, known as phase-II enzymes, are crucial components of endogenous anti-oxidative systems.

Table 1. Determination of Malonyldialdehyde

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/mgprot)</th>
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<tbody>
<tr>
<td>Sham (n=6)</td>
<td>2.62±0.65</td>
</tr>
<tr>
<td>MCAO/R (n=8)</td>
<td>3.86±0.69*</td>
</tr>
<tr>
<td>OMT (n=11)</td>
<td>2.62±0.36**</td>
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*p<0.05 vs. Sham group, **p<0.05 vs. MCAO/R group.

been presented recently by Yang (2009) in our laboratory, and suggested that Nrf2/HO-1 pathway played an important role in reducing ischemic injury caused by permanent MCAO. Data obtained in this study also proved the notably neuroprotective effect of Nrf2/HO-1 pathway in transient cerebral ischemia in vivo, and highlighted the potential of Nrf2/HO-1 activation by ischemic injury such as CI/R as a prophylactic strategy for stroke.

Nrf2 and the adaptor protein, Kelch-like ECH-associated protein 1 (Keap1), are centre regulators of cellular anti-oxidative responses. Under normal-physiological conditions, Nrf2 signaling is repressed by Keap1 and mainly sequestered in the cytoplasm. However, Nrf2 could be activated by oxidative stress, and then translocated into the nucleus to perform tasks. The intermolecular correlation between activated Nrf2 and ARE induced the expressions of antioxidant proteins and phase II enzymes which act actively in cellular defending systems. Our findings showed the characteristic expression of Nrf2 and HO-1 over time during CI/R. The
dynamic expression of Nrf2 and HO-1 in ischemic cortices clearly indicated the response to ischemic injury at the early stage of CI/R. The gradually increased nuclear translocation of Nrf2 and constant enhancement of total Nrf2 protein further confirmed the pivotal role of transcriptional activation of Nrf2 in CI/R for the neuroprotection. Moreover, HO-1 increased continuously early in ischemia over time, suggesting a biological significance of HO-1 as an endogenous defending factor.

Cerebral ischemia induces complex cascade reactions, including the production of ROS/RNS, Ca²⁺ overload, inflammatory reactions, excitotoxicity, and apoptosis etc. Ischemic cascades after stroke usually go on for hours but can last for days. Reperfusion of ischemic brain tissue is critical for restoring normal neuron function, but paradoxically resulting in secondary damage. The most studied cytokines exacerbating tissue injury are tumor necrosis factor-α, interleukins (IL), cellular adhesion molecules, matrix metalloproteinase and others. Studies also find that neuroprotective factors also act actively during ischemia. The rapid and constant increase of Nrf2 and HO-1 from 6 to 72 h after ischemia/reperfusion suggested that minimal stress stimuli could initiate the activation of Nrf2 pathway, and that ischemic injury also initiated the natural defense mechanism in vivo to avoid the excessive damage of ischemic brain tissue.

Activation of Nrf2 pathway is a major mechanism of cellular defense against oxidative stress. Administration of tert-butylhydroquinone (tBHQ), a well characterized Nrf2 inducer, significantly improved behavioral and histological outcomes in models of CI/R in vivo. Moreover, prophylactic treatment with tBHQ could improve functional recovery up to 1 month after reperfusion, suggesting that previous Nrf2 activation may reduce neuronal cell death during delayed apoptosis long after stroke onset. Conversely, Nrf2-deficient mice were susceptible to severe neurological deficit and exacerbating ischemic injury, and susceptible to be abrogated the neuroprotection of tBHQ. Studies using transgenic and gene-knockout mice have demonstrated the significance of HO-1 as a cellular antioxidant in endogenous defense against oxidative stress. Researches revealed that up-regulating HO-1 expression decreased infarct sizes in stroke and protected brain against excitotoxicity. Previous study also showed that electrophilic drugs (such as neurite outgrowth-promoting prostaglandin, NEPP) can afford neuroprotection by activating Keap1/Nrf2/HO-1 pathway. Thus, compounds increasing Nrf2/HO-1 activity may be a promising candidate for limiting oxidative stress after stroke. The present study proved that oxymatrine treatment increased expression of Nrf2 and HO-1, as well as nuclear translocation of Nrf2 in ischemic cortex at the early stage of CI/R, suggesting that the neuroprotective effect of oxymatrine in cerebral ischemia-reperfusion might be associated with the regulation of Nrf2/HO-1. It also suggested that oxymatrine might have a potential efficacy in anti-oxidative reactions. This statement was verified by the reduction of lipid peroxide production, malondialdehyde, in OMT group. Although regulation of Nrf2/HO-1 by oxymatrine lasted for few days after ischemia-reperfusion, from 6 to 72 h, oxymatrine therapy didn’t change the regularity of dynamic expression of Nrf2/HO-1 for the no advanced-peak-value time in OMT group. These results suggested that oxymatrine increased the activity of Nrf2 and HO-1 in ischemic cortex at the early stage of CI/R, and that oxymatrine may be a potential antioxidant in cerebral ischemia.

The previous work on the pharmacological effect of oxymatrine has proved the protective effect of oxymatrine in the permanent acute cerebral ischemia, and showed the possible mechanism of oxymatrine in this protection, as indicated by the down-regulation of NF-kB, toll-like receptor-4 (TLR4), toll-like receptor-2 (TLR2) and myeloid differentiation factor 88 (MyD88). The present study also demonstrated the efficacy of oxymatrine in CI/R, with significantly reduced neurological deficit scores, infarct volume and brain edema in OMT group at different times after operation, which is consistent with the effect of oxymatrine on Nrf2/HO-1 pathway. Furthermore, the degree of lipid peroxidation was also decreased by oxymatrine, directly suggesting a notably antioxidative action of oxymatrine during CI/R. There are some limitations to this study in its present stage, but these will be overcome by further work.

In conclusion, our findings suggested that oxymatrine protected the brain against CI/R injury, and that this effect may be related to Nrf2-mediated antioxidant response. Up-regulating Nrf2/HO-1 expression may be one of the promising therapeutic targets of oxymatrine during CI/R. Treatment of stroke with oxymatrine may prevent severe consequences after brain attack.

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REFERENCES