African Journal of Pharmacy and Pharmacology Vol. 6(7), pp. 459-463, 22 February, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.407
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Protective effect of Naoshuning in a rat brain ischemia-reperfusion damage model

Mei-kui Zhang[1], Shao-qing Shen[2], Feng-hua Xu[2]*, Ruo-zhuo Liu[3] and Ting-ting Chen[1]

[1]Telemedicine Center, Chinese PLA General Hospital, Beijing 100853, China.
[2]Division of Pharmaceutics, Chinese PLA General Hospital, Beijing 100853, China.
[3]Neurology, Chinese PLA General Hospital, Beijing 100853, China.

Accepted 27 January, 2012

To evaluate the protective effects of Naoshuning Chinese traditional medicine on the brain ischemia-reperfusion (IR) injury in rats and explore the possible mechanism of IR injury, rats were divided into 4 groups: sham-operation group, model group as negative control group, Naoshuning group as treatment group and ginaton group as positive control group. The brain IR model was established in rats. Reperfusion was carried out 2 h after ischemia. Neurological evaluation was assessed by scores when the rats came around. Brain infarct volume, superoxide dismutase (SOD) activity and malondialdehyde (MDA) content in serum were examined, respectively at 1st day, 7th day and 14th day after reperfusion. Behavioral abnormities could be seen after the surgery. Compared with sham-operation group, the general scores was much higher in the IR group but was significantly lower in Naoshuning group and ginaton group ($p<0.01$). After surgery, rats in IR groups appeared much more infarcts than those in sham-operation group ($P<0.01$). The infarct volume in the Naoshuning group and ginaton group was markedly decreased and the decrease was more obvious at the 7th day after reperfusion (taking drug for 10 days) ($P<0.01$). When compared with sham group, the activity of SOD in IR group dramatically decreased and the MDA content increased significantly after injury ($p<0.01$). Within IR groups, the SOD activity at the 3rd and 7th day after reperfusion (taking drug for 6 and 10 days, respectively) was markedly increased in Naoshuning and ginaton group, and the MDA content was decreased significantly in Naoshuning and ginaton groups compared with sham-operation group. In conclusion, Naoshuning treatment significantly improved the neurological functions, reduced the infarct volume, decreased the MDA content and improved the SOD activity in rats undergoing IR injury, which suggests that Naoshuning can exert neuroprotective effects via inhibiting free radical generation and improving the activity of antioxidant enzymes.

Key words: Naoshuning, ischemic encephalopathy, reperfusion damage, superoxide dismutase, malondialdehyde.

INTRODUCTION

Ischemic cerebrovascular disease is seriously threatening human health featured as high incidence rate, high mutilation rate and high mortality. The positive and effective prevention and treatment of ischemic cerebrovascular disease is profound and socially necessary. One of the main reasons for ischemic cerebrovascular disease is acute ischemia induced by middle cerebral artery occlusion (MCAO). In present clinical treatment, thrombolytic medicine is widely used to restore cerebral blood flow and relieve ischemic encephalopathy. But the injury induced by ischemic encephalopathy reperfusion is still a problem which is concerned by numerous scholars. Previous studies (Sui and Zhou, 2007; ArunaDevi et al., 2010) showed that peroxidation and inflammatory cascade are the key events in the ischemia-reperfusion (IR) damage.

*Corresponding author. E-mail: xufh@yahoo.com.

Compared with those thrombolytic medicines, traditional Chinese medicine has fewer side effects.The effectiveness of certain Chinese medicine has been reported in some studies, for instance, the *Ginkgo biloba* extract (Louajrj et al., 2001) has good effect on eliminating free radicals and antioxidation. Naoshuning is a compound traditional Chinese medicine studied in our group. It is composed of *Hirudo nipponica*, pseudo-ginseng, Motherwort, *Cyathula* root, *Rheum officinale,* Oriental Waterplantain Rhizome and *Imperata cylindrica*. Previous studies showed that Naoshuning can alleviate the brain edema and improve microcirculation to protect the brain injury to some degree, which may be related to the bioactivities of *H. nipponica* and pseudo-ginseng, the two important ingredients (Cui et al., 2008; Zhang et al., 2006). Nevertheless, the neuroprotective effects of Naoshuning have not been reported so far. From the point of free radicals and lipid peroxidation, the study aimed to investigate the protective effects of Naoshuning on the brain IR injury by monitoring the superoxide dismutase (SOD) activity and malondialdehyde (MDA) content in serum of rat.

MATERIALS AND METHODS

Experimental animals

Male SD rats (SPF grade, 8 months old, body weight 260±20 g) were purchased from the Experimental Animal Center of Chinese People's Liberation Army (PLA), Academy of Military Medical Science. The license number is SCXK-(army) 2007-004.

Main reagents and drugs

Naoshuning Chinese medicine is composed of *H. nipponica,* pseudo-ginseng, *Leonurus heterophyllus* sweet extract, *Cyathula officinalis Kuan,* Rhizoma Rhei, Rhizoma Alismatis and Cogongrass Rhizome and was purchased from Chinese medicine dispensary of PLA. Ginaton (*G. biloba* extract) was from Dr. Willmar Schwabe Pharmaceuticals (batch number: H20040335). 2, 3, 5 triphenyltetrazolium chloride (TTC) (Shanghai lingmian fine chemical Co, Ltd), SOD kit and MDA kit (Nanjing jiancheng Bioengineering Research Facility) were used in this study.

Instruments

800-1 centrifuge (Jiangsu Jintan Medical Instrument Company), TW8 thermostatic water bath (You Lebo Company; Germany) UV-2550 ultraviolet and visible spectrophotometer (Shimadzu Corporation) were used in the present study.

Grouping and treatment

Rats were housed in the Animal Center for 4 days for accommodation and then randomly divided into sham group, IR group, IR + Naoshuning group and IR + ginaton group. Furthermore, each group was subdivided into 1 day group, 7 days group, 14 days group with 6 rats in each subgroup. In the Naoshuning group, rats received Naoshuning (1.5 g/100 g body weight) intragastrically. In the ginaton group, rats were given ginaton (5 mg/100 g body weight) in the same way. Rats in sham and IR groups were administered with normal saline (1 ml/100 g body weight). Treatment was performed twice daily for 3 consecutive days. On the 4th day, 1 h after treatment, MCAO model was established and treatment was performed once 6 h later and then once daily. After operation, all rats were allowed to fast for 12 h, and then given *ad libitum* access to food and water. Rats in each group were sacrificed at 1, 7 and 14 days after reperfusion.

Preparation of ischemic encephalopathy reperfusion model

MCAO was established according to modified method described by Longa et al. (1989). Briefly, rats were intraperitoneally anesthetized with 10% choral hydrate (0.40 ml/100 g body weight) and fixed on a table. After skin preparation, a 2 cm incision was made in the middle line of neck, the subcutaneous fascia and muscles were separated; and the left common carotid artery (CCA) was exposed. After exposure of CCA, external carotid artery (ECA) and internal carotid artery (ICA), CCA and ECA were ligated with 10 cm stitches. The ICA was clamped and a small hole was made in the bifurcation of ICA followed by insertion of a nylon monofilament into ICA. Then, the clamp was released and the monofilament was inserted forward until felling a slight resistance, which indicates that the nylon monofilament reached the starting point of anterior cerebral artery. The suture in ICA was fastened and occlusion was performed for 2 h followed by removal of the monofilament. Then, the wound was closed. For rats in sham group, operation was performed without occlusion.

Neurological function testing

Neurological function was detected at 22 h after IR as previously described (Longa et al., 1989). The score system was as follows: no impairment, 0; incomplete extension of forefoot offside the focus reported 1; rotation toward paralysis signed for 2; falling toward focus was signed 3; no spontaneous action and consciousness was signed for 4.

Infarct volume by TTC staining

After 22 h of reperfusion damage, 6 rats from each group were anesthetized and sacrificed. The brain was collected, stored at -20°C for 4 to 5 min, and then cut into 5 sections from antiunion (2 mm in thickness). These sections were put into TTC solution followed by incubation for 30 min at 37°C (normal brain tissues: red and ischemic tissues: white). These sections were put on the glass slide. Photographs were taken and infarct area was detected by using Image-pro Plus Software. The infarct volume was calculated as follow: $V\% = (\sum s_i d / \sum s_j d) \times 100$ (s_i, and s_j represents the area of infraction and that of brain tissue, respectively; d represents the thickness of brain sections [d =2]).

Detection of MDA

After 22 h of reperfusion, rats were anesthetized and the heart was exposed. Then, 3 ml of heparin anti-coagulated blood sample was collected from the right atrium and centrifuged at 3500 rpm for 15 min. The serum was collected and stored at -20°C (Wu et al., 2011). The activity of SOD and the content of MDA were detected by xanthine oxidase method and thibabituric acid (TBA) methods, respectively.

Table 1. Neurological scores of rats in different groups ($\bar{\chi} \pm s$).

Group	N	Neurological score
Sham operation group	12	0**
IR group	12	2.33 ± 0.39△△
IR + Naoshuning group	12	1.50 ± 0.37**
IR + ginaton group	12	1.51 ± 0.36**

*P<0.05 and **P<0.01 versus IR group; △P<0.05 and △△P<0.01 versus sham group.

Statistical analysis

Data were expressed as mean ± standard deviation (±s) and statistical analysis was performed with SPSS version 11.5 statistic software package. Analysis of variance (ANOVA) was used for comparisons between groups. P< 0.05 was considered statistically significant.

RESULTS

Effects of Naoshuning on neurological function

The behaviors of rats in the three treatment groups were all abnormal after IR injury and mainly characterized by right rotation or falling toward right side when walking with bucking. When compared with IR group, the neurological scores were significantly decreased in Naoshuning and ginaton groups, but not different from those in sham group (Table 1).

Effects of Naoshuning on the infract volume

After IR, infarction was observed in all rats. When compared with IR group, the infarct volume in the Naoshuning and ginaton groups markedly decreased and the decrease was more obvious with the prolongation of reperfusion. There was no significant difference in the infarct volume between the Naoshuning and the ginaton groups (Table 2 and Figure 1)

Effects of Naoshuning on the SOD activity and MDA content

When compared with sham group, the activity of SOD in IR group dramatically decreased and the MDA content increased significantly after IR. When compared with IR group, the SOD activity at 7th and 14th days after reperfusion markedly increased in Naoshuning and ginaton groups and the MDA content decreased significantly in Naoshuning and ginaton group at all time points. In addition, the SOD activity and MDA content in Naoshuning and ginaton groups were not different from those in sham group (Tables 3 and 4).

DISCUSSION

In this study, it is found that Naoshuning significantly decreases the infract volume of brain IR rats. Taking Naoshuning can also markedly increase SOD activity and decrease MDA activity, the effect of which is similar to that of ginaton. Brain IR damage refers to the injury secondary to reperfusion, and represents an important physiologic event in cerebrovascular disease (Eser et al., 2010), which is a complex process with various factors. However, the pathogenesis and the outcome of IR injury are not completely understood. It has been shown that metabolic dysfunction in brain tissues, excessive oxygen radical production, calcium overload, excessive release of excitatory amino acid (EAA), apoptosis of neurons and inflammatory response play important roles in the IR injury. Peroxidization of lipid caused by free radicals is one important reason that leads to IR injury, and its adverse effects are summarized as follows: 1) act on polyunsaturated fatty acid and cause peroxidization of lipid; 2) induce the crosslinking of macromolecular DNA, RNA, polysaccharides and amino acid to lost their activities or functions; 3) promote conglomerate and degradation of polysaccharides to make free radicals widely attack neurolemma and blood vessels, cause inflammatory cascade, protein denaturation, polynucleotide breakage, base remodification, damage the integrity of cell structure, seriously influence the permeability, ion transport, membrane barrier and evenly result in cell death. In addition, free radicals can promote EAA release and brain IR injury generating. Therefore, free radicals induced by brain IR injury causing lipid peroxidization are the point of this study.

A large number of free radicals are produced after brain IR injury and a variety of antioxidants are consumed, causing lipid peroxidization, and finally resulting in damages in structure and functions of the brain. As an important antioxidase, SOD can catalyze superoxide anion and eliminate the toxic effects of superoxide anion and protect the cells from damaging. Oxygen radicals can destroy cell membrane and result in production of a large number of lipid peroxide-MDA. The content of MDA reflects the degree of lipid peroxidation (Bloomer et al., 2010). So inhibiting the free radicals and lipid peroxidation induced by this and eliminating the free radicals may be a favorable strategy in the protection of brain IR injury. Studies have showed that the *G. biloba* extract (Rong et al., 1996) is a kind of antioxidant whose active component has strong ability to eliminate free radical. Standard pharmaceutics about *G. biloba* extract have existed and have been applied to treat diseases correlated with memory disorders caused by age, peripheral blood vessel occlusion. Therefore, the *G. biloba* extract (*jinnaduo*) was elected as the positive control drug.

Naoshuning is an important Chinese traditional medicine and used in promoting blood circulation by removing blood stasis. It is mainly composed of *H.*

Table 2. Infarct volume in rats of different groups (n=6, $\bar{x} \pm s$).

Group	Infarct volume (%)		
	1st day	7th day	14th day
Sham group	0**	0*	0**
IR group	22.33 ± 4.78$^{\triangle\triangle}$	24.49 ± 4.66$^{\triangle}$	18.83 ± 4.61$^{\triangle\triangle}$
IR + Naoshuning group	13.49 ± 3.77*$^{\triangle\triangle}$	10.12 ± 2.63*$^{\triangle}$	8.01 ± 2.43**$^{\triangle}$
IR + ginaton group	12.09 ± 2.97*$^{\triangle}$	8.16 ± 2.75*$^{\triangle}$	7.10 ± 2.46**$^{\triangle}$

*P<0.05 and **P<0.01 versus IR group; $^{\triangle}$P<0.05 and $^{\triangle\triangle}$P<0.01 versus sham group.

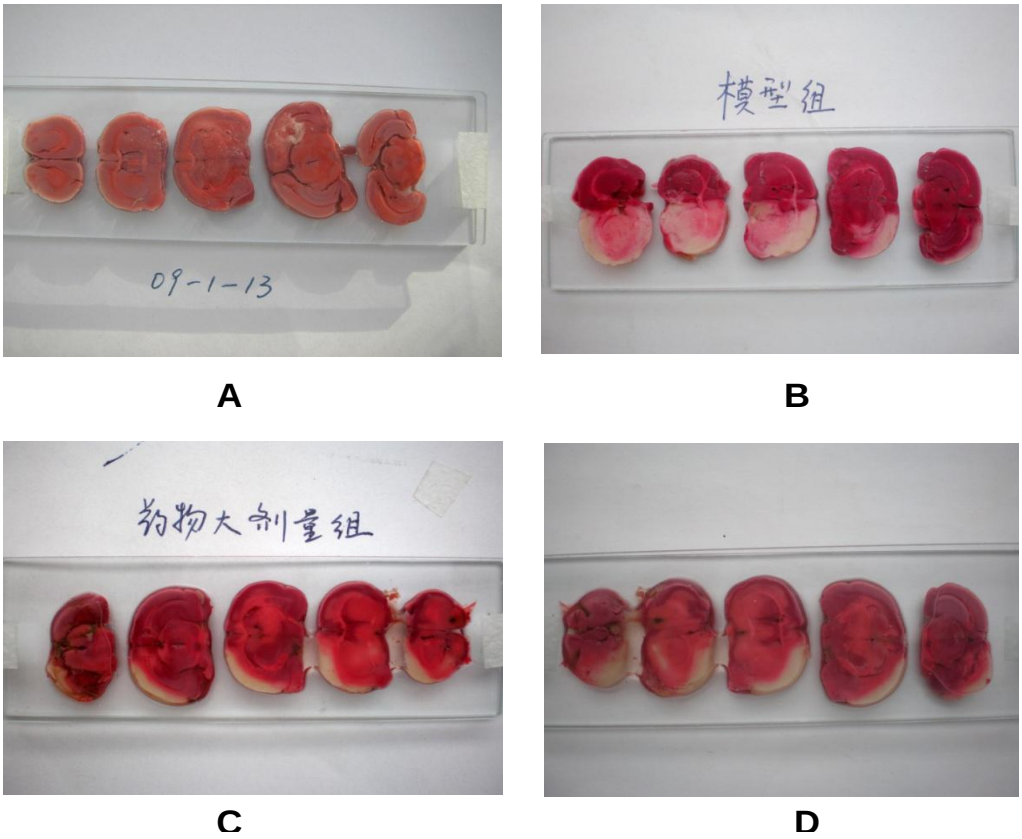

Figure 1. Brain slice of rat by TTC staining: A) Sham group; B) IR group; C) IR + naoshuning group; D) IR + ginaton group.

Table 3. Activity of SOD in rat brain of different groups (n=6, $\bar{x} \pm s$).

Group	Activity of SOD (U/ml)		
	1st day	7th day	14th day
Sham group	166.69 ± 7.02**	164.59 ± 8.07**	168.54 ± 7.77**
IR group	150.21 ± 9.81$^{\triangle\triangle}$	140.00 ± 7.87$^{\triangle\triangle}$	142.28 ± 9.28$^{\triangle\triangle}$
IR + Naoshuning group	158.30 ± 4.20	159.46 ± 9.25**	160.47 ± 6.13**
IR + ginaton group	159.06 ± 7.23	154.36 ± 9.41*	162.05 ± 6.77**

*P<0.05 and **P<0.01 versus IR group; $^{\triangle}$P<0.05 and $^{\triangle\triangle}$P<0.01 versus sham group.

Table 4. Content of MDA in rat brain of different groups (n=6, $\bar{x} \pm s$).

Group	Content of MDA (nmol/ml)		
	1st day	7th day	14th day
Sham group	8.64 ± 0.93	5.99 ± 0.93**	6.32 ± 2.19*
IR group	9.88 ± 1.52	13.45 ± 1.61△△	9.44 ± 0.99△
IR + Naoshuning group	6.62 ± 0.26**	6.56 ± 1.71**	6.31 ± 0.45**
IR + ginaton group	6.18 ± 0.55**	5.79 ± 1.36**	6.63 ± 2.13*

*P<0.05 and **P<0.01 versus IR group; △P<0.05 and △△P<0.01 versus sham group.

nipponica, pseudo-ginseng and *L. heterophyllus* sweet extract. Pseudo-ginseng is an important herb that has been widely used in promoting blood circulation by removing blood stasis. Study has shown that Panax Notoginseng Saponins (PNS) has protective effects on myocardial IR injury and can expand cerebral vessels, increase brain blood flow and counteract with the toxic effects of free radicals in brain ischemia (Li et al., 1991). The pharmacological functions of PNS are wide including the cerebralvascular activity (Hartung et al., 1992). Recent study (Li et al., 1991) indicates that PNS can reduce the infarction volume, improve the neurological function, alleviate the ischemic injury of neurons and improve the survival of neurons exerting neuroprotective effects in an ischemic encephalopathy model (Ma et al., 1997; Ma et al., 1998). Compound leeches mixture (Liu et al., 2001) can increase the activity of SOD, decrease the MDA content and combat with the toxic effects of free radicals after brain IR injury. The micro-powder of leeches (Noda et al., 1996) was also shown to increase the activity of SOD, decrease the MDA content and improve the brain IR injury.

Our results showed that Naoshuning could significantly decrease the neurological scores and improve the motor function of rats after brain IR injury. Naoshuning could markedly decrease the infarct volume and protect the brain IR injury. The MDA content in IR group was markedly higher than that in sham group and the activity of SOD slightly decreased. It was indicated that Naoshuning have free radical-scavenging activity against hydroxyl radicals and alleviate the brain IR injury induced by free radicals, which may be an important mechanism underlying the neuroprotective effects of Naoshuning in a rat brain IR injury model. The neurological functions were evidently improved by Naoshuning in the range of experimental dosage over time, accompanied by the stronger elimination of free radicals and enhanced tissue protection. The exact mechanism of the protective effects of Naoshuning on brain IR injury is still required to be further studied.

REFERENCES

ArunaDevi R, Lata S, Bhadoria BK, Ramteke VD, Kumar S, Sankar P, Kumar D, Tandan SK (2010). Neuroprotective effect of 5, 7, 3', 4', 5'-pentahydroxy dihydroflavanol-3-O-(2''-O-galloyl)-beta-D-glucopyranoside, a polyphenolic compound in focal cerebral ischemia in rat. Eur. J. Pharmacol., 626(2-3): 205-212.

Bloomer RJ, Kabir MM, Marshall KE, Canale RE, Farney TM (2010). Postprandial oxidative stress in response to dextrose and lipid meals of differing size. Lipids Health Dis., 27(9): 79.

Cui XN, Yi L, Wang YL (2008). Experimental study on protective role of naoshuning on icro-cerebral blood vessels in brain-injured rats. C. J. Basic. Med. Tradit. Chin. Med., 14(2): 110-112.

Eser O, Songur A, Yaman M, Cosar M, Fidan HX, Sahin O, Mollaoglu H, Buyukbas S (2010).The protective effect of avocado soybean unsaponifilables on brain ischemia/ reperfusion injury in rat prefrontal cortex. Br. J. Neurosurg., p. 28.

Hartung HP, Jung S, Stoll G, Zielasek J, Schmidt B, Archelos JJ, Toyka KV (1992). Inflammatory mediators in demyelinating disorders of the CNS and PNS. J. Neuroimmunol., 40(2-3): 197-210.

Li LX, Wang Y, Huang ZH (1991). Effects of panax notoginseng saponins on acute cerebral ischemia. Chin. Pharmacol. Bull., 7(1): 56.

Liu YH, Ling ZY, He B (2001). Effects of complex hirudo on the ischemia reperfusion injury in a rat model. Chin. J. Nerv. Men. Dis., 27(2): 142-143.

Longa EZ, Weinstein PR, Carlson S, Cummins R (1989). Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke, 20(1): 84-91.

Louajrj A, Harraga S, Godot V, Toubin G, Kantelip JP, Magnin P (2001). The Effect of Ginkgo Biloba Extract on Free Radical Production in Hypoxic Rats. Biol. Pharm. Bull., 24(6): 710-712.

Ma LY, Han J, Xiao PG (1997). Protective effect of saponins of panax notoginseng on cerebral ischemia. Acta Lab. Anim. Sci. Sin., 5(2): 98-102.

Ma LY, Xiao PG, Liang FQ (1998). Protective effects of panax notoginseng saponins on primary cortical cultures of rats. Chin. Pharm. J., 33(3): 143.

Noda N, Tanaka R, Miyahara K, Sukamoto T (1996). Six trigalactosylceramides from the leech (*Hirudo nipponica*). Chem. Pharm. Bull. (Tokyo), 44(5): 895-899.

Rong Y, Geng Z, Lau BH (1996). Ginkgo biloba attenuates oxidative stress in macrophages and endothelial cells. Free Radic. Biol. Med., 20(1): 121-127

Sui Y, Zhou MX (2007). Immunologic mechanisms in the inflammatory response to cerebral ischemic- reperfusion injury. Anat. Clin., 12(6): 436-438.

Wu JT, Yang GW, Wen WJ, Zhang FM, Yuan JD, An LG (2011). Cholesterol metabolism regulation and antioxidant effect of platycodin D on hyperlipidemic emulsion-reduced rats. Afr. J. Pharm. Pharmacol., 5(22): 2444-2453.

Zhang MK, Yi L, Cui X (2006). Protective effects and mechanism research of naoshuning on brain hemorrhage injury in rats. Tianjin J. Tradit. Chin. Med., 23(6): 490-492.