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Research Article

Ginsenoside RH1 Improves Neurological Function By Inhibiting Oxidative Stress and Promoting the Expression of Neurotrophic Factors in Cerebral Ischemia-Reperfusion Rats

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ABSTRACT

Ginseng, a valued herb in China, showed efficacies for treatment or prevention of a series of conditions in patients. Ginsenoside Rh1 is the major active component of ginseng. The aim of this study was to investigate the neuroprotective effects of ginsenoside Rh1 on injury of cerebral ischemia-reperfusion in rats. Middle cerebral artery occlusion and reperfusion were performed in male Sprague-Dawley rats. The ischemic stroke rats were treated with ginsenoside Rh1 at dose of 5 mg/kg and 10 mg/kg by gavage. After 7-day treatment, neurological functions of animals were evaluated by adhesive removal tape and beam walking tests. Neuron injury was assessed by immunohistochemical staining. The levels of MDA, BDNF, NGF and activities of SOD, CAT in the ischemic hemisphere were assayed. Treatment with ginsenoside Rh1 for 7 days could significantly improve the neurological functions ($P < 0.05$) and increase the number of NeuN-positive cells in rats with cerebral ischemia ($P < 0.01$). Ginsenoside Rh1 decreased significantly MDA level ($P < 0.01$) and enhanced activities of SOD, CAT ($P < 0.05$ or $P < 0.01$). Further measurement showed that ginsenoside Rh1 also increased the levels of BDNF, NGF of the ischemic hemisphere ($P < 0.05$ or $P < 0.01$). The present findings demonstrated that ginsenoside Rh1 improved neurological function by inhibiting oxidative stress and promoting the expression of neurotrophic factors after cerebral ischemia-reperfusion.

Keywords: Ginsenoside Rh1, Ischemic stroke, Neurological function, Oxidative stress

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INTRODUCTION

Stroke is the leading cause of death and long-term disability in adults worldwide. Stroke can be either ischemic or hemorrhagic. In developing countries, ischemic stroke is the most common type of stroke, and it accounts for around 87% of stroke cases⁽¹⁾. Ischemic strokes

are due either to local thrombus formation or to embolic phenomenon, resulting in occlusion of a cerebral artery. The blood flow of brain of ischemic stroke patients is reduced, which will lead to cell necrosis. Then the cell contents may release and the nerve function impairs, which is accompanied by the damage to the blood-brain barrier⁽²⁾, inflammation⁽³⁾,

and oxidative stress⁽⁴⁾. Thrombolytic therapy (< 4.5 hours from onset) with intravenous recombinant tissue plasminogen activator (tPA) has been shown to reduce the ultimate disability due to ischemic stroke⁽⁵⁾. In large artery occlusion, the patients can be treated with mechanical thrombectomy within 6 hours of onset. However, the strategies mentioned above will result in reactive oxygen species (ROS) production, generating oxidative stress and inflammation, which is responsible for the consequence of brain tissue damage⁽⁶⁾. The recovery of nerve function after ischemic stroke is a complex and important process. NGF and BDNF are the main neurotrophic factors that play an important role in nerve repair and regeneration. Previous study demonstrated that NGF and BDNF can promote nerve repair and regeneration after ischemic stroke⁽⁷⁾.

Traditional Chinese medicine (TMC) has been used for treating various diseases including cerebral vascular diseases. Recent studies focused on investigating the neuroprotective and antioxidant effects of TMC on ischemic stroke^(8, 9). Ginseng (*Panax ginseng*) is one of the famous TMC. Ginseng displayed a promising property to ameliorate neurological disorders including stroke. Ginsenosides are the main component of ginseng⁽¹⁰⁾. It has been reported that ginsenoside Rb1 had neuroprotective property by regulating the function of astrocytes, promoting neuronal survival after ischemic stroke⁽¹¹⁾. In addition, further studies have found that ginsenoside Rb1 can increase axon regeneration after ischemic stroke and therefore improve neurobehavioral function⁽¹²⁾. Angiogenesis plays a key role in the recovery and remodeling of nerve function after ischemic stroke. In ischemic stroke, ginsenoside Rg1 enhanced angiogenesis by promoting the expression of VEGF and regulating the PI3K/Akt/mTOR signaling pathway⁽¹³⁾. Ginsenoside Rg1 could also ameliorate nerve damage after ischemic stroke and attenuate the breakdown of the blood-brain barrier by down-regulating aquaporin 4⁽¹⁴⁾.

Rh1, a component of ginsenosides, is a natural antioxidant. Ginsenoside Rh1 inhibited the accumulation of ROS and then attenuate the damage of cells caused by oxidative stress⁽¹⁵⁾. Ginsenoside Rh1 also increased the activity of SOD and enhanced the antioxidant capacity of cells⁽¹⁶⁾. Another studies demonstrated that ginsenoside Rh1 exerted antioxidant property and therefore inhibited tumor growth or improved myocardial injury and heart function^(17, 18).

This study established a rat model of cerebral ischemia-reperfusion injury. Then the rats were treated with ginsenoside Rh1 and the neuroprotective effects of ginsenoside Rh1 and its mechanism of action were investigated.

MATERIALS AND METHODS

Animal Models

Male Sprague-Dawley rats, weighing 280 ± 10 g, were from Pengyue Co. Ltd. (Jinan, China). All the experimental procedures were performed in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals and approved by the Ethics Committee of Yantai University (Approval number, YTU20180520). The rats were allowed to access freely to food and water on a 12 h light/dark cycle. Cerebral ischemia/reperfusion was performed according to the previous study⁽¹⁹⁾. Briefly, rats were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). The left common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were dissected. A suited filament with a rounded tip was inserted into the ICA and therefore occlude the middle cerebral artery. At 90 min after ischemia, the filament was withdrawn to implement reperfusion. The rats of sham group were subjected to the same surgery but without occluding the middle cerebral artery.

Grouping and Treatment

The rats were randomly assigned to 4 groups: Sham, Model, ginsenoside Rh1 5 and 10 mg/kg groups and there were 13 rats in each group. The rats in the ginsenoside Rh1 groups were orally treated with ginsenoside Rh1 at dose of 5 or 10 mg/kg after cerebral ischemia/reperfusion. The animals in sham and model groups were administered with vehicle (0.5% sodium carboxymethyl cellulose).

Adhesive Removal Test

According to previous report, an adhesive removal test was performed to evaluate the sensorimotor deficit⁽²⁰⁾. Adhesive tape (6 mm in diameter) was placed on the palmar surface of the front paw with the same pressure. The time which the rats remove the adhesive tape was recorded. The rats were allowed to perform the task within 2 min.

Beam Walking Test

A beam walking test was applied to assess the coordination of motor movements. The rats were trained twice daily for 2 days before cerebral ischemia/reperfusion. The apparatus consisted of a wooden beam (2.5×120 cm). A box (30×30×30 cm) was installed at the end of the beam. The rats were placed at the starting point of the beam. The time of passing beam which the rats walk from the starting point to the box was recorded. The cut-off time was taken as 60 sec⁽²¹⁾.

Immunohistochemical Staining

After the behavioral tests, 3 rats in each group were anesthetized with isoflurane. The brains were collected and fixed with 4% paraformaldehyde. The brains were subsequently dissected and post-fixed in paraformaldehyde overnight at 4°C. Then they were transferred into 20% sucrose at 4°C for 24 h. Paraffin-embedded specimens were cut at 5- μ m sections and subjected to immunohistochemistry. The brain sections were quenched with 3% hydrogen peroxide and incubated in anti-neuronal nuclear antigen (NeuN) antibody (1:400, Millipore, Billerica, MA, USA) at 4°C for 24 h. The sections of brain were incubated with

biotinylated secondary antibody for 1 h at 37°C. The samples were incubated with 3, 3'-diaminobenzidine for 5 min. The results were analyzed with an Olympus microscope (IX-70; Olympus Corp. Tokyo, Japan). The number of NeuN-positive cells in the ischemic hemisphere of rats was counted.

MDA, SOD and CAT Assay

Anesthetized with isoflurane, 10 rats in each group were used for the MDA, SOD and CAT assay after behavioral experiment. The ischemic hemisphere was collected. The brain tissue was homogenized with normal saline, centrifuged at 4°C at 4500 g for 15 min, and the supernatant was collected to detect MDA, SOD and CAT. MDA level was determined using MDA kit (A003, Nanjing Jiancheng Bioengineering Institute, China). SOD kit (A001) and CAT kit (A007) were from Nanjing Jiancheng Bioengineering Institute and used to assay the activities of SOD and CAT. The experimental analysis process was carried out in accordance with the manufacturer's instructions. The protein concentration in the supernatants was measured using kit of protein assay (Beyotime Institute of Biotechnology, Shanghai, China).

BDNF and NGF Assay

The supernatant was prepared as mentioned above. The levels of BDNF (Lot. Number: SEKR-0015) and NGF (Lot.

Number: SEKR-0076) in the supernatants were assayed using ELISA commercial kits (Solarbio Co., Ltd., Beijing, China) according to the manufacturer's protocol.

Statistical Analysis

The data were expressed as mean \pm standard deviation. Statistical comparisons were carried out using one-way ANOVA followed by Tukey's multiple comparisons test. GraphPad Prism 9 software was used to make statistical analysis of experimental results. Data were considered significant when $P < 0.05$.

RESULTS

Effect of ginsenoside Rh1 on time of removing adhesive tape

The sensorimotor defects of rats were evaluated by adhesive removal test. Compare with the sham group, the time of removing adhesive tape of rats in model group significantly increased ($F_{(1, 12)} = 79.76$, $P < 0.01$). Compare with the model group, ginsenoside Rh1 reduced the time of removing adhesive tape in ischemic stroke rats (Rh1 5 mg/kg: $F_{(1, 12)} = 12.71$, $P < 0.01$; Rh1 10 mg/kg: $F_{(1, 12)} = 23.38$, $P < 0.01$), Figure 1.

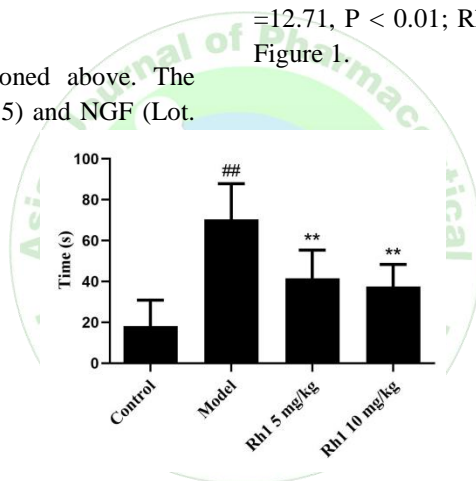


Figure 1: Effect of ginsenoside Rh1 on time of removing adhesive tape. Data are expressed as mean \pm standard deviation (n=13). ### $P < 0.01$ vs. control group. ** $P < 0.01$ vs. model group.

Effect of ginsenoside Rh1 on time of passing beam

Beam walking test was employed to investigate the motor coordination of ischemic stroke rats. Compared with the sham group, the time of passing beam of rats in model

group was significantly increased ($F_{(1, 12)} = 90.02$, $P < 0.01$). Compared with the model group, the time of passing beam of rats in ginsenoside Rh1 groups was markedly decreased (Rh1 5 mg/kg: $F_{(1, 12)} = 8.313$, $P < 0.05$; Rh1 10 mg/kg: $F_{(1, 12)} = 40.57$, $P < 0.01$), Figure 2.

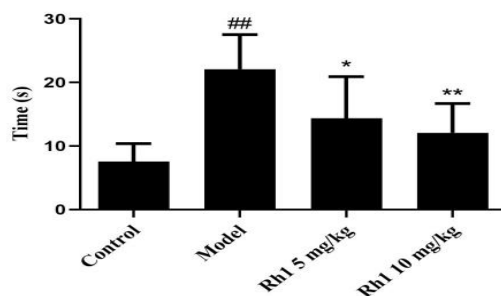


Figure 2: Effect of ginsenoside Rh1 on time of passing beam. Data are expressed as mean \pm standard deviation (n=13). ### $P < 0.01$ vs. control group. * $P < 0.05$, ** $P < 0.01$ vs. model group.

Effect of ginsenoside Rh1 on number of NeuN-positive cells

The number of NeuN-positive cells was observed using immunohistochemical staining. Compared with the sham group, the number of NeuN-positive cells in model group

was significantly reduced ($F_{(1, 14)} = 143.0$, $P < 0.01$). Compared with the model group, ginsenoside Rh1 increased the number of NeuN-positive cells in ischemic stroke rats (Rh1 5 mg/kg: $F_{(1, 14)} = 94.77$, $P < 0.01$; Rh1 10 mg/kg: $F_{(1, 12)} = 56.51$, $P < 0.01$), Figure 3.

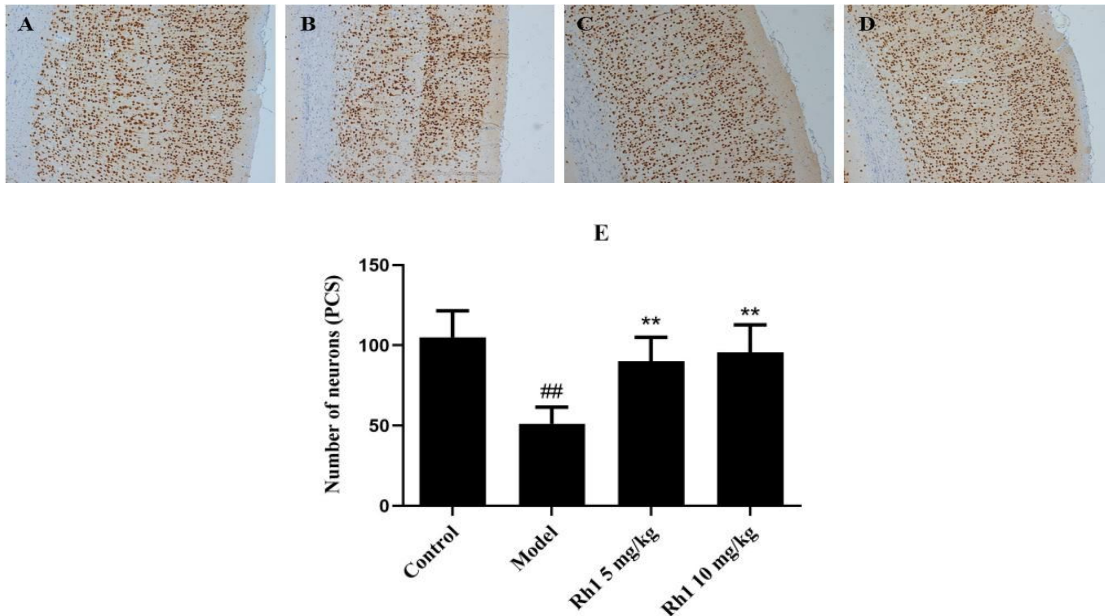


Figure 3: Effect of ginsenoside Rh1 on number of NeuN-positive cells. Immunohistochemical staining of NeuN-positive cells and quantitative data of NeuN-positive cells in ischemic hemisphere. (A) Control group; (B) Model group; (C) Ginsenoside Rh1 5 mg/kg group; (D) Ginsenoside Rh1 10 mg/kg group; (E) Bar graphs of quantitative analysis of NeuN-positive cells. Data are expressed as mean \pm standard deviation ($n=3$). $^{##}P < 0.01$ vs. control group. $^{**}P < 0.01$ vs. model group.

Effect of ginsenoside Rh1 on MDA content

Compared with the sham group, MDA content in ischemic hemisphere of rats in model group was significantly increased ($F_{(1, 9)} = 129.7$, $P < 0.01$).

Compared with the model group, MDA content of rats in ginsenoside Rh1 groups reduced markedly (Rh1 5 mg/kg: $F_{(1, 9)} = 24.36$, $P < 0.01$; Rh1 10 mg/kg: $F_{(1, 9)} = 63.28$, $P < 0.01$), Figure 4.

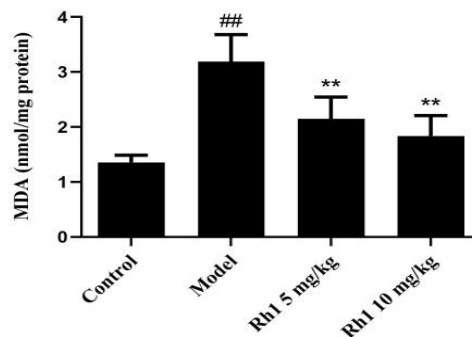


Figure 4: Effect of ginsenoside Rh1 on MDA content. Data are expressed as mean \pm standard deviation ($n=10$). $^{##}P < 0.01$ vs. control group. $^{**}P < 0.01$ vs. model group.

Effect of ginsenoside Rh1 on activities of SOD and CAT

The antioxidant enzymes (SOD and CAT) in ischemic hemisphere were assayed in this study. It demonstrated that SOD and CAT activities of rats in model group were decreased when compared with that of sham group (SOD:

$F_{(1, 9)} = 127.4$, $P < 0.01$; CAT: $F_{(1, 9)} = 86.89$, $P < 0.01$). Compared with the model group, ginsenoside Rh1 treatment increased significantly SOD and CAT activities of ischemic stroke rats (SOD: Rh1 5 mg/kg, $F_{(1, 9)} = 13.19$, $P < 0.05$; Rh1 10 mg/kg: $F_{(1, 9)} = 23.88$, $P < 0.01$. CAT: Rh1 5 mg/kg, $F_{(1, 9)} = 21.19$, $P < 0.05$; Rh1 10 mg/kg: $F_{(1, 9)} = 92.02$, $P < 0.01$), Figure 5.

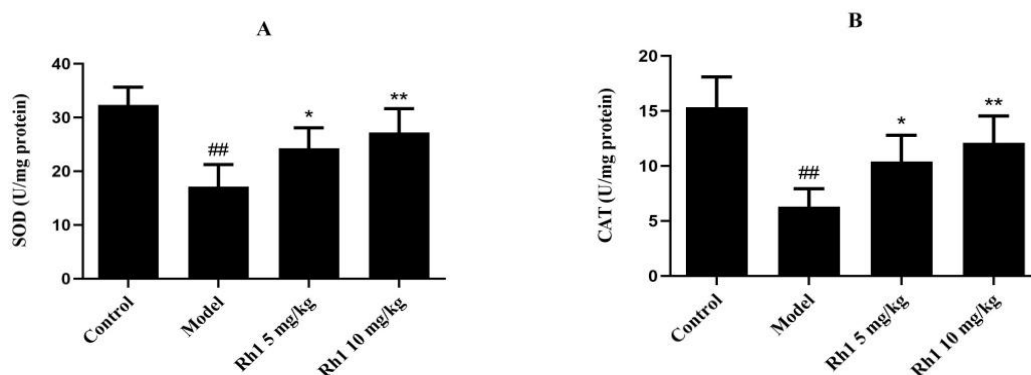


Figure 5: Effect of ginsenoside Rh1 on the activities of (A) SOD and (B) CAT. Data are expressed as mean \pm standard deviation (n=10). ##P < 0.01 vs. control group. *P < 0.05, **P < 0.01 vs. model group.

Effect of ginsenoside Rh1 on levels of BDNF and NGF

Compared with the sham group, there was no significant difference observed in the levels of BDNF and NGF in the ischemic hemisphere of the model group. Compared

with the model group, ginsenoside Rh1 treatment could significantly augment the levels of BDNF and NGF in the ischemic hemisphere of rats (BDNF: Rh1 5 mg/kg, $F_{(1,9)} = 25.47$, $P < 0.05$; Rh1 10 mg/kg: $F_{(1,9)} = 33.94$, $P < 0.01$. NGF: Rh1 10 mg/kg: $F_{(1,9)} = 34.01$, $P < 0.01$), Figure 6.

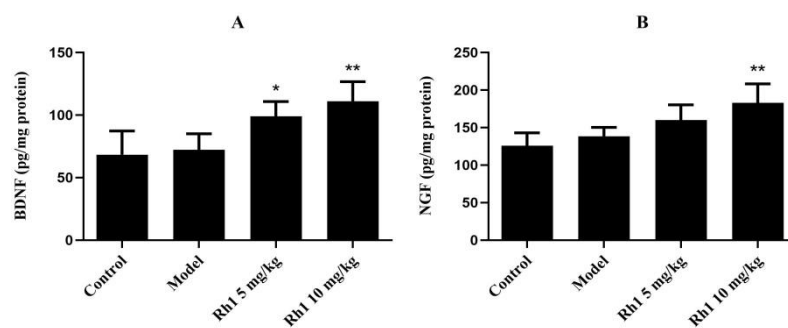


Figure 6: Effect of ginsenoside Rh1 on levels of BDNF (A) and NGF (B). Data are expressed as mean \pm standard deviation (n=10). *P < 0.05, **P < 0.01 vs. model group.

DISCUSSION

Ischemic stroke is a complex disease that causes neuronal injuries after the onset of ischemia. The damage of neurons can affect the stability and integrity of brain anatomy, and the loss of neurons can cause defects of neurological function⁽²²⁾. This study demonstrated that ginsenoside Rh1 improved neurological function after cerebral ischemia-reperfusion. And the mechanisms of action of ginsenoside Rh1 were related to inhibiting oxidative stress and promoting the expression of neurotrophic factors.

Ischemic stroke occurs after the brain is hypoxic, and along with neuronal damage and neurological dysfunction. Behavioral animal research in pharmacology is designed to provide a model for human disease, and great effort is given toward the development of animal models that reflect behavioral function shared by animals and humans. The search for treatment and cure of stroke will continue to lean upon behavioral tests. In this study, neurobehavioral function was evaluated by removing

adhesive tape and the beam walking. Removing adhesive tape have been reported to be an useful method in assessing sensorimotor in ischemic stroke rats⁽²⁰⁾. The beam walking test is a common approach for evaluating motor balance and coordination in rats. And it is often used to investigate the degree of recovery of motor coordination after ischemic stroke in rats⁽²¹⁾. The results of this study showed that treatment with ginsenoside Rh1 increased the time for the rats to move the tape and pass the beam after ischemic stroke. Furthermore, the number of NeuN-positive cells of ischemic stroke rats decreased. However, ginsenoside Rh1 augmented the number of NeuN-positive cells. These data demonstrated that ginsenoside Rh1 had a neuroprotective effect in cerebral ischemia.

Oxidative stress plays an important role in brain injury caused by ischemic stroke. Ischemia results in a situation of hypoxia, and central nervous system is easily affected by oxidative stress⁽²³⁾. In this study, the level of MDA in ischemic cerebral hemisphere of rats increased, the activities of antioxidant enzymes such as SOD and CAT

decreased. These findings indicated that the loss of neurons of rats was associated with oxidative stress, which was consistent with previous studies⁽²⁴⁾. However, ginsenoside Rh1 displayed an anti-oxidative effect. Ginsenoside Rh1 not only increased the activities of SOD and CAT but also reduce the level of MDA in the ischemic hemisphere of rats. These data suggested that the neuroprotective effect of ginsenoside Rh1 was related to its antioxidant capacity, at least in part.

BDNF and NGF are key neurotrophic factors in the repair process of the central nervous system after cerebral ischemia. Previous study showed that BDNF and NGF could decrease the risk of adverse outcomes after ischemic stroke, and high levels of BDNF and NGF could reduce the infarct size⁽²⁵⁾ and avoid the occurrence of severe disability⁽²⁶⁾. The findings of this study demonstrated that ginsenoside Rh1 increased the levels of BDNF and NGF in the ischemic hemisphere of rats, suggesting that the up-regulation of BDNF and NGF might be one of the mechanisms of the neuroprotective effect of ginsenoside Rh1.

In conclusion, ginsenoside Rh1 exerts a neuroprotective effects in ischemic stroke, and the mechanism action is related to inhibiting oxidative stress and promoting the expression of neurotrophic factors after cerebral ischemia-reperfusion.

CONFLICTS OF INTEREST

The authors declare there are no competing interests.

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