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Rhodiola rosea Stimulates Brain Microcirculation in Rats with Hypoxic Brain Injury at High Altitude

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QC, CY and DW conducted the experiments in this study. SL and SM contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

Key words
***Rhodiola rosea*, Brain injury, Oxidative stress, Microcirculation, Sprague-dawley rats, Small microvessels**

ABSTRACT

Acute hypobaric and hypoxic environment can reduce the activity of peroxidase in brain tissue and increase the accumulation of peroxide products, so as to enhance the risk of cerebral microcirculation disorders. *Rhodiola rosea* (RR) is a classic prescription of herbal medicine and has been widely used in Chinese clinics to treat hypoxic brain injury at high altitude (HBIHA). However, the molecular pharmacological activity of RR in HBIHA is largely unknown. The objective of this study was to evaluate the protective effect of RR on malonaldehyde (MDA), superoxide dismutase (SOD) and brain microcirculation in rats with HBIHA. 150 rats were randomly divided into 5 groups (normal control, model control, dexamethasone control, high-dose RR, low-dose RR), 30 rats each group. The rats in each group were fed in a low-pressure oxygen environment control system for 48 h. After anesthesia, blood flow images of focused vessels and oxidative stress indexes in brain tissue were collected to be compared. It was shown that RR could enhance the activities of catalase (CAT), glutathione peroxidase (GPX) and SOD in brain tissue, and remove the peroxide product MDA. In the brains of the normal rats, cortical blood vessels had regular shapes and the flow was continuous in small, medium and large vessels. The proportion of PPV was 99.12±0.48%, and the density of PVD index was 6.54±0.11 mm². The index of MFI decreased in the model group ($P<0.01$). RR can protect the brain tissue of rats suffering from HBIHA through the dual mechanisms of antioxidant and improvement of cerebral blood flow, so as to enhance the hemoperfusion of small microvessels.

INTRODUCTION

High altitude exposure disrupts the efficiency of antioxidant systems and leads to oxidative damage in various organs and tissues (Zhao *et al*., 2009). It can be seen that under the condition of low oxygen at high altitude, the blood-brain barrier is damaged and the oxidative stress reaction is increased, which leads to brain tissue injury. This may be an important obstacle to drug treatment of brain injury in high altitude areas (Aboouf *et al*., 2023).

In recent years, traditional Chinese medicine has been highly regarded by scholars as a treatment plan to alleviate brain injury, brain hypoxia, brain edema and other problems. Some scholars applied ethanol extract of hedyotis diffusa to hypoxia rats at high altitude and found that it could enhance the antioxidant capacity of the body, reduce the damage of free radicals to the body, and alleviate the energy metabolism disorders which was caused by hypoxia (Ma *et al*., 2014). Furthermore, some scholars applied chamomile B to rats with cerebral edema at high altitude and found that it had antioxidant properties and inhibited Caspase-dependent apoptotic pathway

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(Botao *et al*., 2013). *Rhodiola rosea* (RR), a traditional Tibetan medicine, has shown promising prospects in treating brain damage caused by hypoxia. However, the underlying mechanism remains unclear (Wang *et al*., 2019). Therefore, it is of great significance to study the effect of traditional Chinese medicine on prevention and treatment of altitude encephalopathy and to find effective drugs that can reverse altitude brain injury, and RR may be a good candidate.

This study hypothesized that RR could improve the antioxidant status, inhibit malonaldehyde (MDA) and superoxide dismutase (SOD), protect the blood-brain barrier, and improve brain microcirculation, thus playing a protective role in brain injury caused by altitude hypoxia. Therefore, this study intended to establish an animal model of plateau hypoxic brain injury. By being observed the effects of RR on antioxidant stress indexes, blood-brain barrier permeability and brain histopathological changes in the model animals, the protective effect and possible mechanism of RR on plateau encephalopathy can be explored. This study can provide experimental basis for the development and utilization of RR and expand the drug treatment options for altitude encephalopathy. At the same time, it can further elucidate the mechanism of brain injury caused by altitude hypoxia, and provide reference for prevention and treatment of altitude encephalopathy.

MATERIALS AND METHODS

Laboratory animals and drugs

Fifty SD rats, SPF grade, 170-190g, and half male and half female were purchased from Beijing Weitonglihua Experimental Animal Technology Co., LTD. Rhodiola Oral Liquid is product of Tibet Medicine Group Co., LTD., and its batch number 211111 was used in this study. Dexamethasone acetate tablets are produced by Zhejiang Xian Ju Pharmaceutical Co., LTD., and its batch number is LB2239. Main reagents are shown in Table I.

Experiment groups

One hundred fifty rats were randomly divided into normal control group, model control group, dexamethasone control group, high-dose RR group and low-dose RR group, 30 rats each group. In the normal control group, the rats were fed according to conventional standard. In the model control group, the rats were fed under control system of hypoxic environment. In dexamethasone control group, the rats were treated with dexamethasone for preventing hypoxic brain injury, 5mg/ time, two times/ day, 1 day before being entered the hypoxic environmental control system. In the high-dose RR group, the rats were treated with RR, 10ml/ time, two times/day, 7 days before being entered the hypoxic environmental control system, and in the low-dose RR group, the rats were treated with RR, 5ml/ time, two times/day, 7 days before being entered the plateau.

Establishment of model and administration

The normal control group and the model control group were given distilled water by intragastric administration under the same conditions. Except for the normal control group, the other groups were fed in a low-pressure oxygen environmental control system (simulating the altitude of 6000m, atmospheric pressure of 47.2kPa, oxygen concentration of 10.3%) for 12 h and 2 consecutive days. In the dexamethasone control group, the rats were given 0.46mg/kg dexamethasone acetate by intragastric administration at 0.5ml/100g and twice a day before 1 day of founding models. Rats in the high-dose and low-dose RR groups were given Rodiola Oral Liquidby intragastric administration of 0.5ml/100g, 0.9ml/kg/time and 0.45ml/ kg/time before 7 consecutive days of founding model. Seven days later, with the end of the last day of feeding in low-pressure oxygen environment, 50mg of brain tissue was taken immediately after microcirculation image for ELISA detection, and the harmless treatment was carried out immediately after the experiment was completed.

Table I. Main reagents used in this study.

Name	Production Enterprise	Type
MDA ELISA kit	Jiangsu enzyme free industrial Co., LTD	MM-0385R2
SOD ELISA kit	Jiangsu enzyme free industrial Co., LTD	M-0386R2
GPX ELISA kit	Jiangsu enzyme free industrial Co., LTD	MM-20251R2
CAT ELISA kit	Jiangsu enzyme free industrial Co., LTD	MM-20447R2
Control system under low pressure oxygen environment	Shanghai yuyan scientific instrument Co., LTD	Type LP-1500
Enzyme labeling analyzer	Shenzhen leidu life science Co., LTD	Type Rayto RT-6100
Control system under low pressure oxygen environment	Shanghai Yuyan scientific instrument Co., LTD	Type LP-1500
MiroSee microcirculation image system	Guangzhou medical soft intelligent technology Co., Ltd	Type V100

Cerebral microcirculation detection

After the induction box was filled with 4% isoflurane, the rats were placed in the induction box for about 3 min. After the animals were completely anesthetized, the animals were removed from the induction box, and their mouths and snouts were fixed in an anesthesia mask. The air pump of the anesthesia machine was adjusted to 600 ml/min, and a mixture of 2% isoflurane or air was used to maintain anesthesia. After checking that the animals were fully anesthetized (by being penched the paw or tail by two fingers, if the animals did not respond, it indicated that the animals were fully anesthetized), surgery and brain microcirculation testing are performed. SDF image, a stroboscopic light-emitting diode ring based on image mode, is an optical technology for observing microcirculation that is integrated into handheld devices. The device consists of a light guide with a magnifying glass that can be placed over an area of interest to be illuminated and images are taken by means of an analog camera in the device. Illumination is achieved by selecting green light for the wavelength of hemoglobin absorption, such as 530nm, so that the red blood cells appear dark spheres against a white background. At the top of the head of the rat, a cranial window with a diameter of 10mm×10mm was drilled by a dental drill. After the bleeding was completely stopped, the dural membrane was carefully cut to expose the pia, and the blood flow image was obtained by focusing on the probe and the blood vessels on the meninges immediately for video record. The video clips were obtained with a duration of at least 10 sec and were stored by a portable computer and an analog-to-digital video converter. The video clips were stored as AVI files for frame-by-frame image analysis. Vascular density measurements were quantified to determine perfusion vascular density (PVD; mm vessel /mm2) and microvascular flow index (MFI); Based on the main traffic type in the four quadrants, it is defined as nonexistent (0), intermittent (1), slow (2), or normal (3). The proportion of PPV was calculated that perfused vessels which were observed accounted for the percentage of the total number of vessels.

ELISA detection

50mg of brain tissue was cut and 20mg/ml tissue suspension was obtained by PBS homogenate. The samples were centrifuged at 3000 r/min for 20 min and the supernatant was collected for testing. ELISA assay was performed according to the instructions.

Statistical analysis

Statistical analyses were performed with Graph Pad Prism 8.0.2. To determine differences between groups, Bonferroni multiple comparison test was used, which showed the values such as mean ± standard division. $P<0.05$ was considered as statistical significance.

RESULTS

Table II shows that the low-pressure oxygen environment resulted in the significant increase of MDA in the brain tissue of rats compared with the normal control group ($P<0.01$). While the levels of SOD, CAT and GPX were significantly decreased ($P<0.01$). Compared with the model control group, dexamethasone led to decreasing MDA ($P<0.01$) and increasing SOD and GPX ($P<0.01$). High dose of RR decreased MDA ($P<0.01$), and increased SOD, CAT and GPX ($P<0.01$, $P<0.05$). Low dose of RR increased CAT ($P<0.05$).

Table II. Effects of *Rhodiola rosea* on oxidative stress-related factors and microvascular variables ($\bar{x}\pm s$).

Indicator	NCG (n=30)	MCG (n=30)	DCG (n=30)	HDRG (n=30)	LDRG (n=30)	P-value
Oxidative stress-related factors						
MDA (nmol/g)	4.09±0.55	6.41±0.33	5.32±0.74	5.42±0.44	5.93±0.55	0.002
SOD (ng/g)	88.19±7.85	56.54±7.43	70.95±4.83	76.01±5.40	64.18±7.00	0.008
CAT (ng/g)	551.58±58.18	368.52±99.48	431.87±52.21	473.40±57.35	454.96±61.51	0.006
GPX (ng/g)	137.22±12.27	88.71±14.53	120.67±12.49	102.57±8.36	92.78±8.61	0.005
Microvascular variables						
MFI small vessels	3±0	2.34±0.21	2.80±0.16	2.94±0.09	2.85±0.13	0.042
Vessels MFI medium vessels	3±0	2.96±0.09	3±0	3±0	3±0	0.112
MFI large vessels	3±0	3±0	3±0	3±0	3±0	0.101
Vessels PPV (%)	99.12±0.48	98.68±0.51	98.64±0.56	98.92±0.50	98.86±0.45	0.224
PVD (mm/mm²)	6.54±0.11	6.50±0.16	6.52±0.13	6.60±0.10	6.56±0.11	0.132

NCG, normal control group; MCG, model control group; DCG, dexamethasone control group; HDRG, high-dose RR group; LDRG, low-dose RR group; MDA, malonaldehyde; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; MFI, microvascular flow index; PPV, proportion of perfused vessels; PVD, perfusion vascular density.

The results also showed the regular shape of cortical blood vessels in the brains of the normal rats and cortical blood vessels were continuous flow in small, medium, and large vessels (MFI is 3) (Table II). The PPV was 99.12%±0.48%, and the PVD was 6.54± 0.11mm/mm². The MFI of cortical microvessels in the model group was decreased (P<0.01) while the data in the dexamethasone and *Rhodiola* groups were significantly increased compared with the model group (P<0.01). However, in the allowable area of craniotomy, microcirculation parameters in the model group and the administration group did not change obviously. The middle and large vessels in MFI were normal, and PPV and PVD were not significantly different from those in the normal group (P>0.05).

Since the surgical window can only accommodate the probe size, the probe cannot be moved, and only images of the parietal lobe can be obtained. In addition, due to the contamination of subarachnoid hemorrhage which was caused by the operation, enough high-quality images could not be gotten. According to the results of images from the permitted areas of surgery, the quality of blood perfusion of small microvessels in the cerebral cortex of rats in the model group decreased after 48h under hypoxia environment. Prophylactic administration of RR can obviously increase the perfusion of small microvessels, improve cerebral microcirculation, and reduce ischemic hypoxic injury of nerve cells (Fig. 1).

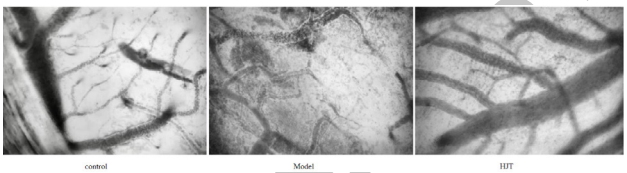

Fig. 1. Comparison of typical images in the parietal lobe of rats. Control, normal control group; Model, model control group (relatively small blood vessels); HJT, high-dose RR group.

DISCUSSION

This study showed that RR can improve the activity of CAT, GPX, SOD and other antioxidant enzymes through antioxidant mechanism, and remove the oxidation product MDA, so as to increase the oxidative stress state of plateau hypoxic rats. This is consistent with the results of previous studies reporting that RR has antioxidant effects (Cao *et al*., 2013; Zhao *et al*., 2019). In plateau environment, reactive oxygen species increase and the over-activation of antioxidant system leads to oxidative stress in the body (Li *et al*., 2022). The flavonoids in RR may be the effective components for its antioxidant effect (Zhang *et al*., 2023). In addition, it was also found that RR could increase the blood perfusion index of cerebral surface microvascular of model animals, so as to improve cerebral microcirculation, and reduce autophagy (Gao *et al*., 2021). This may be another important mechanism for RR to play a role in protecting brain tissue. RR can prevent mountain disease, dilate cerebral vessels and increase cerebral blood perfusion (He *et al*., 2016; Ma *et al*., 2011). Therefore, RR plays a dual protective role on HBIHA through anti-oxidative stress and improving brain microcirculation.

In this study, it was also found that RR could improve the antioxidant indexes of hypoxic rats at high-altitude, which was consistent with the results of Wang *et al*. (2022). They also found that *Rhodiola* reduced oxidative damage in the liver of plateau mice. But this study looked at the effect of RR on the antioxidant system of brain tissue. In addition, Liu *et al*. (2013) explored that *Rhodiola* ameliorates hypobaric hypoxia effect by regulating energy metabolism and choline metabolism of mice in high-altitude areas. In this study, the role of RR was observed in the pathological state of hypoxia at high altitude, and its modeling method was similar. Li *et al*. (2022) found that the extract of compound Astragalus water had the best influence in improving hypoxia tolerance by comparing with compound salvia miltiorrhiza, compound RR and compound *Astragalus*. Previous studies have shown that *Rhodiola* can significantly enhance animal tolerance to hypoxia (Li *et al*., 2014), but it is unclear whether *Rhodiola* improves brain oxygen utilization and function by regulating cerebral microcirculation so as to reduce the incidence of acute cerebrovascular disease. In terms of improving cerebral microcirculation with *Rhodiola*, Fan *et al*. (2018) conducted a meta-analysis and found that *Rhodiola* can improve cerebral hemorheology of patients with unstable angina. The laser confocal technique in this study was used to directly observe the effect of RR on the hemodynamic parameters of cerebral surface microvessels, so the results were more intuitive. It has also been confirmed by mouse models that RR has significant anti-fatigue activity, and its anti-fatigue effect is related to reducing oxidative stress damage, so as to decrease accumulation of adverse metabolites and enhance energy material reserves (Qin *et al*., 2022). However, only cortical blood flow changes were found, and no other brain regions were involved. This study strongly confirmed the protective effect of RR on HBIHA, but its protective mechanism needs to be further studied, and can be compared with other related Chinese medicines to enrich the selection of traditional Chinese medicine intervention for altitude encephalopathy.

This study provides an experimental basis for the application of RR in plateau encephalopathy. Further

studies on the effects of RR on brain tissue and neural function of other plateau animal models can be carried out to further clarify its protective mechanism. It is found that RR can effectively regulate the level of oxidative stress through the blood-brain barrier. This experiment also revealed the prominent role of RR in inhibiting oxidative stress, which may provide a new means for clinical treatment of high grade encephalopathy. But this study only researched the effect of RR on cerebral cortex blood flow, did not involve the deep brain region and did not study the improvement of RR on neurological defects as well as the active components and targets of RR.

DECLARATIONS

Acknowledgments

In this study, the model of acute HBIHA was established. Animal experiments confirmed that RR could increase the activity of SOD, CAT and GPX in brain tissue and remove MDA, so as to improve cerebral microcirculation. Different doses of RR have different effects on brain protection, and there is a dose correlation. However, it is still unclear whether there is a dose-dependent protective effect of RR on HBIHA. Further quantitative studies on the effect and mechanism of RR can be conducted in the future.

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IRB approval

This study was approved by the Advanced Studies Research Board of Qinghai Provincial People's Hospital, Xining, China.

Ethical approval

The study was carried out in compliance with guidelines issued by Ethical Review Board Committee of Qinghai Provincial People's Hospital, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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