Protective Effects of Danhong Decoction on Cerebral Ischemia-reperfusion Injury

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Abstract [**Objectives**] To observe the protective effect of Danhong Decoction on cerebral ischemia-reperfusion injury in Sprague-Dawley (SD) rats. [**Methods**] Eighteen SD rats were randomly divided into blank control group, MCAO group, and observation group ($n=6$ each). In the MCAO and observation groups, the cerebral ischemia-reperfusion model was established by the suture occlusion method (ischemia for 60 min followed by reperfusion). After successful modeling, the observation group received 2% Danhong Decoction (2 mL/rat) by intragastric administration twice daily for 1 week, while the blank control and MCAO groups received an equal volume of normal saline. After treatment, the expression levels of endothelial nitric oxide synthase (eNOS), nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD), vascular endothelial growth factor (VEGF), and endothelin-B (ET-B) receptor in brain tissue were measured. [**Results**] Compared with the MCAO group, the observation group showed significantly decreased levels of MDA, ET-B protein, and ET-B mRNA, and significantly increased levels of NO, eNOS, SOD, VEGF, and VEGF mRNA (all $P<0.05$). [**Conclusions**] Danhong Decoction can attenuate cerebral ischemia-reperfusion injury in SD rats, and its mechanism may be related to inhibition of oxidative stress and promotion of angiogenesis.

Key words Danhong Decoction, Sprague-Dawley (SD) rats, Cerebral ischemia-reperfusion injury, Protective effects

1 Introduction

Acute cerebral infarction, also known as ischemic stroke, is one of the cerebrovascular diseases that seriously endangers human health. Due to the activation of oxidative stress response, neurological damage is often aggravated[1]. In traditional Chinese medicine, Carthami Flos (Honghua) is warm in nature, while Salviae Miltiorrhizae Radix et Rhizoma (Danshen) is cool; both herbs are pungent in taste. When used in combination, they exhibit the effects of promoting blood circulation, unblocking meridians, dispelling blood stasis, and alleviating pain[2]. In this study, we used self-made Danhong Decoction to treat MCAO rats and observed whether it exerts a protective effect against cerebral ischemia-reperfusion injury.

2 Materials and methods

2.1 Experimental animals and grouping Eighteen Sprague-Dawley (SD) rats with body weight of (275 ± 5) g were selected. The laboratory animal permit number was SCXK (Hubei) 2025-0008. The rats were randomly divided into blank control group, MCAO group, and observation group, with 6 rats in each group. The animal room was maintained at a temperature of (22 - 24) ℃ and a relative humidity of 70% - 80%. The rats were housed in separate cages under natural light, with free access to water and food.

2.2 Drugs and reagents Danhong Decoction was prepared by the Pharmacy Department of our hospital (batch No.: THYF20251203). Chloral hydrate was obtained from Yangzhou Aoxin Auxiliary Factory. ELISA kits for SOD, MDA, NO, VEGF, and eNOS were provided by Shanghai Enzyme-Linked Biotechnology Co., Ltd. (batch No.: SH02501, SH02519, SH025210, H2604, and H19083, respectively). The endothelin-B (ET-B) polyclonal antibody kit was purchased from AmyJet Scientific Technology Co., Ltd. (batch No.: A202503).

2.3 Methods

2.3.1 Model establishment. After anesthesia with 10% chloral hydrate, the SD rats were placed in the supine position and fixed. The neck region was disinfected, and sterile surgical drapes were applied. Following shaving, a midline incision was made along the anterior cervical skin, and the fascial tissues were bluntly dissected with a glass rod to expose the external carotid artery, internal carotid artery, and pterygopalatine artery. Subsequently, the external carotid artery was ligated, and the proximal end of the internal carotid artery as well as the distal end of the pterygopalatine artery were temporarily clipped. A small incision was made on the internal carotid artery, and a pre-prepared monofilament suture was gently inserted. When the suture reached the clamped site of the pterygopalatine artery, the arterial clip was released. The insertion was continued until a slight resistance was felt upon tactile feedback, indicating successful occlusion. After 60 min of ischemia, the suture was withdrawn to restore cerebral blood flow[2]. Rats in the blank control group underwent only anesthesia and neck incision without modeling.

2.3.2 Administration methods. The blank control group and the MCAO group received intragastric administration of 0.9% NaCl (2 mL/rat), while the observation group received 2% Danhong Decoction (2 mL/rat) by gavage, twice daily, for 1 consecutive week [2].

2.4 Detection indicators and methods All measurements were performed according to the manufacturer's instructions provided with the corresponding kits for SOD, MDA, NO, VEGF, eNOS, and ET-B.

2.5 Statistical methods All data were analyzed using SPSS 27.0 statistical software. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Comparisons among groups

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were performed by one-way analysis of variance (ANOVA), and pairwise comparisons were conducted using the least significant difference (*LSD*)-*t* test. A P value of <0.05 was considered statistically significant.

3 Results and analysis

3.1 Effects on SOD, MDA, NO and eNOs Compared with the blank control group, the levels of eNOS, NO, and SOD in both the MCAO group and the observation group were decreased, while the level of MDA was increased (all $P<0.05$). Compared with the MCAO group, the levels of eNOS, NO, and SOD in the observation group were significantly increased, whereas the MDA level was decreased (all $P<0.05$). The results are shown in Table 1.

Table 1 Comparison of SOD, NO, eNOs and MDA in each group ($\bar{x}\pm s$, $n=6$)

Group	NO // μmol/L	eNOs // μmol/L	MDA // μmol/L	SOD // μg/L
Blank control	56.21 ± 4.85	60.25 ± 5.02	19.51 ± 2.01	203.85 ± 13.54
MCAO	42.36 ± 5.14[a]	34.40 ± 2. 71[a]	47.70 ± 2.28[a]	70.41 ± 5.02[a]
Observation	53.27 ± 3.85[b]	57.20 ± 3.36[b]	22.50 ± 1.89[b]	202.74 ± 16.86[b]

NOTE [a] indicates $P<0.05$ compared with the MCAO group; [b] indicates $P<0.05$ compared with the MCAO group. The same below.

3.2 Effects on expression of VEGF, ET-B, VEGF-mRNA and ET-BmRNA Compared with the blank control group, the observation group showed increased levels of ET-B and ET-B mRNA, and significantly decreased levels of VEGF and VEGF mRNA ($P<0.05$). Compared with the MCAO group, the observation group exhibited decreased levels of ET-B and ET-B mRNA, and significantly increased levels of VEGF and VEGF mRNA ($P<0.05$). The results are shown in Table 2.

Table 2 Comparison of expression of VEGF, ET-B, VEGFmRNA and ET-BmRNA in each group ($\bar{x}\pm s$, $n=6$)

Group	ET-B // pg/mL	ET-B-Mrn // pg/mL	VEGF // ng/mL	VEGF-mRNA // pg/mL
Blank control	6.53 ± 1.48	2.32 ± 0.97	51.32 ± 3.57	21.36 ± 3.21
MCAO	18.04 ± 1.48[a]	9.55 ± 1.84[a]	30.29 ± 2.44[a]	12.48 ± 2.50[a]
Observation	7.04 ± 0.81[b]	2.48 ± 0.51[b]	50.24 ± 4.09[b]	20.16 ± 1.51[b]

4 Discussion

Under physiological conditions, nitric oxide (NO) is a vasodilatory factor, produced under the catalysis of endothelial nitric oxide synthase (eNOS), and exerts a relaxing effect on blood vessels[3]. Endothelin-B (ET-B) receptors are widely distributed on vascular endothelium and can be abundantly induced under ischemia and hypoxia. Therefore, high expression of ET-B protein and ET-B mRNA indicates severe vascular endothelial injury, whereas decreased expression of both suggests improvement and correction of cerebral ischemia[4]. Malondialdehyde (MDA) is an important indicator reflecting the degree of oxidative stress. Accordingly, increasing SOD activity and the expression of the pro-angiogenic factor VEGF, scavenging oxygen free radicals and the lipid peroxidation product MDA, inhibiting oxidative stress, and promoting angiogenesis in the ischemic region to enhance cerebral blood flow are all of great significance for promoting synaptic proliferation and neural remodeling of the brain after ischemia[5-6].

This experiment revealed that Danhong Decoction plays an important protective role in inhibiting the oxidative stress response induced by cerebral ischemia-reperfusion injury. The underlying mechanism may be associated with increasing the activities/levels of SOD, NO, and eNOS, as well as suppressing the oxidative stress response and the production of MDA, which are crucial steps in eliminating oxygen free radicals and lipid peroxidation. Meanwhile, Danhong Decoction also elevated the expression levels of VEGF and VEGF-mRNA, while reducing the expression levels of ET-B and ET-B-mRNA. This is conducive to angiogenesis in the ischemic region and post-ischemic neural remodeling, thereby exerting a protective effect against cerebral ischemia-reperfusion injury in SD rats.

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