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EXPERIMENTAL STUDY

Effect of brain functional recovery decoction on expression of vascular endothelial growth factor and Ang-1 protein in a rat cerebral ischemia reperfusion model

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Abstract

OBJECTIVE: To investigate the effect of brain functional recovery decoction (BFRD) on expression of vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang-1) protein in rats with cerebral ischemia reperfusion injury, and to explore the mechanism of action of BFRD.

METHODS: Using the suture-occlusion method, a Wistar rat model of focal cerebral ischemia reperfusion was established. The rats were randomly divided into treatment group, model group, and sham operation group. The treatment group was administered BFRD. In situ hybridization was used to detect VEGF mRNA expression. Immunohistochemistry was used to observe expression of Ang-1 protein.

RESULTS: VEGF mRNA expression was greater in the model group compared with the sham operation group ($P < 0.05$); Ang-1 protein expression was more obvious in the treatment group than the model group ($P < 0.05$).

CONCLUSION: BFRD promoted VEGF mRNA and Ang-1 protein expression in the brains of rats with cerebral ischemia, suggesting increased angiogenesis.

Key words: Vascular endothelial growth factors; Brain ischemia; Angiopoietin-1; Stroke; Brain functional recovery decoction

INTRODUCTION

Stroke is a disease that causes serious harm to human health, and with tod's aging population, the incidence rate of stroke increases every year. Stroke is categorized into hemorrhagic and ischemic stroke, with ischemic stroke accounting for 60%-80% of stroke cases. Blood supply should be restored to the ischemic area as soon as possible to preserve neurons, vascular endothelial cells, and glial cells.[1] VEGF plays a role in the prevention and treatment of ischemic stroke. Angiopoietin-1 (Ang-1) is an angiogenic growth factor and plays an important role in microvascular remodeling;[2,3] it improves tissue perfusion around ischemic regions, induces angiogenesis, and promotes brain ischemia area functional recovery. Molecular biological methods have been used to show the effects of focal cerebral ischemia reperfusion and Traditional Chinese Medicine on VEGF and Ang-1 expression in the rat brain.

MATERIALS AND METHODS

Animal grouping

The rats were randomly divided into a focal cerebral ischemia reperfusion model group (model group, 30 rats), model group of focal cerebral ischemia reperfusion treated with Chinese medicine (Traditional Chinese Medicine group, 30 rats), and sham operation group (sham group, 30 rats). Animals were replaced in groups where there was accidental death. The groups were further subdivided into focal cerebral ischemia 1 h reperfusion groups after 2 h, 24 h, 3, 7, and 14 d, with a total of 5 groups and 6 rats in each group. The sham operation group did not undergo additional treatment.

Model establishment

A total of 90 healthy adult rats, weighing 200-300 g, were provided by the Animal Experimental Center of Xi'an Jiaotong University, Department of Medicine. All experimental rats were housed at room temperature (25 ± 1) ℃ and (50% ± 1%) humidity. The rats were allowed free access to food and water, and the feeding experiment was performed after a week of maintenance. For the Traditional Chinese Medicine group and model group, middle cerebral artery occlusion was established according to previously described methods.[4, 5] Briefly, the rats were fixed in a supine position on the operating table and were abdominally injected with 10% chloral hydrate anesthesia (0.3 mL/100 g), and a neck incision was made to reveal the carotid artery, external carotid artery, and internal carotid artery extracranial end. A ring groove was made in the external carotid artery stump, and a 0.22-mm-diameter nylon rope was inserted around the stump with polyurethane treatment, which was inserted 11-20 mm along the incision to the intracranial internal carotid artery. When resistance of the thread tip at the origin of the middle cerebral artery was encountered, ischemia was induced for 1 h, after which the suture was pulled back to the external carotid artery end to achieve perfusion. Intramuscular gentamicin was administered to prevent infection. For the sham operation group, the suture was only inserted to 5 mm, which did not induce middle cerebral artery occlusion, and the remaining steps were the same as the other groups. Successful model establishment was characterized; a 5-grade score was applied after the rats awoke from surgery according to the Longa standards.[6]

Effect of brain ischemia reperfusion on the neurological behavior scores of rats

The sham operation group did not exhibit symptoms of ischemic injury. The model group and the drug group, however, exhibited symptoms of neurological dysfunction.

Treatment

The sham operation group and model group were treated with distilled water (2 mL) two times daily in the First Affiliated Hospital of Xi'an Jiao Tong University (see basic components of drug production in the Chinese medicine group discussion section). The Chinese medicine treatment group was administered 2 mL BFRD two times each day (three times the equivalent adult dosage).

VEGF mRNA in situ hybridization

In situ hybridization was performed using the VEGF mRNA in situ hybridization kit from Wuhan Boshide Company (Wuhan, China). Cells were cultured on polylysine slides, frozen, and then fixed with a 4% paraformaldehyde solution. Citric acid was used to expose the mRNA nucleic acid fragments, after which the cells were incubated in 3% fresh pepsin. Pre-hybridization was performed by incubating overnight in pre-hybridization solution at 38 ℃. A protective film was placed over the glass to cover the slices. The glass slides were biotinylated mouse anti-digoxin antibody was added to the slide and incubated overnight in hybridization solution at 38 ℃, and then washed and followed by the addition of Strept Avidin-Biotin Complex (SABC), and then the color reaction was initiated with 3, 3′-diaminobenzidine (DAB). The slides were then dehydrated through an alcohol gradient, cleared in xylene, and coverslipped.

Immunohistochemical detection of Ang-1 protein

The paraffin sections were incubated in 3-Aminopropyl-Triethoxysilane (APES) at 58 ℃-60 ℃ for 30-60 min to detect the antigen. The sections were dewaxed in 30% H_2O_2. Ten copies were mixed with distilled water, 3 times distilled water. The sections were immersed in 0.01 M citrate buffer (pH 6.0) and microwaved in 5-minute intervals, which was repeated twice. After cooling, the sections were washed twice in poly butylenes succinate (PBS) (pH 7.2-7.6), and then biotinylated goat anti-mouse IgG was diluted with PBS and the sections were incubated at 30 ℃ for 20 min and subsequently washed three times in PBS for two min each. A color reaction was obtained with DAB (1 mL; DAB Kit; AR1022). Then, the ABC reagent was mixed with distilled water and added to the section, incubated at room temperature for 10 min, and the amount of staining was monitored under a microscope.

Positive cell count

Positive cells (× 400) with a brown-stained cytoplasm were randomly selected from non-consecutive sections from 3 rats, and each section was quantified with an average of 10 discontinuous fields of view.

Statistical analysis

Data were expressed as mean ± standard deviation ($\bar{x}$ ± s), and analyzed using SPSS 12.0 (SPSS Inc. SPSS Statistics for Windows, Version 12.0. Chicago, IL, USA). $P < 0.05$ was the level of statistical significance. The differences between groups were tested with analy-