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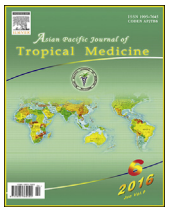

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Expression and significance of angiostatin, vascular endothelial growth factor and matrix metalloproteinase-9 in brain tissue of diabetic rats with ischemia reperfusion

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ABSTRACT

Objective: To discuss the expression and significance of angiostatin, vascular endothelial growth factor and matrix metalloproteinase-9 in the brain tissue of diabetic rats with ischemia reperfusion.

Methods: A total of 60 male Wistar rats were randomly divided into the normal group, sham group, diabetic cerebral infarction group and single cerebral infarction group according to the random number table, with 15 rats in each group. The high sucrose diet and intraperitoneal injection of streptozotocin were performed for the modeling of diabetic rats, while the thread-occlusion method was employed to build the model of cerebral ischemia reperfusion. The immunohistochemical staining was performed to detect the expression of angiostatin, vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) in the brain tissue.

Results: The expression of angiostatin after the reperfusion in the brain tissue of rats in the single cerebral infarction group and diabetic cerebral infarction group was increased 6 h after the reperfusion, reached to the peak on 1 d and then decreased gradually. The expression of angiostatin in the diabetic cerebral infarction group 6 h, 1 d, 3 d and 7 d after the reperfusion was significantly higher than that in the single cerebral infarction group ($P < 0.05$). VEGF began to be increased 1 h after the reperfusion in the single cerebral infarction group and diabetic cerebral infarction group, reached to the peak at 6 h and then decreased gradually. The expression of VEGF in the diabetic cerebral infarction group at each time point after the reperfusion was significantly lower than that in the single cerebral infarction group ($P < 0.05$). MMP-9 began to be increased 1 h after the reperfusion in the single cerebral infarction group and diabetic cerebral infarction group, reached to the peak on 1 d and then decreased gradually. The expression of MMP-9 in the diabetic cerebral infarction group at each time point after the reperfusion was significantly higher than that in the single cerebral infarction group ($P < 0.05$).

Conclusions: The high glucose environment in which the diabetic cerebral infarction is occurred is to induce the formation of MMP-9 at first and then activate and increase the expression of angiostatin. Afterwards, the expression of VEGF is inhibited, resulting in the poor angiogenesis after cerebral infarction, which thus makes the injury of brain tissue after cerebral infarction even worse than the non-diabetes mellitus.

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1. Introduction

The impairment of cerebral blood supply can cause the ischemia and hypoxia of brain tissue and then result in the cerebral infarction. The focus of cerebral infarction mainly consists of the ischemic penumbra and central necrotic area. The central area would induce the apoptosis of brain cells because of ischemia, while the collateral circulation in which the penumbra exists could provide the blood for the focus and thus there would still be a great number of alive neuronal cells [1,2]. If repairing the metabolic function of brain after the injury as soon as possible, the function of some neuronal cells can be recovered. Accordingly, the building of effective collateral circulation would be of critical significance for reversing the injury of neuronal cells and improving the neural function [3]. The prognosis of patients with diabetes mellitus and cerebral infarction is even poorer than that of patients with single cerebral infarction. The animal experiment proved that [4] the combined diabetes mellitus could significantly reduce the collateral circulation of cardiac muscular tissue and focus of cerebral infarction and thus aggravated the tissue injury after the cerebral ischemia. There have been many researches that reported the diabetes mellitus could aggravate the injury of cerebral infarction tissue, but no definite conclusion could be drawn. Therefore, in this study, by building the model of diabetic rats with ischemia reperfusion injury and observing the expression of angiostatin, vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) in the focus tissues, it was to discuss the possible pathophysiological mechanism of diabetic rats with ischemia reperfusion injury, in order to provide the new thought for the further clinical treatment and pharmaceutical development. The findings were summarized as follows.

2. Materials and methods

2.1. Materials

A total of 60 male Wistar rats were provided by Laboratory Animal Center of School of Medicine, Shandong University, with the weight of (180–230) g and average weight of (206.3 ± 13.8) g; 60 rats were randomly divided into 4 groups according to the random number table: normal group, sham group, diabetic cerebral infarction group and single cerebral infarction group, with 15 rats in each group. Rats in each group were divided into 5 subgroups according to 1 h, 6 h, 1 d, 3 d and 7 d of ischemia reperfusion, with 3 rats in each subgroup. The feeding conditions were as follows: the temperature of feeding room was maintained at (20–22) °C, the room humidity was 50% and rats were given the diet and water freely, with 12 h of lighting and 12 h of darkness by turns. The experiment was performed 1 week after the feeding. The experimental protocol, operation and animal ethics of this study were reviewed and approved by School of Medicine, Shandong University.

2.2. Methods

2.2.1. Modeling of diabetic rats

Rats in the diabetic cerebral infarction group were given the high sucrose diet for 2 months and then the intraperitoneal injection of streptozotocin (STZ, which was purchased from Sigma). The blood glucose and weight were measured 1 month and 2 months after the injection. The modeling standards were as follows: the weight of rats was (290–360) g and the blood glucose level was (16.7–25.6) mmol/L (not fasting).

2.2.2. Modeling of cerebral ischemia reperfusion

The model of cerebral ischemia reperfusion was built using Zea-Longer thread-occlusion method [5] for rats in the diabetic cerebral infarction group and single cerebral infarction group: rats were placed on the operating table at 25 °C in a supine position. After being fixed, they were given the intraperitoneal injection of 35 mg/100 g 10% chloral hydrate. An incision was done in the middle of neck to separate the external carotid artery, internal carotid artery and common carotid artery. The proximal part of external carotid artery and internal carotid artery were ligated. The eye scissors were used to cut an incision at the distal part of common carotid artery (about 5 mm to the bifurcation) and the nylon thread was inserted, with the depth of about 25 mm. The thread occlusion was then fixed. The skin and muscle were sutured and disinfected layer by layer. After 1.5 h of thread occlusion, the thread was pulled out to realize the ischemia reperfusion. The rectal temperature of rats was maintained at (36.5–37.5) °C during the operation and the right middle cerebral artery was chosen as the embolization artery. The nylon thread was inserted in rats of sham group with the depth of about 16 mm and the left operations were the same as above. The modeling standard of ischemia reperfusion was: when the rats were awakened from the anesthesia, the Longa score [6] was employed to evaluate the neural function of rats, where a score of 0 indicated no neurologic deficit, a score of 1 failure to extend left forepaw fully, a score of 2 circling of forepaws when walking, a score of 3 falling to the left when walking ahead and 4 no spontaneous walk and loss of consciousness. The score of 1–3 indicated the successful modeling.

2.2.3. Sampling

The detection was performed at 5 time points of 1 h, 6 h, 1 d, 3 d and 7 d after the ischemia reperfusion. The head of rats was broken and the brain was collected. After being placed in the liquid nitrogen, they were fixed and dehydrated. Afterwards, they were treated with the common paraffin embedding, with the slice thickness of 4 μm. The immunohistochemical staining was employed to detect the expression of angiostatin, VEGF and MMP-9 in the brain tissue. The slices were deparaffinized using the regular method. After adding 3% H_2O_2, it was placed in the microwave for 10 min to repair the antigen. After 15 min of adding the goat serum blocking solution and 3 h of adding the primary antibody, it was washed with 0.1 mol/LPBS for 3 times, with 5 min each time. Afterwards, the secondary antibody was added for 1.5 h and it was washed with 0.1 mol/LPBS for 3 times, with 5 min each time. After the DAB staining, it was restained with hematoxylin. The primary antibody was replaced by PBS in the negative control, with the remained steps same as above. The antibodies of angiostatin, VEGF and MMP-9 were all purchased from Sigma.

2.3. Outcome evaluation

The angiostatin and VEGF with positive staining were mainly in the cell membrane and cytoplasm, while MMP-9 was