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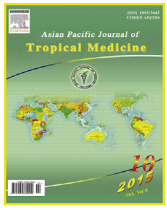

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Effect of microRNA-155 on angiogenesis after cerebral infarction of rats through AT1R/VEGFR2 pathway

Ying-Chun Meng[1*], Zhen-Ying Ding[2], Hai-Quan Wang[1], Li-Ping Ning[1], Chao Wang[1]

[1]*Department of Rehabilitation, Affiliated Shandong Provincial Hospital of Shandong University, Ji'nan 250021, Shandong, China*

[2]*Department of Neurology, Shandong Qixia People's Hospital, Qixia 370686, Shandong, China*

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ABSTRACT

Objective: To explore the function and mechanism of microRNA-155 to regulate the angiogenesis after the cerebral infarction of rats through the angiotensin II receptor 1 (AT1R)/vascular endothelial growth factor (VEGF) signaling pathway.

Methods: Female SD rats were chosen for the construction of cerebral infarction model of rats using the modified right middle cerebral artery occlusion. The real-time PCR (RT-PCR) method was employed to detect the expression of microRNA-155 in each group at different time points after the cerebral infarction (1 h, 1 d, 3 d and 7 d). SD rats were randomly divided into four groups (n = 20 rats): sham operation group (Sham group), MACO group, MACO+microRNA-155 mimic group, and MACO+microRNA-155 inhibitor group. Sham group was given the free graft, while MACO+microRNA-155 mimic group and MACO+microRNA-155 inhibitor group were treated with microRNA-155 mimic and microRNA-155 inhibitor respectively. The Zea Longa 5-point scale was used to score the neurologic impairment of rats in each group; 2, 3, 5-triphenyl tetrazolium chloride staining to evaluate the volume of cerebral infarction of rats in each group; the immunohistochemistry to detect the expression of CD31; Western blot and RT-PCR to detect the expression of AT1R and VEGF receptor 2 (VEGFR2).

Results: The expression of microRNA-155 was increased in the cerebral ischemia tissue after the cerebral infarction. It was significantly increased at 1 d of ischemia and maintained at the high level for a long time. Rats in the Sham group had no symptom of neurologic impairment, while rats in the MACO group had the obvious neurologic impairment. After being treated with microRNA-155 inhibitor, the neural function of MACO rats had been improved, with the decreased area of cerebral infarction. But after being treated with microRNA-155 mimic, the neural function was further worsened, with the increased area of cerebral infarction. Results of immunohistochemical assay indicated that microRNA-155 inhibitor could up-regulate the expression of CD31, while microRNA-155 mimic could down-regulate the expression of CD31. The RT-PCR found that, after being treated with microRNA-155 inhibitor, MACO rats had the increased expression of *AT1R* and *VEGFR2* messenger RNA (mRNA); but after being treated with microRNA-155 mimic, the expression of *AT1R* and *VEGFR2* mRNA was decreased. Results of Western blot showed that, after being treated with microRNA-155 inhibitor, MACO rats had the increased expression of *AT1R* and *VEGFR2* mRNA; but after being treated with microRNA-155 mimic, the expression of *AT1R* and *VEGFR2* mRNA was decreased.

Conclusions: The inhibition of microRNA-155 can improve the neurologic impairment of rats with the cerebral infarction, reduce the volume of cerebral infarction and effectively promote the angiogenesis in the region of ischemia, which may be mediated through AT1R/VEGFR2 pathway.

*Corresponding author: Ying-Chun Meng, Department of Rehabilitation, Affiliated Shandong Provincial Hospital of Shandong University, No. 324 Nanjingwu Road, Ji'nan 250021, Shandong, China.
Tel: +86 15168889866
E-mail: myc9866@163.com

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1. Introduction

The epidemiological survey shows the upward trend of incidence of cerebral infarction in China, which has attracted a wide attention because of its high disability rate and mortality rate. The cerebral infarction can cause the irreversible degeneration and necrosis of brain neurons and thus lead to a series of neurological dysfunction. Therefore, the pursuit of effective means to early promote the local angiogenesis in the region of cerebral infarction to recover the tissue perfusion will be of critical significance for the prognosis of patients with cerebral infarction. But there are still no effective therapeutic programs.

The angiogenesis could improve the perfusion in the region of cerebral infarction and thus promote the regeneration of central nerve, as the basis for the recovery of brain neurons [1,2]. The vascular endothelial growth factor (VEGF) is one of the most important vascular growth factors in the process of angiogenesis. It's reported that its specific effect on the endothelial cells could be involved in the angiogenesis [3]. VEGF plays its role through three receptors, including VEGF receptor 1 (VEGFR1), VEGF receptor 2 (VEGFR2) and VEGF receptor 3 (VEGFR3), where VEGFR2 is of most important for the early angiogenesis. Angiotensin II (Ang II) is a multifunctional bioactive peptide, which can promote the angiogenesis and plays the key role in the angiogenesis. Angiotensin II receptor 1 (AT1R)/VEGFR2 signaling pathway can promote the proliferation and migration of vascular endothelial cells and play a role in regulating the angiogenesis both in the physiological and pathological condition. According to researches, the angiogenesis began at the time of cerebral infarction, and the regulation of AT1R/VEGFR2 pathway on the angiogenesis has been widely recognized [4]. However, when the cerebral infarction occurred, such reaction of the body still could not improve the revascularization in the region of cerebral infarction and the perfusion of brain tissue. Therefore, it would be necessary to seek the effective measures to enhance the role of AT1R/VEGFR2 pathway in such process.

microRNA is some kind of small non-coding RNA molecule with about 22 nucleotides, which can be widely found in the plants, animals and some viruses [5]. Based on the complementary pairing with messenger RNA (mRNA) of the target gene, the microRNA can play a critical role in the gene regulation after the silencing and transcription of RNA. Some researchers found that microRNA could be widely involved in the biological processes of proliferation, apoptosis and differentiation of cells and play a key role in the regulation of onset and development of many diseases (cardiovascular diseases and tumors) [6,7]. It's reported that there was the close relationship between microRNA-155 and angiogenesis and the target gene of microRNA-155 was *AT1R*, which could target at reducing the expression of AT1R [8] to inhibit Ang II/AT1R signaling pathway and thus result in the inhibition of angiogenesis. A research found that the expression of microRNA-155 was significantly increased in the condition of cerebral ischemia, while the inhibition of microRNA-155 could protect the nerves and thus improve the damage of cerebral ischemia [9].

Consequently, this study was to build the cerebral infarction model of rats. According to the *in vivo* over-expression and the inhibition against the expression of microRNA-155, it was to observe the effect of microRNA-155 on the angiogenesis of rats with cerebral infarction and further explore the role of AT1R/VEGFR2 pathway in such process.

2. Materials and methods

2.1. Materials

Adult female SD rats with the weight of about 200–250 g were chosen as the subjects in this study (which were purchased from Shanghai SLAC Laboratory Animal Co. Ltd.); other materials included RNA extraction kit (Invitrogen, USA); reverse transcription kit (Takara, Japan); Real-time fluorescent quantitative PCR kit (TaKaRa, Japan); microRNA-155 mimic (Life Technologies, USA); microRNA-155 inhibitor (Life Technologies, USA); triphenyl tetrazolium chloride (TTC) (Sigma, USA); Rat CD31, AT1R, VEGFR2, β-actin antibody (Santa Cruz, USA); horseradish peroxidase-labeled goat anti-rabbit IgG (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.).

2.2. Methods

2.2.1. Construction of cerebral infarction model of rats

Adult female SD rats (200–250 g) were fed in the single cage at the room temperature of about 25 °C and humidity of about 50%, with the light/dark cycle of 12 h. They were given with diet and water freely. After one week of adaptive feeding, rats were given the intraperitoneal injection of pentobarbital sodium (0.3 mL/100 g) for the anesthesia. The right middle cerebral artery occlusion was performed on rats under sterile conditions. Referring to the modified Zea-Longa method [10], the specific procedures were as follows: the incision was taken in the middle of neck and the tissues were separated layer by layer to expose and free the right common carotid artery, internal carotid artery and external carotid artery. A small incision was taken in the external carotid artery close to the heart. The suture was inserted in the common carotid artery through the incision and then taken back to pass by the bifurcation of common carotid artery and enter in the internal carotid artery. Afterwards, the suture was slowly delivered to the starting point of middle cerebral artery, with the insert length of about (8.0 ± 0.5) cm from the bifurcation of common carotid artery. The suture was fixed and the neck skin was stitched. Rats in the sham operation group (sham group) were only freed the vessels. According to Zea-Longa 5-point scale (Table 1), the neurologic impairment of rats in each group was scored [11].

2.2.2. Grouping

Laboratory rats were randomly divided into four groups (n = 20 rats): Sham group, MACO group, MACO+microRNA-155 inhibitor group and MACO+microRNA-155 mimic group. At 5 min of sham operation for rats in the sham group, they were injected with the equal normal saline through the lateral ventricle; at 5 min of cerebral ischemia, rats in the MACO group were injected with the equal normal saline through the lateral ventricle; at 5 min of cerebral ischemia, rats in the MACO+microRNA-155 mimic group were injected with 10 μg miRNA-155 mimic through the lateral ventricle; at 5 min of cerebral ischemia, rats in the MACO+microRNA-155 inhibitor group were injected with 10 μg microRNA-155 inhibitor through the lateral ventricle.