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The decrease of Tie-2 receptor phosphorylation in microvascular endothelial cells is involved in early brain injury after subarachnoid hemorrhage

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Abstract *Purpose:* Loose endothelial cells and the destruction of the blood—brain barrier (BBB) are one of the pathophysiological mechanisms of early brain injury (EBI) after subarachnoid hemorrhage (SAH). Tie-2 receptor phosphorylation is important for maintaining integrity of microvascular endothelial cells and BBB. This study aimed to explore the role and changes of Tie-2 receptor phosphorylation levels in EBI after SAH.

Methods: This study used an endovascular puncture model of rat to simulate the occurrence and development of aneurysmal subarachnoid hemorrhage. The location of Tie-2 receptor in brain tissues was determined by immunofluorescence. Immunofluorescence and western blot was carried out to observe the expression of Claudin-5 and Occludin in cortex and hippocampus. We chose to observe the Tie-2 receptor phosphorylation level in hippocampus according western blot. Evans blue viability assay was used to evaluate BBB permeability.

Results: The results suggested that Tie-2 receptor mainly expressed around the vascular endothelial cells in brain. Following SAH, the Tie-2 receptor phosphorylation level and expression of tight junction protein (claudin-5 and occluding) decreased. Both of these downtrends were reversed by exogenous Angiopoietin-1 (Ang-1). Finally, injection of exogenous Ang-1 reduced SAH-associated BBB leakage.

Conclusions: Our study indicated that Tie-2 receptor phosphorylation in microvascular endothelial cells was involved in pathophysiological process after SAH, and the decline of Tie-2

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receptor phosphorylation might increase blood—brain barrier permeability by decreasing the tight junction protein expression.
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Introduction

Subarachnoid hemorrhage (SAH) is a common cause of acute cerebrovascular disease, including intracranial aneurysm rupture of spontaneous SAH etiology of about 85%.[1] According to epidemiological statistics, the overall incidence of spontaneous SAH is 10 people/10 million per year, ranking third in cerebrovascular disease, and second only to Cerebral thrombosis and cerebral hemorrhage.[2] The mortality rate of aneurysmal subarachnoid hemorrhage reaches up to 51%, and 46% of survivors remain significant neurological dysfunction.[3,4]

The blood brain barrier (BBB) is a special barrier system in the central nervous system. It plays an important role in selectively absorbing nutrients, blocking pathogens and maintaining the central nervous system steady state.[5] One of the earliest signs of brain injury after SAH is the change in BBB permeability, including microvascular endothelial cell apoptosis, opening of cell—cell tight junctions, and degradation of basal membrane extracellular matrix (ECM) and type IV collagen.[6,7] All of the above pathophysiological process increase the brain edema, microthrombosis, inflammatory response and abnormal brain metabolism, and result in irreversible damage.

Brain microvascular endothelial cells (BMECs) are the important components of BBB, and tight junctions between BMECs are the key factors in ensuring the physical barrier integrity of BBB. A recent animal experiment found that tight connection loosening between the BMECs occurred in the hyperacute period (3h) after SAH, and resulted in BBB leakage and angiogenic brain edema.[8] Angiopoietin (Ang) is an important endothelium-specific angiogenic factor. Unlike vascular endothelial growth factor (VEGF), the Angiopoietin/Tie-2 system mainly acts on the later stages of angiogenesis, inducing and sustaining endothelial cell migration and survival, and plays an important role in vascular stabilization.[9] Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) are the most important proteins in the currently known angiopoietin family. Their binding targets are Tie-2 receptors on the surface of vascular endothelial cells. Ang-1 can make Tie-2 receptor phosphorylation, while Ang-2 cannot. The TIE-2 receptors which bind to ANG-1 promote the maturation and stabilization of endothelial cells, inhibit the inflammatory response and increase the cell—cell tight junction.[10] Ang-2 binds to the Tie-2 receptor and competitively inhibits the effect of Ang-1, allowing endothelial cells to loosen, migrate, promote inflammatory responses, and disrupt cell connections.[11] Under physiological conditions, Ang-1 expression in the brain tissue is higher, and Ang-2 is stored in the Weibel-Palade bodies of endothelial cells. So the highly phosphorylated Tie-2 receptors of BMECs ensure the endothelium Cell stability.

In 2011, Fischer et al.[12] found that the serum concentration of Ang-1 was significantly changed within 1 day in a single-center clinical study of patients with subarachnoid hemorrhage. This finding was confirmed by Yi Wang et al., in 2015,[13] who found that patients with higher Ang-1 serum concentrations had better prognosis in another larger sample size study. Furthermore, our published basic study has demonstrated that, in the early period after SAH, Ang-1/Ang-2 ratio decreased significantly in rat brain tissue.[14]

These all encouraging studies suggest that Angiopoietin/Tie-2 signal path is involved in the pathophysiology of subarachnoid hemorrhage. So it is necessary to explore the mechanism. On this basis, we suspect that Angiopoietin/Tie-2 signal path plays a role in BBB permeability via influencing the stability of microvascular endothelial cells after subarachnoid hemorrhage.

Methods

Animals and experimental tissue acquisition

120 adult male Sprague—Dawley rats weighing 250—300 g (Experimental Animal Center of Huzhou Teachers College) was housed in 12hr light and dark cycles in a temperature and humidity controlled environment.

In the first part, euthanasia was carried out in the Sham-operated group ($n = 10$) at 24h, while the SAH group at 6h ($n = 10$) and 24h ($n = 10$). We washed the brains from the rest in normal saline solution and fixed them overnight at room temperature in 4% paraformaldehyde in 0.01 M phosphate buffered saline. We then dehydrated the tissue blocks with an ascending ethanol solution series, cleared with xylene, and then embedded in paraffin. We cut the paraffin blocks into transverse serial sections of 10-μm thickness. Next, from each perfused rat, we randomly took five sections containing the hippocampus and cortex and mounted them on poly-L-lysine coated slides for immunofluorescence staining.

In the second part, the remaining rats were divided into Sham group ($n = 30$), SAH group ($n = 30$) and SAH + Ang-1 group ($n = 30$). After euthanized with perfused with saline at 24h, two thirds of rats were used for Western Blot (10 per group) and immunofluorescence staining (10 per group). The rest were used for Evans Blue viability assay.

This study followed strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Biomedical Ethics Committee of Medical College of Huzhou Teachers College.

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