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Salvianolic acid A attenuates ischemia reperfusion induced rat brain damage by protecting the blood brain barrier through MMP-9 inhibition and anti-inflammation

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[ABSTRACT] Salvianolic acid A (SAA) is a water-soluble component from the root of *Salvia Miltiorrhiza* Bge, a traditional Chinese medicine, which has been used for the treatment of cerebrovascular diseases for centuries. The present study aimed to determine the brain protective effects of SAA against cerebral ischemia reperfusion injury in rats, and to figure out whether SAA could protect the blood brain barrier (BBB) through matrix metallopeptidase 9 (MMP-9) inhibition. A focal cerebral ischemia reperfusion model was induced by middle cerebral artery occlusion (MCAO) for 1.5-h followed by 24-h reperfusion. SAA was administered intravenously at doses of 5, 10, and 20 mg·kg^{-1}. SAA significantly reduced the infarct volumes and neurological deficit scores. Immunohistochemical analyses showed that SAA treatments could also improve the morphology of neurons in hippocampus CA1 and CA3 regions and increase the number of neurons. Western blotting analyses showed that SAA downregulated the levels of MMP-9 and upregulated the levels of tissue inhibitor of metalloproteinase 1 (TIMP-1) to attenuate BBB injury. SAA treatment significantly prevented MMP-9-induced degradation of ZO-1, claudin-5 and occludin proteins. SAA also prevented cerebral NF-κB p65 activation and reduced inflammation response. Our results suggested that SAA could be a promising agent to attenuate cerebral ischemia reperfusion injury through MMP-9 inhibition and anti-inflammation activities.

[KEY WORDS] Salvianolic acid A; Ischemia; MCAO; Blood brain barrier; NF-κB; MMP-9

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Introduction

With the increasing pressure of life and work, the incidence of cerebrovascular diseases is increasing year by year. Among these diseases, stroke is a great threat to the human health and brings huge burden to society and families. Ischemic stroke is about 87% of the entire stroke cases [1]. Up to date, recombinant tissue plasminogen activator (rt-PA) has been the most effective agent to improve the prognosis of acute ischemic stroke [2]. However, the clinical use of rt-PA is limited by its relatively narrow therapeutic time window and a serious risk of hemorrhagic complications [3-5]. The development of new drug for the treatment of brain ischemic stroke is an important task worldwide.

Matrix metalloproteinase 9 (MMP-9) is a member of the MMP family. Its main substrates are extracellular matrix components maintaining the integrity of blood-brain barrier [6]. Under normal physiological conditions, the expression of MMP-9 in brain is strictly controlled and remains at a low level [7]; while under cerebral ischemia and reperfusion (I/R)

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conditions, the expression and activity of MMP-9 are increased significantly, which leads to the redistribution and degradation of tight junction proteins (ZO-1, claudin-5 and occludin) and causes severe blood-brain barrier injury with neuronal inflammation [8]. Tissue inhibitor of metalloproteinase 1 (TIMP-1) is the endogenous inhibitor of MMP-9 [9]. MMP-9 inhibition or maintaining the MMP-9/TIMP-1 balance may be a new target for the treatment of ischemic stroke.

Salvianolic acid A (SAA, Fig. 1) is one of the main active compounds extracted from *Salvia Miltiorrhiza* Bge., a traditional Chinese medicine. It was first reported that the therapeutic effect of SAA on I/R might be related to the inhibition of brain lipid peroxidation and the scavenging of free radicals [10-11]. Then, other studies showed its protective mechanisms might include the inhibition of ICAM-1 (intercellular cell adhesion molecule-1), CD11b/CD18, soluble epoxide hydrolase and granulocyte adherence [12-17]. A most recent study showed SAA alleviated ischemic brain injury through the inhibition of inflammation and apoptosis and the promotion of neurogenesis [18], but the underlying mechanism remains to be further elucidated. It is reported that salvianolic acid B (SAB), a structurally similar polyphenol compound with SAA, significantly attenuates LPS-induced cell migration through the inactivation of MMP-2 and MMP-9 protein synthesis [19]. By the inhibition of MMP-9, SAA prevents cardiac remodeling in spontaneously hypertensive rats and attenuates aortic aneurysm formation in apolipoprotein E-deficient mice [20-21]. The above results suggest that the anti-ischemia effects of SAA may be due to the inhibition of MMP-9, which results in severe blood-brain barrier injury. In order to clarify the mechanism by which SAA exerts the brain protective effects against ischemia reperfusion injury, a middle cerebral artery occlusion and reperfusion (MCAO/R) rat model was used in the present study, and the MMP-9 inhibition and anti-inflammation effects of SAA were determined.

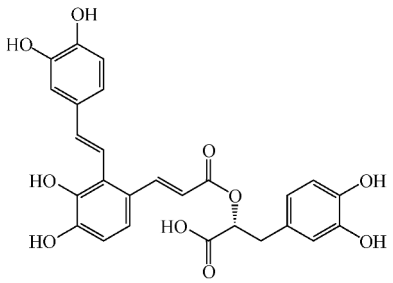

Fig. 1 Chemical structure of salvianolic acid A (SAA)

Materials and Methods

Animals, agents, and chemicals

Male Sprague-Dawley rats , weighing 240–260 g, were obtained from Beijing Vital River Experimental Animal Co., Ltd. (Beijing, China; certificate No. SCXK2012-0001). All the animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College (No. 00000901, 2016. 03.15). Efforts were made to minimize the pain and discomfort of animals.

SAA with a purity of > 98% by HPLC analysis were provided by the Institute of Materia Medica (Beijing, China). Edaravone injection was purchased from Simcere (Nanjing, China) and was set as the positive control. The suture used in MCAO/R model was purchased from Cinontech Co., Ltd. (Beijing, China). 2, 3, 5-Triphenyltetrazolium chloride (TTC) was purchased from Sigma Chemical Co., Ltd. (Shanghai, China). Rat Interleukin 6 (IL-6), Interleukin 1β (IL-1β) and Tumor necrosis factor-α (TNF-α) ELISA Kit were purchased from Cusabio Biotech Co., Ltd. (Wuhan, China). MMP Zymography Assay Kit (for MMP-2 and MMP-9 analyses) was purchased from Applygen Technologies Inc. (Beijing, China). Evans Blue was purchased from Sigma Chemical Co., Ltd. (Shanghai, China). Total Protein Extraction Kit was purchased from Applygen Technologies, Inc. (Beijing, China). BCA Protein Assay Kit was purchased from Cwbio (Beijing, China). The antibodies against MMP-9, MMP-2, TIMP-1, phospho-IκBα (p-IκBα), IκBα, phospho-NF-κB p65 (p-NF-κB p65), NF-κB p65, ZO-1, and β-actin were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The antibodies against claudin-5, occludin, phospho-IKKα/β (p-IKKα/β), IKKα/β and Histone H1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). And the HRP conjugated goat anti-rabbit IgG and HRP conjugated goat anti-mouse IgG were both purchased from Cwbio (Beijing, China). All other chemicals used in the present study were of analytical reagent grade and commercially available.

Preparation of rat middle cerebral artery occlusion (MCAO) model and SAA administration

The rats were randomly divided into sham operation group (n = 35), I/R group (n = 40), I/R + SAA (5, 10, and 20 mg·kg^{-1}) groups (n = 40) and Edaravone (5 mg·kg^{-1}) group (n = 20). The SAA doses were selected based on our previous pharmacokinetic study [22-24]. Because SAA was rapidly eliminated from animal body, relatively high doses (5, 10, and 20 mg·kg^{-1}) were selected to maintain a high plasma and tissue concentration. SAA was diluted with normal saline. Focal cerebral I/R model was established by MCAO for 1.5-h followed by 24-h reperfusion as described before [25]. First, the rats were fasted overnight with free access to tap water. After anesthetized with 10% chloral hydrate (380 mg·kg^{-1}, i.p.), the right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) of the rats were isolated. An 18-mm length of nylon suture (ϕ: 0.2 mm) was introduced into the ECA lumen and advanced into the ICA to block the origin of the middle cerebral artery. Occlusion was performed for 1.5-h, then the nylon suture was withdrawn for reperfusion. The sham operation rats received all surgical procedures but without the suture inserted. Right after the reperfusion, I/R + SAA groups were intravenously given dif-