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Resveratrol protects hippocampal neurons against cerebral ischemia-reperfusion injury via modulating JAK/ERK/STAT signaling pathway in rats

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ABSTRACT

Cerebral ischemia/reperfusion injury (I/R injury) can cause neuronal deficits even death. Recent studies demonstrated that resveratrol (RSV) exerts neuroprotective effects in ischemia and several signaling pathways were involved in the process. However, it is still possible that other signaling pathway participates in the neuronal protective process. Our study examines the possible mechanism underlying RSV treatment. We randomly divided rats into four groups: the sham group, I/R group, I/R group, I/R + RSV group, I/R + vehicle group. Locomotive and cognitive behavior were utilized by open-field and closed-field test and Morris water maze test. Neuronal cell loss was measured by hematoxylin-eosin (HE) staining for hippocampus. Western blot was applied to measure the level of p-JAK, p-ERK, p-STAT and p-JNK. The results indicated that RSV could alleviate cognitive impairment, reduce neuronal loss, downregulate p-JAK, p-ERK, p-STAT and p-JNK expression and inflammatory cytokines. In summary, resveratrol protects hippocampal neurons against cerebral ischemia-reperfusion injury via modulating JAK/ERK/STAT signaling pathway in rats.

1. Introduction

Cerebral ischemia/reperfusion injury (I/R injury) induces numerous pathophysiological reactions and remains high lethality rate (Broussalis et al., 2012). The re-establishment of blood flow following ischemia leads to severe brain damage and causes generation of reactive oxygen species (ROS) (Chouchani et al., 2014). Continually, accumulation of extracellular glutamate increases calcium influx which further triggers several intracellular reactions and causes cell death (Lai et al., 2014).

The janus kinases, extracellular signal-regulated kinases and signal transducers and activators of transcription (JAK/ERK/STAT) pathway is associated with cell proliferation, differentiation and survival (Nicolas et al., 2013). This signaling pathway is dedicated to the regulation of gene expression (Tsurumi et al., 2017) and acts downstream of the binding growth factors, cytokines as well as ROS (Yu et al., 1995). Recent studies have showed that JAK/STAT pathway proteins are widely expressed in brain area such as hippocampus and cerebral cortex (Takeda and Akira, 2000). Other studies suggested that dysregulation of the JAK-STAT pathway in inflammation, tumor and neurodegenerative diseases contributes to the brain damage (Chiba et al., 2009; Yu et al., 2009). Besides, modulation of the JAK/ERK/STAT signaling pathway exhibited inflammatory and apoptotic cascades effects in prolonged myocardial ischemia/reperfusion (Ottani et al., 2013). The JAK/ERK/STAT signaling pathway is also involved in attenuating hippocampus ischemia/reperfusion injury (Jia et al., 2017).

Resveratrol (RSV), a natural stilbene in various edible plants, is known for exerting anti-oxidant activity effect (de Santi et al., 2000). The pharmacokinetics of resveratrol are well understood with high oral absorption but poor bioavailability [24830814]. Moreover, resveratrol has blood-brain barrier permeability and has been reported to defend BBB integrity in autoimmune encephalomyelitis mice [27535376] as well as cerebral ischemia reperfusion rats [25330860]. Evidences have been reported that resveratrol exerts neuroprotective properties in both in vitro and in vivo models of stroke. In an in vitro model of hypoxia-ischemia, resveratrol reduced oxygen and glucose deprivation (OGD) induced cell death via activation of phosphatidylinositol 3′-kinase/Akt (PI3K/Akt) pathway (Zamin et al., 2006). Other study demonstrated that resveratrol enhances BK channel and decreases AMPA/NMDA receptor mediated excitatory postsynaptic currents in hippocampus slices with OGD treatment (Zhang et al., 2008). In an in vivo model of stroke, resveratrol administration showed neuroprotective effect of attenuating brain damage and improving cognitive impairment (Della-Morte et al., 2009; Wang et al., 2002). Furthermore, resveratrol attenuates infarct volume after middle cerebral artery occlusion (Sinha et al., 2002) and

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prevents cognitive impairment after chronic cerebral hypoperfusion (Anastacio et al., 2014). Thus, resveratrol protects brain damage from stroke through multiple pathways. However, the role of JAK/ERK/STAT signaling pathway in the neuroprotective effect of resveratrol is still unknown.

The aim of this study is to investigate the effect of resveratrol in ischemia/reperfusion injury and the underlying mechanism using a rat model of middle cerebral ischemia/reperfusion. In our study, we hypothesize that JAK/ERK/STAT signaling pathway could be involved in the neuroprotective effect of resveratrol against the inflammatory response, oxidative stress as well as neuronal apoptosis in hippocampus.

2. Methods

2.1. Animals

Sprague-Dawley rats weighing 260–300 g were obtained from the Shanghai Experimental Animal Center (Chinese Academy of Science, Shanghai, China). All rats were maintained in a pathogen-free environment at 25 ± 1 °C, on a 12 h light/12 h dark cycle, with free access to food and water. All the experimental procedure and animal care were approved by the University Ethics Committee for Animal Experimentation.

2.2. Experimental procedure

All rats were randomly divided into four groups: the sham group, I/R group, I/R group, I/R + RSV group, I/R + vehicle group. Trans-resveratrol (R5010, Sigma, St. Louis, USA) was dissolved in 50% propylene glycol and administrated in dose of 20 mg/kg intraperitoneally once a day for 7 consecutive days. Open-field and closed-field assessment were conducted after resveratrol treatment. One week later, rats were performed Morris water maze test. Rats were then sacrificed to conduct histology method, lipid peroxidation and antioxidant enzyme assays and western blot assay three weeks after surgery.

2.3. Ischemia model

Middle cerebral ischemia/reperfusion model was established according to previous studies (Tu et al., 2014). After anesthesia with isoflurane, rats were positioned in a stereotaxic frame (Shanghai Ruanlong Science and Technology Development Co., Ltd.). An incision was made through the neck midline and the right common carotid artery and the right external carotid artery were exposed. Then, a 4-0 monofilament nylon suture (Ethicon Nylon Suture; Ethicon Inc., Osaka, Japan) coated with a silicone was inserted through the bifurcation of the common carotid artery to the internal carotid artery and advanced to occlude the origin of the middle carotid artery. After 2 h ischemia, the monofilament was removed to reperfusion and the incision was sutured. Rats from the sham group received the same surgery without the occlusion.

2.4. Open-field test and closed-field test

Open-field test and closed-field test were utilized to measure the locomotive activity of the rats (Zhang et al., 2017). The rats were placed in the center of an open field apparatus and closed field apparatus (both W50 cm × D50 cm × H30 cm, divided into 9 squares by line grids) to acclimate for 3 min. Then they were allowed to move freely for 5 min. Their behavior was analyzed using ANY-maze video tracking system (Stoelting, Wood Dale, IL, USA) with a CCD camera. The total distance and number of crossings were measured.

2.5. Morris water maze (MWM) testing

Morris water maze testing was applied to evaluate learning and memory ability (Zhou et al., 2015). A circular pool (diameter: 120 cm; height: 50 cm, filled with depth of 30 cm water at 21.5 ± 1 °C) was placed in an independent darkened room. The pool was divided into four quadrants (N: north; S: south; E: east; W: west) and the wall was attached with different patterned cards to allow the rats to distinguish the spatial orientation. The tank was monitored with a CCD cameral on the top. A platform with a diameter of 4 cm was fixed invisible 2 cm beyond the water surface. Then, rats were trained 3 swimming trials per day for 4 consecutive days before test. The starting position of each trial was chosen randomly with their heads facing the wall, with the platform kept in the same position. Each trial was ended once the rat reached the platform in 120 s or automatically terminated when 120 s elapsed. Rats were gently guided to swim to the platform if they failed the trial. Animals were allowed to remain on the platform for 20 s and the escape latency was measured. On the testing day, the hidden platform was removed from the pool, while rats were placed in the place farthest from the platform and allowed to swim for 120 s. The swimming distance and number of crossings were measured. The MWM behavior was analyzed using the ANY-maze video tracking system (Stoelting, Wood Dale, IL, USA).

2.6. HE staining

Neuronal cell loss was measured by hematoxylin-eosin (HE) staining for hippocampus (Wang et al., 2014). After deeply anesthetized by 1% pentobarbital sodium, rats were perfused with 0.9% normal saline, followed by infusion of 4% paraformaldehyde. Brains were isolated and maintained in 4% paraformaldehyde for 24 h. Then, brains were dehydrated with sucrose solution and coronal dissected at a thickness of 20 μm. Sections were digitalized using Nikon Eclipse E-600 microscope (Nikon, Tokyo, Japan) coupled to a Pro-Series High Performance CCD camera. The number of necrotic neurons in hippocampus was counted in a blind manner in 3 different slides for each animal.

2.7. Lipid peroxidation and antioxidant enzyme assays

Approximately 24 h after MCAO surgery, the rats were sacrificed and their brains were quickly isolated. Then, in an ice-cold environment, the hippocampus was quickly removed. Samples were used to measure the activities of superoxide dismutase (SOD) and the malondialdehyde (MDA) content.

2.8. Western blot analysis

The hippocampus tissue was homogenized in ice-cold tissue lysis buffer (W:V 1:5) with protease inhibitor. Then, the lysed tissue was centrifuged and the supernatant was collected. The total protein concentration was measured by bicinchoninic acid (BCA) protein assay kit (Beyotime, Haimen, China). The protein was then subjected to 10% SDS-PAGE electrophoresis and electro-transferred to polyvinylidenedifluoride (PVDF) membranes. After blocking with 5% skimmed milk, the membranes were incubated with primary antibodies for p-JAK2, JAK2, p-STAT3, STAT3, p-ERK, ERK, p-JNK, JNK, TNF-α, IL-6, BAX, Bcl-2 (Cell Signaling Technology, USA) and GAPDH (Santa Cruz Biotechnology, USA) overnight at 4 °C. After washed 3 times, the membranes were incubated with secondary antibodies (1:5000) at room temperature for 2 h. Subsequently, the membranes were washed three times with TBST. Membrane was treated with ECL reagent (ThermoFisher, Waltham, USA) to detect protein expression. The density of the bands was visualized with X-ray film (Kodak, Shanghai, China) and analyzed using the Image-Pro Plus software (Media Cybernetics, Bethesda, USA).

2.9. Data analysis

The data of each experiment were presented as the mean ± S.E.M.,