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Research article

Astragaloside IV attenuates cognitive impairments induced by transient cerebral ischemia and reperfusion in mice via anti-inflammatory mechanisms

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HIGHLIGHTS

- Astragaloside IV significantly ameliorates cognitive impairments induced by transient cerebral ischemia and reperfusion injury.
- Astragaloside IV regulates inflammatory responses by inhibiting TLR4 signaling pathway and NLRP3 inflammasome overactivation.
- Astragaloside IV attenuates cerebral ischemia and reperfusion injury by suppressing the overactivation of microglia.

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ABSTRACT

Astragaloside IV (AS-IV) is the main active component isolated from the traditional Chinese medicinal herb *Astragalus membranaceus*. Studies have demonstrated that AS-IV has neuroprotective effects in cerebral ischemic models. In this study, we aimed to investigate the effects of AS-IV on memory impairment induced by transient cerebral ischemia and reperfusion in mice, as well as the associated signaling mechanisms. Severe memory deficits were induced by bilateral common carotid artery occlusion (BCCAO) in mice as indicated in the Morris water maze test in this study. Oral administration of AS-IV (10 and 20 mg/kg, once per day, started 7 days before surgery and continued for 7 days after surgery) significantly attenuated memory impairment and neuroinflammation. Moreover, AS-IV treatment significantly reduced the expression of toll-like receptor-4 (TLR4) and its downstream adaptor proteins, including myeloid differentiation primary response gene 88 (MyD88), toll/interleukin-1 receptor-domain containing adaptor-inducing interferon-β (TRIF) and tumour necrosis factor receptor associated factor-6 (TRAF6), and subsequently inhibited NF-κB phosphorylation. It is well-known that cerebral ischemia and reperfusion injury enhances the formation of reactive oxygen species (ROS) and further neuroinflammation. Importantly, we found that AS-IV suppressed NLRP3 inflammasome activation by controlling ROS production. In addition, AS-IV markedly reduced overactivation of microglia and the overexpression of inflammatory cytokines in the hippocampus compared with the transient cerebral ischemia and reperfusion group. These results suggest that AS-IV might possess neuroprotective effects against transient cerebral ischemia and reperfusion partly through its anti-inflammatory effects by inhibiting TLR4 signaling pathway and NLRP3 inflammasome overactivation.

1. Introduction

Among all kinds of dementia, vascular dementia ranks second only to Alzheimer's disease (AD), presenting in 20% of cases [1]. Cognitive impairments are largely attributed to cerebrovascular pathologies, and cerebral ischemia has been regarded as the most important cause of vascular dementia [2]. According to reports, cerebral ischemia can induce many deleterious effects including brain injury. The mechanism of cerebral ischemic injury is complex and embraces excitotoxicity, inflammation, apoptosis and free radical overproduction, ionic imbalance, metabolism and blood-brain barrier (BBB) integrity dysfunction [3]. Current studies have found that inflammation plays a crucial role in the pathobiology of cerebral ischemia [4]. Inflammatory signaling, which is important from the early damaging stages to late post-ischemic tissue repair, plays an essential role in the progression of brain

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ischemia and reperfusion injury [5]. Microglia, which compose 10% to 15% of all the brain cells, are the resident macrophages of the central nervous system (CNS) and they are usually maintained in a quiescent state as the first line of defense [6,7]. During cerebral ischemia states, the microglia are activated and lead to the production and secretion of inflammatory factors, such as IL-1β and TNF-α. Cerebral ischemia also evokes a strong inflammatory response, and TLR4 is the first mammalian TLR to be characterized and promotes the activation of the inflammatory signaling response [8]. Thus, after cerebral ischemia, TLR4 signaling pathways are activated and play important roles in the ischemic injury. TLR4 is expressed in microglia and astrocytes after inflammation in the CNS [9]. TLR4 signalling pathways are usually classified into MyD88-dependent and −independent pathways based on the separate recruitment of two adaptors, MyD88 and TRIF, and the downstream adaptor TRAF6, which interacts with TRIF and is involved in crosstalk between two pathways that mediate of NF-κB activation [10]. Moreover, TLR4 facilitates the NF-κB-dependent transcription of pro-inflammatory cytokines and NLRP3 inflammasome, which regulates caspase-1 maturation and the secretion of IL-1β [11]. Astragalus membranaceus, a traditional Chinese medicine, has been widely used to treat the chronic debilitating, immune, cardiovascular disorders, aging, and hepatic diseases in China [12]. AS-IV (3-0-beta-D-xylopyranosyl-6-0-beta-D-glucopyranosylcycloastrag- enol), one of the major active components purified from Astragalus membranaceus, is a small molecular ($C_{41}H_{68}O_{14}$, molecular weight = 784) saponin [13]. AS-IV has been used to prevent and treat diabetes, cardiovascular, hepatic and renal disorders due to its wide variety of therapeutic effects. It has been reported that AS-IV exerts neuroprotective effects in cerebral ischemia. Sooyong found that AS-IV ameliorated cognitive deficits induced by chronic cerebral hypoperfusion by suppressing neuronal apoptosis and oxidative damage [14]. Recent studies have demonstrated that AS-IV has anti-inflammatory, anti-oxidative, antiviral, antiapoptotic and immuno-regulatory pharmacological activities, as well as protective effects against myocardial hypertrophy by inhibiting the TLR4/NF-κB signaling pathway [15,16]. However, it is unclear whether AS-IV can suppress inflammation by inhibiting the TLR4 signaling pathway in cerebral ischemia. Therefore, the present study is aimed at investigating the ameliorative effects of AS-IV on cognitive impairments induced by cerebral ischemia and reperfusion injury by inhibiting inflammation signaling pathways.

2. Materials and methods

2.1. Materials

Astragaloside IV (purity 98%) was purchased from Nanjing Spring & Autumn Biological Engineering Co. Ltd. (Nanjing, China). SOD and MDA assay kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and IL-1β, TNF-α and ROS ELISA kits were purchased from Shenzhen Dakewe Biotech Co. Ltd. (Shenzhen, China). The bicinchoninic acid (BCA) kit was purchased from the Beyotime Institute of Biotechnology Co. Ltd. (Shanghai, China). The following primary antibodies were used in the Western blot analysis: (1) TLR4 (S441), MyD88 (V220), TRIF and TRAF6 (H154) were purchased from Bioworld Technology, Inc. (St. Paul, MN, USA); (2) anti-p65 (BS1560) and phospho-p65 (BS4138) were purchased from Bioworld Technology (St. Paul, MN, USA); (3) anti-NLRP3 (NBP2-12446) was purchased from Novus Biologicals (Littleton, CO, USA); (4) anti-caspase-1 (ab108362) and anti-Iba1 (ab178680) were purchased from Abcam (Cambridge, MA, USA); and (5) anti-β-actin (sc-130656) and anti-rabbit IgG (HRP) (sc-45101) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

2.2. Experimental animals

Male ICR mice weighing 20 ± 2 g were procured from the Experimental Animal Center, Jiangsu Province (Nanjing, China). The animals were maintained under controlled environmental conditions at 25 ± 1 °C and 60–65% air humidity under a 12 h light/12 h dark cycle with free access to food and water. All of the animal care and experimental procedures were handled strictly according to the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation and were approved by the Science and Technology Department of Jiangsu Province.

2.3. Surgeries and drug administration

Transient cerebral ischemia and reperfusion was prepared by BCCAO, as BCCAO is considered an ideal model to study transient cerebral ischemia and reperfusion injury-mediated inflammatory response [17]. Mice were randomly divided into the Sham, Model, AS-IV (10 mg/kg) and AS-IV (20 mg/kg) treatment groups. The AS-IV treatment groups were intragastrically administered 7 days before the surgery and terminated on the day of sacrifice. On the day of the surgery, AS-IV was administrated 2 h prior to ischemia. The Sham-operated and Model groups were treated with distilled water. After the mice were anesthetized with an intraperitoneal injection of chloral hydrate (350 mg/kg), the bilateral common carotid arteries were exposed and carefully separated with a small ventral neck incision and occluded twice (20 min each) with ligated surgical silk as described previously with minor modifications [18]. There was a 10 min reperfusion period between the two occlusion periods (ischemia 20 min – reperfusion 10 min – ischemia 20 min). Sham-operated mice were subjected to the same surgical operation without the surgical silk ligation. Mouse body temperature was maintained at 37 ± 0.5 °C during the surgery with heating equipment until recovery from the anesthesia.

2.4. Morris water maze test

The spatial learning and memory abilities of the mice were examined by the Morris water maze (MWM) test as described previously [19]. The MWM test began on the eighth day after the surgery, as the neurobehavioural and neuropathological consequences of BCCAO in mice are established after that period of time [20]. In this experiment, a swimming pool (100 cm in diameter and 50 cm in height) was divided into four quadrants. A platform (10 cm in diameter) was located in the center of target quadrant 1 cm below the surface of the water, which was at 22 ± 1 °C. Mice were gently placed into the water in one of the four starting positions facing the pool wall in a different order each time. Each mouse was subjected to two trials per day for four consecutive days. The time interval between training in each quadrant should be more than 30 min. Once the mouse reached the platform, it was permitted to stay on it for 15 s and the escape latency was recorded. If a mouse failed to find the platform within 90 s, it was guided to the platform for 15 s and the escape latency was recorded as 90 s. On the fifth day of the probe trial test, the platform was removed and the mice were allowed to swim freely in the pool for 90 s to search for the platform. The time spent in the target quadrant and frequency of crossing the platform were measured.

2.5. IL-1β, TNF-α and ROS measurements

The levels of IL-1β, TNF-α and ROS in the supernatant from homogenized hippocampus were quantified by ELISA assay using