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Asiaticoside attenuates memory impairment induced by transient cerebral ischemia–reperfusion in mice through anti-inflammatory mechanism

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ABSTRACT

Asiaticoside (AS) is isolated from *Centella asiatica* (L.) which has been using for a long time as a memory enhancing drug in India. This study was to investigate the effects of AS on memory impairment and inflammatory cytokines expression induced by transient cerebral ischemia and reperfusion in mice, as well as the potential signaling pathway. Transient bilateral common carotid artery occlusion (tBCCAO) induced severe memory deficits in mice according to the Morris water maze task and the step-down passive avoidance test. Meanwhile the microglial activation and the gene expression of inflammatory cytokines including interleukin (IL)-1β, interleukin (IL)-6 and tumor necrosis factor (TNF)-α were increased in the hippocampus of the mice with cerebral ischemia and reperfusion. Oral administration of AS (40 and 60 mg/kg, once per day, started the day after surgery and lasted for 7 days) significantly ameliorated the memory impairment and the inflammation. Moreover, AS (20, 40 and 60 mg/kg) markedly reduced the microglial overactivation and the phosphorylation of p38 MAPK in hippocampus compared with the transient cerebral ischemia and reperfusion group. These results suggested that AS showed the neuroprotective effect against transient cerebral ischemia and reperfusion in mice, and this effect might be associated with the anti-inflammation effect of AS via inhibiting overactivation of p38 MAPK pathway.

1. Introduction

Cerebral ischemia is regarded as the most important cause of vascular dementia (VaD), which results in insufficient oxygen and glucose delivery to support cellular homeostasis, followed by excitotoxicity, ionic imbalance, peri-infarct depolarization, oxidative and nitrative stress, inflammation and apoptosis (Doyle et al., 2008; Ohtaki et al., 2005). Both clinical and experimental studies have found that inflammation is a key factor in the pathobiology of cerebral ischemia (Denes et al., 2010; Wang et al., 2007; Xia et al., 2010). Inflammatory signaling is involved in all stages of the ischemic cascade, from the early damaging events triggered by arterial occlusion to the late post-ischemic tissue repair (Costantino and Josef, 2011). Brain ischemia and reperfusion process causes inflammatory damages including blood brain barrier (BBB) disruption, mitochondria dysfunction and cytoskeleton damaged by releasing oxygen-free radicals and proteolytic enzymes, edema and apoptosis (Doyle et al., 2008; Love, 2003; Wang et al., 2007). Microglia, as the "first line of defense guardians" of central nervous system (CNS), are serve as scavenger and antigen presentation cells, as well as important producers of a range of immunoregulatory molecule, when the brain is infected or damaged (Tambuyzer et al., 2009). Cytokines such as interleukin (IL)-1β, interleukin (IL)-6 and tumor necrosis factor (TNF)-α produced by activated microglia, are increased and related to ischemic damage degree (Doyle et al., 2008; Yenari et al., 2010). Thus, appropriately inhibiting activation and inflammatory response of microglia in ischemic brain may be a potential therapeutic strategy for the treatment of ischemic VaD. Various mechanisms of cell death and survival are evoked during cerebral ischemia. Among these mechanisms, the mitogen activated protein kinase (MAPK) signaling pathways, including c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and p38 MAPK are activated after cerebral ischemia and play an important role in ischemic injury (Centeno et al., 2007; Irving and Bamford, 2002; Irving et al., 2000; Kovalska et al., 2012).

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Asiaticoside (AS) is isolated from *Centella asiatica* (L.) which has been using as a memory enhancing and psychoactive drug for a long time in India. In China, *C. asiatica* is widely used as a folk medicine for several therapeutic indications including treatment of dermal disorders and leprosy, epidemic hepatitis and acute glomerulonephritis (Zheng and Qin, 2007). Many studies have shown that *C. asiatica* indeed has many biological activities in CNS (Dhanasekaran et al., 2009; Mook Jung et al., 1999; Orhan, 2012; C. L. Xu et al., 2012). Tabassum found that ethanolic extract of *C. asiatica* prevented neuronal injury induced by middle cerebral artery occlusion by its antioxidant and free-radical scavenging potentials (Tabassum et al., 2013), but the role of individual component in ischemic injury remained to be evaluated. In addition to neuroprotective effect of AS, it has been reported to have many other activities including anti-inflammation (Yun et al., 2008; Zhang et al., 2010). However, few studies have shown the effect of AS on VaD and the inflammatory response caused by cerebral ischemia–reperfusion.

It is reported that transient bilateral common carotid artery occlusion (tBCCAO) can cause hippocampal injuries and memory impairments (Kim et al., 2006). In this study, we investigated the influence of AS on the impairments induced by tBCCAO in mice. Cognitive function was evaluated by using the Morris water maze test and step-down passive avoidance test. Levels of inflammatory cytokines were detected by Quantitative real-time PCR (qPCR). In addition, the microglial activation, phosphorylation of p38MAPK and MAPK/ERK signal pathways were investigated via western blot methods.

2. Materials and methods

2.1. Animals

Male ICR mice weighing 20 ± 2 g were procured from Experimental Animal Center in Jiangsu Province (Nanjing, China). The animals were housed under controlled environmental conditions with 23 ± 1 °C, 12 h light/dark cycle and ad libitum access to food and water. All the experiments and animal care were handled 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.

2.2. Drugs

Asiaticoside was purchased from Nanjing Zelang Medical Technology Company Limited (Nanjing, China) and its purity was 90%.

2.3. Surgeries and drug administration

Mice were anesthetized with 10% chloral hydrate (350 mg/kg, i.p.), and subjected to a transient cerebral hypoperfusion as described by Wang et al., (2006), with slight modifications. Transient cerebral hypoperfusion was induced by tBCCAO with aneurysm clips for 10 min followed by 10 min of reperfusion. Then aneurysm clips were clipped again for 10 min. Blood flow was restored by removing clips. Sham-operated controls were subjected to the same surgical operation without clipping of the carotid arteries.

Mice were treated orally with 20, 40, or 60 mg/kg of AS 24 h after operation, and then once a day for a week. The sham-operated control group and model control group were treated with distilled water instead.

2.4. Morris water maze test

Morris water maze (MWM) test which widely used to assess spatial learning ability of rodents was carried out according to the previously described method (Lan et al., 2012). The MWM test began at the eighth day after the surgery. The MWM is a black circular pool (100 cm in diameter and 50 cm in height) with a featureless inner surface, which is filled to a depth of 30 cm with water at 22 ± 1 °C. The pool was divided into four equal quadrants. A submerged platform (1 cm below the surface of the water) was placed in the target quadrant. The mice were placed in the water facing the pool wall at one of the pool quadrants in a different order per day for five days, with time interval of 30 min between training in each quadrant. Once the mouse located the platform, it was permitted to remain on it for 10 s and the escape latencies were recorded. If a mouse failed to find the platform within 90 s, it was placed on the platform for 15 s and the escape latency was recorded as 90 s. On the sixth day, the platform was removed and the mice were subjected to a probe trial session in which mice were allowed to swim freely in the pool for 90 s to search for the platform. The mean time spent by the mice in target quadrant was measured.

2.5. Step-down passive avoidance test

Step-down passive avoidance test was carried out in accordance with the described method (Zhong et al., 2009). On the first day of the test, the mice were placed in the box to adapt for 3 min. Then electric currents were delivered for 5 min and the mice would jump onto the platform to avoid the electric shock. After a 24 h interval, the mice were again placed on the platform, and the latency to step down on the grid for the first time and the number of errors subjected to shocks within 5 min were measured as learning performances.

2.6. Assay of nitric oxide (NO) content

The NO concentration was detected by nitrate reductase method, using NO colorimetric kit (Nanjing Jiancheng Institute of Biological Engineering, China) as described by the manufacturers. NO content was expressed as μmol of nitrite/g protein.

2.7. Assay of inducible nitric oxide synthase (iNOS) activity

The NOS activity was measured by NOS colorimetric kit (Nanjing Jiancheng Institute of Biological Engineering, China) as described by the manufacturers. iNOS activity was measured as the capacity of catalyzing NO production from L-arginine (Arnaiz et al., 1999). Data was expressed as U/mg protein. One unit was defined as generating 1 nmol NO per minute at 37 °C per microgram protein.

2.8. Quantitative real-time PCR for IL-1β, IL-6 and TNF-α

The hippocampus of the mice were used for extracting total RNA by using RNA isolater Total RNA Extraction Reagent (Vazyme Biotech, Nanjing, China) according to the instructions of the manufacturer, and the RNA concentration was determined by measuring the absorbance at 260 nm in a spectrophotometer. The extracted RNA was treated with gDNA wiper Mix (Vazyme Biotech, Nanjing, China) to eliminate DNA contamination and reverse-transcribed using HiScript™ Q RT SuperMix for qPCR (Vazyme Biotech, Nanjing, China) according to the manufacturer. Quantitative real-time PCR was performed on CFX96 System (Bio-Rad) by using FastStart Universal SYBR green Master (ROX)

Table 1
Sense and antisense sequences of primers used in the RT-PCR reactions.

Gene	5′ (sense) and 3′ (antisense) primer
β-Actin	5′-TCT GGC ACC ACA CCT TCT A-3′ (sense) 5′-AGG CAT ACA GGG ACA GCA C-3′ (antisense)
IL-1β	5′-CTG TGT CTT TCC CGT GGA CC-3′ (sense) 5′-CAG CTC ATA TGG GTC CGA CA-3′ (antisense)
IL-6	5′-CCA GAA ACC GCT ATG AAG TTC CT-3′ (sense) 5′-CAC CAG CAT CAG TCC CAA GA-3′ (antisense)
TNF-α	5′-ATC CGC GAC GTG GAA CTG-3′ (sense) 5′-CAG CTC ATA TGG GTC CGA CA-3′ (antisense)