Orientin Attenuates Cerebral Ischemia/Reperfusion Injury in Rat Model through the AQP-4 and TLR4/NF-κB/TNF-α Signaling Pathway

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Background: Orientin has been reported to have extensive pharmaceutical effects of antioxidant, anti-inflammatory, antithrombosis, antiapoptosis, and so on. In the present study, we tried to investigate the protective effects of orientin on cerebral ischemia-reperfusion (I/R) injury and explored the possible mechanisms. *Methods:* Middle cerebral artery occlusion rat model was established and then treated with low, middle, and high concentrations of orientin, respectively, with edaravone as a positive control. The treatment effect of orientin was evaluated by measuring the neurological deficit score, cerebral infarction, brain edema, oxidative stress, excitatory amino acids release, the expression levels of aquaporin-4 (AQP-4), and related inflammatory molecules using different methods including immunohistochemistry, enzyme-linked immunosorbent assay, real-time PCR, and western blot. Moreover, morphological and structural changes were also observed by hematoxylin-eosin staining and transmission electron microscope. *Results:* Orientin provided a significant reduction on neurological deficits, cerebral infarction, cerebral edema, oxidative damage, and neurotoxicity of excitatory amino acids compared to model group ($P < .05$) in a dose-dependent manner. In addition, orientin substantially downregulated AQP-4 and inflammatory factors expression ($P < .05$) and improved cell morphology and structure in rats following I/R injury. *Conclusion:* Orientin was able to mediate noticeable protection against cerebral I/R injury through the attenuation of oxidative stress and neurotoxicity of amino acids and inhibiting the upregulation of AQP-4 and inflammatory cytokines. **Key Words:** Orientin—ischemia-reperfusion—oxidative stress—AQP-4—amino acids—anti-inflammation.

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Received March 5, 2017; revision received April 30, 2017; accepted May 5, 2017.

Grant support: This study was supported by grants from the Major Scientific Projects of Hebei North University (No. ZD1314) and Innovation Fund Project of Graduate Student of Hebei Province (No. 2015-203).

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1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2017.05.002

Introduction

Ischemic cerebrovascular disorder is one of the most common diseases leading to high mortality and severe long-term disability worldwide, which gravely threatens human health and has become an important medical problem to be urgently solved.[1] Cerebral ischemia due to loss of blood supply induces brain damage and results in destructive neurological sequelae along with a variety of alterations.[2] Although utilizing thrombolysis agent is recognized as an effective approach to be capable of managing this disease, the therapy has its disadvantage of time limitation or unexpected adverse side effects.[3] Moreover, the immediate recovery of blood flow after cerebral ischemia may induce secondary injury, namely ischemia-reperfusion (I/R) injury.[4]

Currently, numerous studies proposed that the factors including oxidative stress, blood–brain barrier (BBB) dysfunction, glutamate (Glu) neurotoxicity, inflammatory response, and cell apoptosis are involved in brain I/R injury.[5-9] These factors interact with each other and affect themselves and then form the pathological cascade reaction and cause cell apoptosis or death. Previous studies displayed that cerebral edema is rapidly developed in brain I/R model and the destruction of BBB integrity is caused by edema.[10] Aquaporin-4 (AQP-4) is a water channel protein with the capacity of mediating water through BBB and regulating water accumulation in brain.[11] In addition, numerous inflammation-associated factors including membrane toll-like receptor 4 (TLR4) and nuclear factor kappa B (NF-κB) participate in I/R-induced brain damage.[12] TLR4 could activate transcription factor NF-κB, which transfers into nucleus and regulates the expression of inflammatory genes, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). Preinflammatory cytokine TNF-α could in turn stimulate the activation of NF-κB and aggravate inflammatory damage.[13]

Orientin is a monomer of flavonoid C-glycosides and the main active component in Chinese traditional medicines, such as *Trollius chinensis* Bunge and *Polygonum orientale* L.[14,15] Several reports indicate that orientin has wide pharmacological activities including anti-inflammation, antithrombosis, and antioxidation.[16-18] Our previous studies have shown that orientin provides health benefit on human erythrocytes and D-galactose-aged mice.[19,20] Pharmacokinetic studies in rabbits display that distribution and elimination of orientin were fastest with no accumulative toxication.[21] However, the protective effect of orientin on brain ischemic rats has not been reported. This study investigated the neuroprotective effects and possible mechanisms of orientin on cerebral I/R injury using middle cerebral artery occlusion (MCAO) rat model to provide some novel therapeutic targets for ischemic cerebrovascular diseases.

Materials and Methods

Preparation of Animals and Drug Solutions

Adult male Sprague-Dawley rats, weighing 200 ± 20 g, were obtained from Beijing HFK Bioscience Co., Ltd (Beijing, China). Animal permit number was SCXK (Beijing) 2014-0004. The procedures of experimental animals complied with the Guide for the Care and Use of Laboratory Animals. The rats, with access to water and food ad libitum, were reared in the same room at humidity of 50% ± 2% and temperature of 24°C ± 2°C with a 12-hour light-dark cycle. All of them were kept for a 1-week acclimation period before initiation of the experiment.

Orientin (2.9 mg) was dissolved in 100 μL of N,N-dimethylformamide and Tween-80 (v/v = 1:1), and diluted to 6.48 μmol·kg^{-1} with normal saline (1.0 mL) as stock solution. Then the stock solution was further diluted to different concentrations (1.62 and 3.64 μmol·kg^{-1}) of orientin with normal saline. Edaravone solution was prepared by using the same procedure as orientin.

MCAO Model Establishment

MCAO model was established in this study as previously described by Longa et al.[22] with slight modifications. Briefly, the experimental rats under anesthesia were placed in a supine position, and then an incision was made on the right neck of the rats to expose the right common carotid artery, external carotid artery, and internal carotid artery (ICA). A focal cerebral ischemia was conducted in the ICA with a 50-mm nylon monofilament, which was pulled out carefully to induce reperfusion 2 hours later. During the whole process, the rectal temperature of rats was maintained almost at 37°C using a heating pad. The same procedure was performed in sham rats except the ones that no nylon filament was inserted into the right carotid artery to induce brain ischemia and reperfusion.

Experiment Grouping and Administration

One hundred and ninety-two experimental rats were equally divided into 6 groups, 32 for each group: sham group; model (I/R) group; 3 groups with low, middle, and high doses (1.62, 3.24, and 6.48 μmol·kg^{-1}) of orientin and 3.24 μmol·kg^{-1} of Edaravone as a positive control group. After 1 hour of reperfusion, the treated rats were performed intraperitoneal injections of 1.0 mL of different concentrations of orientin and edaravone solutions, respectively. The rats in sham group and model group were administrated the same volume of normal saline including 100 μL of N,N-dimethylformamide and Tween-80. The administrated times were 24 and 72 hours (once a day).

Assessment of Neurological Deficit Score

After 24 and 72 hours of reperfusion, all rats were evaluated for neurological deficits using a 5-point scoring system