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Original article

Protective effect of chlorogenic acid on the focal cerebral ischemia reperfusion rat models

Mingsan Miao *, Lihua Cao, Ruiqi Li, Xiaoyan Fang, Yanyan Miao

Department of Pharmacology, Henan University of TCM, Zhengzhou 450046, China

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ABSTRACT

Objective: The aim of the study was to investigate the protective characteristic of chlorogenic acid, a natural glucosyl xanthone found in Lonicera Japonica on the cerebral ischemia reperfusion injury and the underlying mechanism.
Methods: Focal cerebral ischemia reperfusion model was built by blocking the left middle cerebral artery in rats by using the suture-occluded method. Before operation, the corresponding drugs were given for each group once a day for 7 days. After 1 h of final administration, the model was built, after operation, reperfusion was conducted for 22 h, Before the reperfusion 10 min tail vein injection of large, medium and small dose of chlorogenic acid and then mortality was calculated, and Neurological deficit score (NDS) was conducted, and serum was collected to measure the NSE level; a 2 mm thick brain slice located at the intersection of optic nerves was collected for TTC staining, and the percentage of cerebral infarction area was calculated; brain homogenate was collected to measure the ICAM-1, VCAM-1, EPO and HIF-1α levels in brain tissue of cerebral ischemia reperfusion rat models; NGF was detected using immunohistochemical method; the morphological changes in brain tissue was observed with HE staining.
Results: All focal cerebral ischemia reperfusion rat models were duplicated successfully. Every chlorogenic acid group with different dosage can significantly reduce the mortality, NDS and cerebral infarction area of rats, and significantly increase the EPO, HIF-1α and NGF levels in brain tissue; significantly improve the pathological lesions of hippocampus and cortex in brain tissue.
Conclusion: The results showed that chlorogenic acid could protect the focal cerebral ischemia reperfusion injury rat models by adjusting the inflammatory factor, hypoxia factor and nerve growth factor.

1. Introduction

Cerebral ischemia is a common disease in the elderly, with high mortality and morbidity, severely threatening human health. Cerebral ischemia reperfusion injury refers to the phenomenon that after blood supply is restored after a certain time of cerebral ischemia, its function not only fails to restore, but more serious brain dysfunction appear (Zhu and Wang, 2010; Thomas et al., 2013). The effective ingredients of Chinese traditional medicine and single Chinese traditional medicine can prevent and treat various diseases through acting on the multiple links and multiple targets by using multiple ways, and have achieved some success in reducing the cerebral ischemia reperfusion injury, can reduce the injury caused by the reperfusion, showing the advantages of traditional Chinese medicine for prevention and treatment of cerebral ischemia reperfusion injury (Jean et al., 2016; Abbas et al., 2017). "Activating blood to resolve stasis" is a common method for clinical prevention and treatment of cerebral ischemic injury, with remarkable effect, has been widely recognized, "Clearing away heat and removing toxin" is a new viewpoint of traditional Chinese medicine for prevention and treatment of cerebral ischemia injury, in microcirculation and clinical research fields, traditional Chinese medicine and modern medicine recognize each other in brain ischemia injury, blood-activating and stasis-resolving medicine focuses on improving brain circulation, protecting nerve cells, and scavenging free radical. Heat-clearing and toxicity-removing medicine focuses on reducing inflammatory response, activating the self-protection mechanism of brain cell, and reducing the secondary reaction of cerebral ischemia. The application of "Activating

* Corresponding author.
E-mail address: miaomingsan@126.com (M. Miao).
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blood to resolve stasis" combined with "Clearing away heat and removing toxin" methods for prevention and treatment of cerebral ischemic reperfusion injury allows two theories of traditional Chinese medicine and modern medicine to find the best combination point in clinical practice (Cheng et al., 2012; Ghafar et al., 2017) Preliminary study (Cao et al., 2016; Fang et al., 2016a,b; Miao et al., 2013) finds traditional Chinese medicines with heat-clearing and toxicity-removing and blood-activating and stasis-resolving effects, such as Turmeric, rabdosia rubescens, Maodongqing, trumpet creeper, beggarticks and motherwort, all of which have good effect on resisting cerebral ischemia injury.

Honeysuckle is a commonly used heat-clearing and toxicity-removing medicine, with effects of clearing away heat and removing toxin, quickening the blood and dispersing swelling, anti-inflammation, and tonifying deficiency and treating wind, having obvious effect on the treatment of fullness and ventral disease, warm disease and fever, heat-toxin and large carbuncle, tumor and other disorders. Chlorogenic acid is a kind of phenylpropanoid compound, and the main active ingredient of honeysuckle (Song et al., 2015), having a wide range of biological activities, such as antibacterial, anti-inflammation, antiviral, scavenging the free radicals, reducing blood fat, lowering blood pressure, protecting the liver and gall bladder, anti-tumor.

Studies have shown that chlorogenic acid can achieve anti-inflammatory effect so as to play a role in anti-inflammatory effect by inhibiting the activation of inflammatory factors such as TNF-α and IL-6, affecting the metabolism of AA (Feng et al., 2016), or reducing the level of NF-κB p65 (Guo et al., 2015; Song et al., 2015). Chlorogenic acid, as a kind of phenolic antioxidant, has a certain number of R-OH radicals, which can form the hydrogen free radical with antioxidant activity, and eliminate the activity of free radicals such as superoxide anion, thereby protecting tissue cells from oxidative damage (Zhang et al., 2010). Therefore, chlorogenic acid can create a protective effect on the red blood cell hemolysis and DNA oxidation, so as to work as an antioxidant by capturing free radical cations ABTS+ and DPPH, stabilizing cell membrane and other mechanisms (Wang et al., 2011,). In addition, chlorogenic acid has a good protective effect on the cardiovascular system, and preventive and therapeutic effects on the atherosclerosis, thromboembolic disease, hypertension and other diseases (Lee et al., 2012).

In summary, it can be inferred that chlorogenic acid may have certain effects on the protection of brain tissues and alleviation of cerebral ischemia reperfusion injury. By observing the intervention effect of chlorogenic acid on the focal cerebral ischemia reperfusion rat models, conducting an in-depth study of its pharmacological effect on the cerebral ischemia reperfusion injury, this paper defined the intervention effect and mechanism, laying the foundation for the follow-up study with a view to developing a new drug which could alleviate the cerebral ischemia reperfusion injury.

2. Material and methods

2.1. Animals, drugs and reagents

96 Wistar rats, SPF, male, weight of 230–250 g, provided by Shandong Lukang Pharmaceutical Co., Ltd., certificate number: 0016029; laboratory certificate number: SYXK (Yu) 2010-001.

Chlorogenic Acid, Chengdu Mansite Pharmaceutical Co., Ltd., content of 99.39%, NO. MUST-13031401; Nimodipine Tablets, Shanxi Yabao Pharmaceutical Group Co., Ltd., NO. 130150; ELISA Kit for the detection of ICAM-1, R&D Systems, NO. 20131202B; ELISA Kit for the detection of VCAM-1, R&D Systems, NO. 20131202B; ELISA Kit for the detection of EPO, R&D Systems, NO. 20131202B; ELISA Kit for the detection of HIF-1α, R&D Systems, NO. 20131202B; ELISA Kit for the detection of NSE, R&D Systems, NO. 20131202B.

2.2. Model building and administration

96 Wistar rats, SPF, male, weight of 230–250 g, were normally fed for 3 days. Then they were randomly divided into 6 groups, i.e. sham-operated group, model group, Nimodipine group, low, medium and high dosage of chlorogenic acid groups according to body weight, 16 Wistar rats per group. Intragastric administration of 1 ml/100 g Nimodipine suspension was carried out for the Nimodipine group (positive control drug, dosage of 20 mg/kg, equivalent to 10 times the clinical dosage, drug with a concentration of 2 mg/ml was made with 0.5% CMC before use); intragastric administration of chlorogenic acid suspension was carried out for the low, medium and high dosage of chlorogenic acid groups (dosage of 60 mg/kg, 30 mg/kg and 15 mg/kg, drugs with a concentration of 6 mg/ml, 3 mg/ml and 1.5 mg/ml were made with 0.5% CMC before use); intragastric administration of the same volume of 0.5% CMC was carried out for the sham-operated group and model group, administered once a day for 7 days.

At 8 p.m. of the 6th day, fasting without water deprivation in batches, at 8 a.m. of the 7th day, they were weighted in batches, after 1 h of administration, intraperitoneal injection of 10% chloralic hydras (0.3 ml/100 g) was carried out for anesthetized rats, they were incised in the left of center of the neck, separated layer by layer to expose left CCA, ECA and ICA, CCA and ECA were ligated, ICA was blocked with artery clip, a small 0.2 mm wide opening was sheared at the place where CCA was 5 mm from bifurcation, the suture was inserted, which entered into ICA through CCA bifurcation, and penetrated upward 18–20 mm above CCA bifurcation until there was resistance, namely, the entrance of middle cerebral artery was blocked, the ICA incision and suture were ligated, after 2 h, the suture was taken out lightly, reperfusion was realized, and MCAO reperfusion model was built, for the sham-operated group, only the left-side blood vessels were exposed, no suture inserting treatment.

2.3. Detection index

After 22 h of reperfusion for all rats was conducted, NDS was conducted for rat models: scoring was performed using the Longa standard. Scoring standard: 0 score: no NDS, normal activity; 1 score: not fully extending the forepaws; 2 scores: turning around on the hemiplegic side appear when crawling; 3 scores: body dumping to the hemiplegia side when walking; 4 scores: unable to walk spontaneously, loss of consciousness; 5 scores: death. The rats scoring 0 and 5 were excluded; eyeballs were removed and blood was collected, after standing for half an hour, it was centrifuged for 10 min at 3500 r/min, serum was collected, and NSE level was measured according to the kit instructions; after the rats were decapitated and killed, their brain tissues were taken off rapidly, their cerebellum, olfactory bulb and the remainder of the lower brain stem were removed, a 2 mm thick brain slice at the intersection of optic nerves was removed rapidly, and placed in 1% TTC dyeing solution made from phosphate buffer solution with pH = 7.2 for incubation for 10 min using 37 °C water bath without light. After being taken out, it was placed in 10% formalin for preservation away from light for 24 h. After dyeing, non-ischemic area was rosy and infarction area was white. The pictures were taken using a digital camera, the total area of the brain slice and the area of the infarction area were calculated by the image analysis software respectively, and the percentage of infarction area to total area was calculated. The front left side brain at the intersection of optic nerves was used to make 10% brain homogenates,