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ORIGINAL ARTICLE

Intervention action of total flavonoids from root of *Ilex pubescens* in cerebral ischemic tolerance with blood stasis

Mingsan Miao *, Lihua Cao, Kun Xu, Weiyun Xin, Yan Zheng

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Henan University of Chinese Medicine, Zhengzhou 450000, China

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KEYWORDS

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Expression of Bcl-2 protein;
Expression of Bax protein

Abstract The aim of this study was to explore the action characters of total flavonoids from MDQ on cerebral ischemic tolerance with blood stasis. Fully understanding the mechanism of action of total flavonoids from MDQ is helpful for the development of new drugs and the utilization of resources. Male Wistar rat model of blood stasis was established by injecting dexamethasone into the intramuscular side of the thigh. Then they were given related drugs via an intragastric administration for a successive 10 days. After 7 days, the following occurred: firstly, the method of blocking the bilateral common carotid artery (CCA) was used for 10 min, followed by a restoration of perfusion. After 72 h, we performed a temporary occlusion of the rat's middle cerebral artery for 2 h with an intraluminal thread method. This was followed by reperfusion for 24 h, respectively, to establish the rat model of cerebral ischemic tolerance with blood stasis. Viscosity of the whole blood was measured after the last administration was given blood. Brain was removed, and then the activity of ATP enzyme and T-SOD was determined. To observe the pathological changes of the hippocampus area by HE staining, and the expression of Bcl-2 and Bax were observed by immunohistochemical method. The rat model of cerebral ischemic tolerance with blood stasis was copied successfully. The whole blood viscosity, the activity of NOS, the content of Gluin in the ischemic brain in the IPC model group and the ischemia–reperfusion group were increased significantly. The activity of ATPase was decreased significantly. Compared with the ischemia–reperfusion model group, the activity of ATPase and the whole blood viscosity in the ischemic preconditioning (IPC) group were increased significantly. The activity of NOS and the content of Gluin were decreased significantly. The degree of pathological injury of the brain tissue was also relieved significantly. Total flavonoids of MDQ were used, improving blood circulation, improving

* Corresponding author.
E-mail address: miaomingsan@163.com (M. Miao).
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energy metabolism, activating endogenous anti-oxidative capability, enhancing the antiapoptotic effect, and relieving the injury of the nerve cell. Hence, the use of MDQ flavonoids improves the tolerance ability of cerebral ischemia.

1. Introduction

In China, the dried root of *Ilex pubescens* Hook. et Arn. (MDQ) aqueous extract can be used for parenteral administration for the treatment of cardiovascular diseases. At this point most research is geared toward the exploration of the pharmacological study and clinical application of the MDQ extract. There is only a small part of the total flavonoids of research. The mechanism research and application research have many contents that remain to be studied further.

MaoDongqing (MDQ) is the dried root of *Ilex pubescens* Hook. et Arn. It belongs to the family of Aquifoliaceae. The effect of MDQ is promoting blood circulation, relieving pain, and detoxification (Hao and Yang, 2010). It is a Chinese herbal medicine commonly used in Southern China. Its root has been well-known for its medicinal use in treating cardio cerebral, vascular and arterial thrombosis diseases (Wang, 2006; He and Qiu, 2010) such as stroke, coronary arterial thrombosis, thromboangiitis obliterans, and thrombophlebitis (Zheng et al., 2006). In addition, it has often been used for alleviating upper respiratory infections and other inflammatory diseases. Pharmacological studies showed that extracts of MDQ could significantly enlarge blood vessels, improve blood microcirculation, lower blood pressure, inhibit platelet aggregation (Jiang et al., 2005), prevent thrombosis, and reduce cardiac ischemia .It could also protect brain tissue and has anti-inflammatory properties. Previous studies showed that the total flavonoids from MDQ could improve the cerebral homogenate ATP enzyme activity, decrease the content of LD and reduce the MDA content. It could also reduce cerebral injury caused by ischemia. Furthermore, it had a good effect on the cerebral ischemia of the rat model (Cheng et al., 2012a,b; Miao, 2009). Preliminary experiments have found a more mature extraction process that can be used to purify the total number of flavonoids (Xu et al., 2011). This experiment observed the effect of total flavonoids from MDQ on hemorheology, brain tissue energy metabolism, SOD activity and apoptosis, as well as the anti-apoptotic gene in the rat models of cerebral ischemic tolerance with blood stasis.

2. Material and methods

2.1. Drug agents

Total flavonoids from MDQ (52% of content provided by analytical chemistry laboratory, Henan University of Chinese Medicine, batch number: 20101101); Extract of Ginkgo Biloba Leaves Tablets (Jin Naduo) (DR Willmar Schwabe production, batch number: 0601209); Dexamethasone Sodium Phosphate Injection (DX) (Lianshui Jiangsu Pharmaceutical Co., Ltd. production, batch number: 0912253); Sodium Chloride Injection (Zhengzhou Yonghe Pharmaceutical Co. Ltd., batch number: 10062121); Coomassie blue protein determination kit (batch number:20101214); ATP Adenosine-triphosphate detection reagent box (batch number:20101215); SOD (superoxide dismutase) detection reagent box (batch number: 20101213), both provided by Nanjing built biological engineering institute.

2.2. Instrument

LBY-N6A type rotary viscometer (Beijing precil group); 75-2 Spectrophotometer (Shanghai Third Analytical Instrument Factory).

2.3. Animals

Clean grade male Wistar rats, weight 280 g ~ 300 g, provided by Hebei experimental animal center, certificate number 1010133.

2.4. Methods

A total of 112 male, Wistar rats were randomly divided into 8 groups. They were given large, medium and small doses of total flavonoids from MDQ (0.2 g/kg, 0.1 g/kg, 0.05 g/kg; 10 mg/ml, 5 mg/ml, 2.5 mg/ml). The positive control drug, Ginaton, was given to one group (0.02 g/kg, 1 mg/ml). There was also a cerebral ischemia reperfusion injury group, a cerebral ischemic tolerance group, a blood stasis sham operation group, and a non-blood stasis sham operation group. The rat model of blood stasis was established by an intramuscular injection of dexamethasone (0.2 $mg{\cdot}kg^{-1}\ d^{-1}$) in the side of the thigh. Then they were given related drugs via an intragastric administration for successive 10 days. The Cerebral ischemia reperfusion injury group, cerebral ischemic tolerance group, blood stasis sham operation group and non blood stasis group were treated with the same volume of 0.1% sodium carboxymethyl cellulose. The volume of perfusion was 2 ml/100 g and the positive drug group was prepared with 0.1% (sodium carboxymethyl cellulose) CMC solution.

The non-blood stasis sham operation group was intraperitoneally (ip) injected with an equal volume of normal saline. The rest of the 7 groups were injected with dexamethasone 0.2 mg/kg (1 mL/kg) on the hind leg muscles and given related drugs once per day using the method of intragastric administration. After 7 days the following occurred. Firstly, we used the method of blocking the bilateral common carotid artery (CCA) for 10 min (Fang et al., 2010, 2009), and then restored perfusion. After 72 h, fasting occurred for 12 h. In the last hour of fasting we used the method of blocking the bilateral CCA. Then we used a thread to occlude the left middle cerebral artery for 2 h, followed by reperfusion for 24 h, respectively, to establish the rat model of cerebral ischemic tolerance with blood stasis.