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Neuroprotective effects of emodin-8-*O*-β-D-glucoside *in vivo* and *in vitro*

Chaoyun Wang [a], Dalei Zhang [b], Hongmei Ma [c], Juntian Liu [a,*]

[a] *Xi'an Jiaotong University School of Medicine, Xi'an Shaanxi 710061, PR China*
[b] *National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, PR China*
[c] *China Agriculture University, Yantai 264002, PR China*

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Abstract

Emodin-8-*O*-β-D-glucoside extracted from the traditional Chinese medicinal herb *Polygonum cuspidatum* Sieb. et Zucc is widely used to treat acute hepatitis possibly by antioxidative mechanisms. The present study was designed to investigate whether emodin-8-*O*-β-D-glucoside exerted neuroprotective effects on the focal cerebral injury induced by ischemia and reperfusion *in vivo* and on the neuronal damage induced by glutamate *in vitro*, and to study the possible mechanisms. Male Wistar rats were used to establish the model of ischemia and reperfusion. The behavioral test was performed and the cerebral infarction area was assessed in the brain slices stained with 2% 2,3,5-triphenyl tetrazolium chloride to evaluate the neuroprotective effects of emodin-8-*O*-β-D-glucoside. Superoxide dismutase (SOD) activity, total antioxidative capability and malondialdehyde (MDA) level in the brain tissue were determined with spectrophotometrical methods to probe the primary mechanisms of emodin-8-*O*-β-D-glucoside. *In vitro*, the neuroprotective effects of emodin-8-*O*-β-D-glucoside were tested in the cultured cortical cells of fetal rats exposed to glutamate. Emodin-8-*O*-β-D-glucoside concentration in plasma and brain tissue was also measured to examine distribution of emodin-8-*O*-β-D-glucoside in the brain. The results showed that the treatment of rats with emodin-8-*O*-β-D-glucoside reduced the neurological deficit score and the cerebral infarction area, increased SOD activity and total antioxidative capability, and decreased MDA level in the brain tissue in dose-dependent way. Emodin-8-*O*-β-D-glucoside also inhibited the neuronal damage induced by glutamate. Besides, emodin-8-*O*-β-D-glucoside was able to penetrate blood-brain barrier and distribute in the brain tissue. These findings demonstrate that emodin-8-*O*-β-D-glucoside is able to provide neuroprotection against cerebral ischemia-reperfused injury and glutamate induced neuronal damage through exerting antioxidative effects and inhibiting glutamate neurotoxicity.

Keywords: *Polygonum cuspidatum* Sieb. et Zucc; Emodin-8-*O*-β-D-glucoside; Ischemia and reperfusion; Neuroprotection; Lipid peroxidation

1. Introduction

Polygonum cuspidatum Sieb. et Zucc (*Huzhang* in Chinese), a plant genus of the family Polygonaceae, is a well-known traditional Chinese medicine recorded in Chinese Pharmacopoeia. At present, it is mainly used for the treatment of dermatitis and abscess in clinical practice. Emodin-8-*O*-β-D-glucoside is one of the major components of *P. cuspidatum* and used to treat acute hepatitis (Chen et al., 2000). Previous researches show that reactive oxygen species interact with other signal pathways to regulate hepatocyte apoptosis and proliferation (Purohit and Brenner, 2006; Schwabe and Brenner, 2006), and some antioxidants are able to effectively alleviate symptoms of hepatitis (Levent et al., 2006; Medina and Moreno-Otero, 2005), suggesting that emodin-8-*O*-β-D-glucoside possibly possesses antioxidant activity.

It has been known that reactive oxygen species also participate in the pathogenesis of cerebral injury induced by ischemia and reperfusion, and oxygen free radicals are elevated during cerebral ischemia and reperfusion because of the failure of metabolic reactions (Love, 1999). Antioxidant defenses including free radical scavengers and antioxidative enzymes are considered as the most effective means at attenuating the tissue injury induced by ischemia and reperfusion (Papadopoulos et al., 1998; Truelove et al., 1994). Besides, glutamate plays an

* Corresponding author. Department of Pharmacology, Xi'an Jiaotong University School of Medicine, Xi'an, Shaanxi 710061, PR China. Tel./fax: +86 29 82655188.
E-mail addresses: ytwcy@163.com, ljt@mail.xjtu.edu.cn (J. Liu).

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important role in promoting ischemic cerebral injury. Excessive glutamate causes an intracellular cation imbalance followed by generation of reactive oxygen species, inflammation and cell death via receptor-mediated way (Gunasekar et al., 1995; Schwabe and Brenner, 2006). Blockade of glutamate receptors is able to significantly improve cell injury induced by glutamate through inhibiting generation of reactive oxygen species and inflammatory response (Gunasekar et al., 1995). So, it is interesting to know whether emodin-8-*O*-β-D-glucoside has a neuroprotective effect on ischemic cerebral injury and to investigate its possible mechanisms.

2. Materials and methods

2.1. Plant material

Emodin-8-*O*-β-D-glucoside (Fig. 1) from *P. cuspidatum* was the red amorphous powder with the purity of more than 98% by HPLC (provided by Dr. Dalei Zhang). Its chemical structure (MF: $C_{21}H_{20}O_{10}$) was consistent with other studies by UV, ^{1}H and ^{13}C NMR (Chu et al., 2005; Yang et al., 2001).

2.2. Animal model

Male Wistar rats weighing 300–350 g, from the Experimental Animal Center of Shandong (clean grade, Certificate No. 20020305), were housed in a cage of two to three at 22–24 °C in a 12 h/12 h light/dark cycle with pellet food and tap water. The animal experiment was carried out according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the local authorities. Rats were randomly divided into 7 groups with 13 animals in each group: sham control; model control; emodin-8-*O*-β-D-glucoside-treated groups (2.5, 5, 10 mg/kg) and vitamin C-treated group (20 mg/kg as a positive control, vitamin C from Hulin, P. R. China). All drugs were administered via tail vein injection at 15 min after the occlusion. Rats in sham control and ischemia-reperfusion model groups received vehicle by the same way (0.9% NaCl, pH > 8.0).

Focal cerebral ischemia was produced by a modification of the monofilament method as described previously (Mhairi Macrae, 1992; Longa et al., 1989). Briefly, rats were anesthetized with10% chloral hydrate in 0.9% NaCl (350 mg/kg, i.p.) and placed in dorsal recumbency. Under the sterile condition, a ventral neck incision was made and the external carotid artery and the internal carotid artery were exposed and carefully isolated. A nylon monofilament (40 mm in length and 0.24 mm in diameter) was inserted from the lumen of the right external carotid artery to that of the right internal carotid artery to occlude the origin of the right middle cerebral artery. The sham operated rats received all surgical procedures but without the suture inserted. During the operation, body temperature was monitored with a rectal probe and maintained in the normal range with a heating lamp and a heating pad. The rats with the abnormal blood pressure and temperature were discarded. After the occlusion for 2 h, the nylon suture was withdrawn for reperfusion. Then, the rats were returned into their home cage with free access to food and water. Body temperature was continually maintained within the normal range.

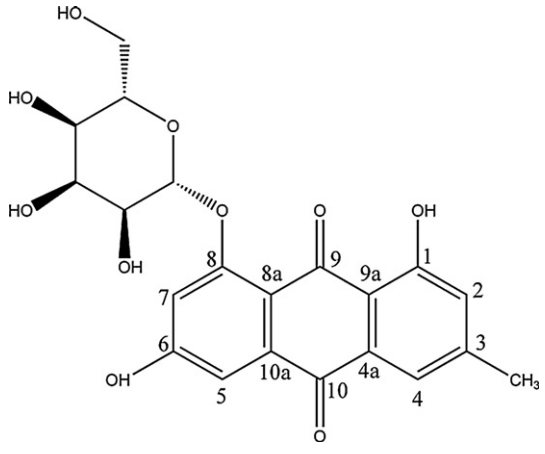

Fig. 1. Chemical structure of emodin-8-*O*-β-D-glucoside.

2.3. Evaluation of neurological deficit

After the reperfusion for 24 h, the neurological deficit of each rat was evaluated according to Longa's method of a 5-point scale (Longa et al., 1989) by the same experimenter, who was blinded to the different treatments in the experiment (no neurological deficit = 0, failure to extend right paw fully = 1, circling to right = 2, falling to right = 3, being unable to walk spontaneously and depression of consciousness = 4).

2.4. Assessment of cerebral infarction area

Following the neurological deficit evaluation, the brain of rats was carefully removed and cut into 5 slices of 2 mm thickness. Then, the brain slices were stained in 2% solution of 2,3,5-triphenyl tetrazolium chloride (TTC, St. Louis, MO, USA) at 37 °C for 10 min and photographed to separately determine the infarcted and total areas of each hemisphere on a Compix System Computer (C Imaging 1280 System, Compix Inc Image System).

2.5. Determination of SOD activity, total antioxidative capability and MDA level in the brain tissue

After the reperfusion for 24 h, the ischemia-reperfused brain hemisphere was removed, blotted and weighed, and then homogenized in 0.1 M phosphate buffer solution (pH 7.4). The homogenate was centrifuged at 3000 ×*g* and 4 °C for 30 min, and the supernatant was used to spectrophotometrically determine malondialdehyde (MDA) level at 532 nm, superoxide dismutase (SOD) activity at 550 nm and total antioxidative capability at 520 nm according to the procedures provided by the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, PR China). Measurement of SOD is based on catalysis of the oxidation of hydroxylamine to nitrite by superoxide anion ($O_2 \cdot -$) from xanthine–xanthine oxidase and then, the nitrite reacts with color developing reagent to form a purple compound. MDA was assayed by the method of Buege and Aust (1978), i.e. the TBA method, which is based on the reaction of MDA with thiobarbituric acid to form a pink chromogen. Total antioxidative capability reflects