ELSEVIER

Pharmacological Research 53 (2006) 303–309

Pharmacological research

www.elsevier.com/locate/yphrs

# Protective effects of isoliquiritigenin in transient middle cerebral artery occlusion-induced focal cerebral ischemia in rats

Chun Zhan, Jing Yang *

*Department of Pharmacology, School of Medicine, Wuhan University, Donghu Road, Wuhan 430071, China*

Accepted 23 December 2005

**Abstract**

Epidemiological studies indicate that the intake of flavonoids is inversely associated with risk of stroke, cardiovascular diseases and cancer. Isoliquiritigenin (ISL), a flavonoid constituent in the root of *Glycyrrhiza glabra*, is known to have vasorelaxant effect, antioxidant, anti-platelet, anti-tumor, anti-allergic, antiviral activities and estrogenic properties. However, there is no report on the effects of ISL in cerebral ischemia. Evidence demonstrate that the impaired energy metabolism and the excessive generation of reactive oxygen radicals (ROS) contribute to the brain injury associated with cerebral ischemia. In the present study, the protective effects of ISL were investigated in transient middle cerebral artery occlusion (MCAO)-induced focal cerebral ischemia–reperfusion injury in rats. Male Sprague–Dawley rats were divided into five groups: sham-operated group, vehicle-pretreated group, and three ISL-pretreated groups (5, 10 and 20 mg $kg^{-1}$, i.g.). ISL were administered once a day, for 7 days prior to ischemia. The rats were subjected to 2 h right MCAO via the intraluminal filament technique and 22 h reperfusion. Pretreatment with ISL significantly reduced the cerebral infarct volume and edema and produced significant reduction in neurological deficits. In this study, in order to clarify the mechanism of ISL's protection against cerebral ischemia damage, cerebral energy metabolism, brain $Na^+K^+$ATPase activity, malondialdehyde (MDA) content and antioxidant enzyme activities were measured. ISL pretreatment increased the brain ATP content, energy charge (EC) and total adenine nucleotides (TAN) in a dose-dependent manner. The brain $Na^+K^+$ATPase activity was protected significantly by pretreatment of ISL for 7 days. Pretreatment with ISL significantly inhibited the increases of brain MDA content and prevented the activities of brain superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) from declines caused by cerebral ischemia–reperfusion. All these findings indicate that ISL has the protective potential against cerebral ischemia injury and its protective effects may be due to the amelioration of cerebral energy metabolism and its antioxidant property.
© 2006 Published by Elsevier Ltd.

*Keywords:* Isoliquiritigenin; Cerebral ischemia; Energy metabolism; Antioxidant

## 1. Introduction

Cerebral ischemia or stroke is a major cause of death and the primary cause of adult disability in many countries. Human cerebral ischemia most often results from a transient or permanent occlusion of the middle cerebral artery (MCA). The brain has an absolute dependence on the blood for its immediate supply of oxygen and energy substrates. Following interruption of the cerebral blood flow, the depletion of energy stores results in several acute metabolic disturbances, such as disruption of ion homeostasis, massive release of excitotoxic amino acids and free radical formation [1]. Energy-rich compounds, ATP in particular, are necessary to maintain cellular structure and such cellular functions as active transport, protein synthesis, protein phosphorylation and processing, synaptic transmission and intracellular ion homeostasis [2,3]. ATP depletion has been suggested to be the critical factor in the determination of cell death [4]. ATP is a critical energy source for maintaining the ion pumping of $Na^+K^+$ATPase, which regulates the ionic concentration gradients necessary to generate action potentials by neurons [3]. The $Na^+K^+$ATPase is very sensitive to even small changes in ATP production [5], so that the early and late stages of tissue injury can be studied by measuring its activity. Cerebral ischemia–reperfusion is accompanied by enhanced formation of reactive oxygen radicals (ROS) such as superoxide anion, hydroxyl radical, and hydrogen peroxide in brain tissues. These ROS can be scavenged by endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glu-

* Corresponding author. Tel.: +86 27 68758665; fax: +86 27 68759339.
*E-mail address:* yangjingliu@yahoo.com.cn (J. Yang).

1043-6618/$ – see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.phrs.2005.12.008

tathione peroxidase (GSH-Px). Increased free radical formation coupled with a reduced antioxidant defense has been postulated to play a pivotal role in brain injury associated with stroke [6,7].

Flavonoids are ubiquitous compounds found in a wide variety of edible plants, fruits, vegetables, grains and red wine. They are an integral part of the human diet, and are important and effective constituents of some medicines, especially of Chinese herbal medicines. Currently, there is growing evidence for flavonoids with a wide range of therapeutic activities of high potency and low toxicity. Epidemiological studies have indicated an association between the consumption of flavonoids and a reduced incidence of diseases such as stroke [8], coronary heart diseases [9,10] and cancer [11,12]. *Glycyrrhiza glabra* (family, Leguminosae) is the licorice plant, which are widely used in Chinese medicine and food, and has a history of consumption for the past 6000 years. Isoliquiritigenin (ISL), 2′,4′,4′-three hydroxy chalcone, one of the components in the root of *G. glabra*, is a member of the flavonoids, which has shown various biochemical activities, such as vasorelaxant effect, antioxidant, anti-platelet, anti-tumor, anti-allergic, antiviral activities and estrogenic properties [13–19]. However, the effects of ISL in cerebral ischemia have not been studied. Therefore, in the present study, the protective effects of ISL were evaluated in MCA occlusion–reperfusion rat model, which is considered to more closely mimic the clinical situation [20]. Its possible mechanism of protection was investigated by measuring brain energy metabolites, $Na^+K^+$ATPase activity, malondialdehyde (MDA) content and antioxidant enzyme activities.

## 2. Materials and methods

### 2.1. Extraction and purification of ISL

The roots of *G. glabra* used in this study were collected in Yili, Xinjiang Province, China, and the identification was performed by Dr. Mingxi Jiang of Wuhan Institute of Botany, Chinese Academy of Sciences, Wuhan, China. The air-dried roots of *G. glabra* were crushed into powder and were extracted with ethyl acetate (EtOAc) three times for 12 h each at room temperature. The EtOAc extract was subjected to silica gel column chromatography and further separated to obtain ISL (a yellow amorphous solid) (Fig. 1) as described previously [21]. The identification of ISL was definitively assessed by spectroscopic data as follows: $^1$H NMR (in acetone-d6, 400 MHz) $\delta$ (ppm) 7.76 (1H, d, $J$ = 15.3 Hz, H-α), 7.84 (1H, d, $J$ = 15.3 Hz, H-$\beta$), 7.39 (2H, d, $J$ = 8.5 Hz, H-2 and H-6), 6.94 (2H, d, $J$ = 8.5 Hz, H-3 and H-5), 6.43 (1H, d, $J$ = 2.1 Hz, H-3′), 6.59 (1H, dd, $J$ = 8.8, 2.1 Hz, H-5′), 8.12 (1H, d, $J$ = 8.8 Hz, H-6′), 13.68 (1H, s, HO-2′), 7.73, 7.75 (2H, s, HO-4 and HO-4′). EI-MS $m/z$: 256 ($M^+$), 255, 239, 163, 137, 120, 107. All the data coincide well with the previous reports [22,23].

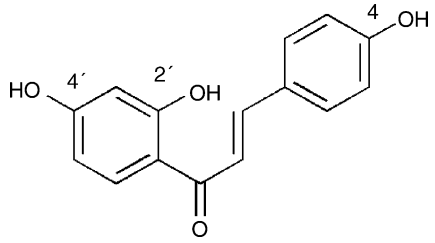


Fig. 1. The structure of isoliquiritigenin (ISL).

### 2.2. Animals and treatments

Male Sprague–Dawley rats weighing 260–270 g were purchased from the Experimental Animal Center of Wuhan University, Wuhan, China. Animals were housed in a room with temperature of 21–25 °C, relative humidity of 50–60%, and a 12-h light/12-h dark cycle (lights on at 07:00 h). They had free access to food and water. All experimental procedures carried out in this study were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Wuhan University, Wuhan, China. Animals were randomly divided into five groups. Rats in ISL-pretreated groups (ischemia–reperfusion + ISL) were given i.g. ISL (5, 10, 20 mg kg$^{-1}$) once a day, for 7 days prior to ischemia. Rats in sham-operated group and vehicle-pretreated group (ischemia–reperfusion + vehicle) were given i.g. 0.5% CMC–saline. ISL was suspended in a 0.5% carboxymenthylcellulose (CMC)-saline solution.

### 2.3. Surgical procedures

After 7 days of pretreatment with ISL, rats were subjected to 2 h right middle cerebral artery occlusion (MCAO) via the intraluminal filament technique [24] and 22 h reperfusion. The rats were anesthetized with chloral hydrate (400 mg kg$^{-1}$, i.p.). The right common carotid artery was exposed at the level of the external and internal carotid artery bifurcation. A 4–0 nylon monofilament, the tip of which was coated with silicone resin/hardener mixture, was inserted into the external carotid artery and advanced into the internal carotid artery for a length of about 18–19 mm until a slight resistance was felt. Such resistance indicated that the filament had passed beyond the proximal segment of the anterior cerebral artery. At this point, the intraluminal filament blocked the origin of the MCA and occluded all sources of blood flow from the internal carotid artery, anterior cerebral artery and the posterior cerebral artery. Body temperature was kept at 37 ± 0.5 °C by a heating pad during the whole procedure. Two hours after the induction of ischemia, the filament was slowly withdrawn until tip reached external carotid artery. Animals were then returned to their cages and closely monitored until they recovered from anesthesia. In sham-operated group, the external carotid artery was surgically prepared for insertion of the filament, but the filament was not inserted.

### 2.4. Neurological deficit

Neurological deficits in the vehicle- and drug-pretreated group were determined after 22 h of reperfusion. Neurological findings were scored on a 5-point scale [24] as follows: no neurological deficit = 0, failure to extend right paw fully = 1, circling

Download English Version:

https://daneshyari.com/en/article/2563022

Download Persian Version:

https://daneshyari.com/article/2563022

Daneshyari.com