European Journal of Pharmacology 636 (2010) 96–101

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural Pharmacology

Osthole improves chronic cerebral hypoperfusion induced cognitive deficits and neuronal damage in hippocampus

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ARTICLE INFO

Article history:
Received 16 November 2009
Received in revised form 22 February 2010
Accepted 14 March 2010
Available online 1 April 2010

Keywords:
Osthole
Chronic cerebral hypoperfusion
Cognition
Oxidative stress
Apoptosis

ABSTRACT

This study is to investigate the effects of osthole on cognitive impairment and neuronal degeneration in hippocampus induced by chronic cerebral hypoperfusion in rats, as well as the potential mechanism. Permanent occlusion of bilateral common carotid arteries (2VO) induced severe cognitive deficits tested by the water maze task, along with oxidative stress and neuronal loss in hippocampus. Oral administration of osthole for 3 weeks markedly attenuated cognitive deficits and neuronal damage. Biochemical experiments revealed that osthole decreased the production of malondialdehyde (MDA) and significantly increased the activities of Glutathione Peroxidase (GPx) and Catalase. Western blot analyses indicated that osthole prevented the downregulation of bcl-2 expression and upregulation of bax expression, which resulted in decreasing bax/bcl-2 ratio in hippocampus of 2VO rats. Additionally, osthole effectively alleviated the activation of caspase-3 induced by permanent occlusion of bilateral common carotid arteries. The observed results in present study suggest that osthole exhibits therapeutic potential for vascular dementia, which is most likely related, at least in part, to its antioxidation and anti-apoptotic actions.

1. Introduction

Chronic cerebral hypoperfusion has been well characterized as a common pathological status contributing to neurodegenerative diseases such as vascular dementia (Hartman et al., 2005; Masada et al., 1997; Pazos et al., 1999). The main clinical outcomes of chronic cerebral hypoperfusion are the cognitive deficits and permanent neural impairment (Sarti et al., 2002). Previous studies have revealed that oxidative injury plays a key role in the pathogenesis of numerous neurodegenerative diseases including stroke, Alzheimer's disease, and vascular dementia, etc. (Chong et al., 2005; Coyle and Puttfarcken, 1993; Markesbery, 1997). Oxygen free radicals and lipid peroxidation may have an aetiological role in the development of lesions induced by chronic cerebral hypoperfusion in the central nervous system. Therefore, antioxidation therapy may be an important strategy for managing vascular dementia.

Osthole (Fig. 1), a component isolated from medicinal plants, such as *Cnidium monnieri* (L.) cusson and *Peucedanum ostruthium*, is a natural coumarin derivative. It exerts a broad spectrum of pharmacological activities including anti-osteoporotic (Kuo et al., 2005; Li et al., 2002; Zhang et al., 2007), anti-proliferative (Chou et al., 2007; Guh et al., 1996), anti-allergic (Chiu et al., 2008; Matsuda et al., 2002), anti-seizure (Luszczki et al., 2009) and antidiabetic (Liang et al., 2009) effects. Besides, the beneficial effects of osthole on learning and memory ability have been explored in recent years. Increasing evidence indicates that osthole ameliorates learning and memory impairment induced by Scopolamine in rodents (Hsieh et al., 2004; Wu et al., 2008) and also ameliorates learning impairment in Kidney-Yang deficiency rats induced by continuous administration of hydrocortisone acetate (Qin et al., 1997). It is well known that hippocampus correlates with learning and memory closely. Most of the neurological deficits, such as behavioral disorders, amnesia and dementia, are associated with structural and functional abnormalities of specific hippocampal neurons or pathway (Amaral and Witter, 1989; Eyre et al., 2003).

In this study, a widely accepted model of chronic cerebral hypoperfusion induced by permanent occlusion of bilateral common carotid arteries in rats was used to evaluate the possible therapeutic potential of osthole on cognitive deficits, and the antioxidation and neuroprotective effects of osthole on the hippocampus of 2VO rats were investigated.

2. Materials and methods

2.1. Drugs

Osthole (purity >98% tested by HPLC) was purchased from the National Institute for the Control of Pharmaceutical and Biological

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doi:10.1016/j.ejphar.2010.03.038

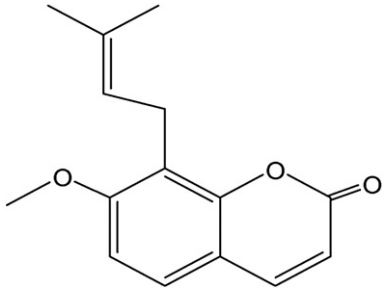

Fig. 1. Chemical structure of osthole.

products (Beijing, China). Osthole was dissolved in olive oil and administrated p.o. at a volume of 5 ml/kg.

2.2. Animals

Wistar rats aged nine weeks (230±20 g) were housed in groups of five per cage at a temperature of 23 ± 1 °C with a 12 h light–dark cycle (light on 7 a.m.–7 p.m.), and had free access to the food and water. All experiments were performed in accordance with the guidelines established by the National Institutes of Health for the care and use of laboratory animals and were approved by the Animal Care Committee of the Peking Union Medical College and Chinese Academy of Medical Sciences(Beijing, China). Behavioral tests including training and probe trials were carried out between 9:00 a.m. and 11:00 a.m.

2.3. Surgical procedure

After rats were anesthetized with choral hydrate (350 mg/kg, i.p.), the bilateral common carotid arteries of the rats were exposed and carefully separated from carotid sheath, cervical sympathetic, and vagal nerves through a ventral cervical incision. The bilateral common carotid arteries were ligated with 4-0 type surgical silk in ischemia rats, whereas not ligated in sham-operated rats. The operation was performed on a heating pad to maintain body temperature at 37.5 ± 0.5 °C, and the animal was kept on the pad until recovery from anesthesia.

2.4. Drug administration and experimental design

In the present behavioral experiment, the Morris water maze was used in two stages. The first stage was carried out from day 26 to day 30 after operation in order to reject the unsuccessful rats. The mean time required to reach the hidden platform of each rat during acquisition stage in consecutive 5 days was calculated. Then the mean time of each 2VO rat was defined as *value 1* and the mean time of all rats in sham group as *value 2*. Screening Criteria (*SC*) was selected as index for evaluating the cognitive deficit of each ischemic rat, $SC = (value\ 1 - value\ 2)/value\ 1$. The rat was considered as cognitive deficit if its *SC* was greater than 0.2 (Zhao et al., 2002). Rats induced by permanent occlusion of bilateral common carotid arteries with a ratio larger than 0.2 were randomly divided into 3 groups: (1) 2VO rats treated with olive oil; (2) 2VO rats treated with osthole 10 mg/kg; (3) 2VO rats treated with osthole 20 mg/kg. The sham–operated rats treated with olive oil were served as Group 4. Each group consisted of 10 rats with identical mean body weights. Daily oral administration of osthole (10, 20 mg/kg) or vehicle (olive oil) started on day 30 post-surgery, and lasted for the termination of the experiment on day 55. The second stage was carried out from day 50 to day 55 after operation to evaluate osthole on improvement of cognitive deficits. Behavioral tests were conducted 60 min after drug administration.

2.5. Morris water maze test

Morris water maze test was performed as previously described (Morris, 1984). The apparatus consisted of a circular water tank 120 cm in diameter and 50 cm in height, filled to a depth of 30 cm with water at 23 ± 1 °C to cover a black platform (10 cm in diameter). The tank was divided into four quadrants called north, east, south and west at equal distances on the rim. The platform was located in the center of the northeast quadrant during training. The top of the platform was approximately 1.5 cm below the surface of water. During the training period of the task, rats were given one trial per day to find the hidden platform for five consecutive days (maximum trial duration 60 s, 20 s reinforcement on the platform). The experimenter conducting the Morris water maze was blinded to the treatment groups. The rats were gently placed into the water, facing the side walls of the maze from one of the four starting position. Swimming paths of the rats were monitored by a video camera linked to a computer through an image analyzer. For training trial, the latency to escape onto the hidden platform and the path length were recorded. The rats were given a maximum of 60 s to find the hidden platform. If the rat failed to find the platform within 60 s, the training was terminated and a maximum score of 60 s was assigned. The rat was then guided to the hidden platform by hand, and it was allowed to stay on the platform for 20 s.

After the last learning trial on training day 5, a probe test, in which the hidden platform was removed, was conducted in the next day. The rats were allowed to swim freely for 60 s in the pool before they were removed from water by hand and a percentage of the time in the target quadrant was recorded as an assessment on spatial memory.

2.6. Biochemical analysis

The oxidation–antioxidation status of the hippocampus subjected to chronic cerebral ischemia was assessed by the level of lipid peroxidation and the activities of Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase. Lipid peroxidation was determined by measuring the levels of malondialdehyde (MDA), a by-product of lipid peroxidation. MDA formation was determined by detecting thiobarbituric-acid reacting substances (Agar et al., 1999). SOD was examined based on its ability to inhibit the oxidation of oxymine by $O2^-$ produced from the xanthine/xanthine oxidase system (McCord and Fridovich, 1988). The determination of GPx activity was performed as previously described (Armstrong and Browne, 1994), and Catalase activity was measured by employing H_2O_2 to generate H_2O and O_2 (Maehly and Chance, 1954). Rats were decapitated 60 min after the probe trial at day 55 post-surgery. The hippocampus was separated on ice and stored at −70 °C. The hippocampus was homogenized with ice-cold saline to be 10% (w/v) homogenates. Protein concentration was determined by the Coomassie blue protein-binding (Bradford, 1976) using bovine serum albumin (BSA) as a standard. The detailed procedures of measurements followed the manufacture instruction in different reagent kits (Nanjing Jiancheng Institute of Biological Engineering, China).

2.7. Morphology

Four rats chosen randomly from each group were anesthetized with chloral hydrate (350 mg/kg, i.p.) after behavior test and then perfused transcardially with normal saline followed by 4% paraformaldehyde. Whole brains were removed and then post-fixed in the same paraformaldehyde at 4 °C, dehydrated and subsequently embedded in paraffin blocks. Coronal sections of 8 μm were stained with 0.5% cresyl fast violet.

2.8. Western blot analysis

The hippocampus homogenate (in 150 mM NaCl, 25 mM Tris–HCl,1 mM EGTA,1 mM EDTA, pH7.4, 1% Triton X-100,1 mM PMSF) was