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Research Report

Neuroprotective effect of osthole against acute ischemic stroke on middle cerebral ischemia occlusion in rats

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ABSTRACT

Osthole, a natural coumarin derivative, has taken considerable attention because of its diverse pharmacological functions. It has been reported to be useful in the treatment of chronic cerebral hypoperfusion and neuronal damage. In the present study, we examined the neuroprotective effect of osthole and its potential mechanisms against acute ischemic stroke induced by middle cerebral artery occlusion (MCAO) in rats. The rats were pretreated with osthole 10, 20 and 40 mg/kg 30 min before MCAO. The neuroprotective effect of osthole against acute ischemic stroke was evaluated by neurological deficit score (NDS), dry–wet weight and 2,3,5-triphenyltetrazolium chloride (TTC) staining. The contents of malondialdehyde (MDA) and glutathione (GSH), activity of myeloperoxidase (MPO) and the level of interleukin (IL)-1β and IL-8 after 2 h of MCAO in rats were detected to investigate its anti-oxidative action and anti-inflammatory property. Pretreatment with osthole significantly increased in GSH, and decreased the volume of infarction, NDS, edema, MDA, MPO, IL-1β and IL-8 compared with rats in the MCAO group at 24 h after MCAO. The study suggests the neuroprotective effect of osthole in the MCAO model of rats. The anti-oxidative action and anti-inflammatory property of osthole may contribute to a beneficial effect against stroke.

1. Introduction

Ischemic stroke causes a very extensive health problem throughout the world. Nearly one-third of patients with acute ischemic stroke develop early neurological deterioration, a situation associated with increased mortality and long-term functional disability (Davalos et al., 1999). Despite the enormous efforts made to identify ways to attenuate neural cell injury induced by cerebral ischemia, the mechanisms underlying neural cell injury in cerebral ischemia are not yet well understood.

However, it has been extensively implicated that acute ischemic stroke enhanced the formation of reactive oxygen species (ROS) in brain tissue. The excessive production of ROS can cause cellular damage and subsequent cell death after ischemia–reperfusion injury, because ROS can oxidize vital

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cellular components such as lipids, proteins and DNA (Sugawara and Chan, 2003). Although many anti-oxidants showed a strong positive effect against acute ischemic stroke in the laboratory, approval for these uses in the treatment of cerebral ischemia is still difficult. Therefore, the need for developing an effective treatment for cerebral ischemia remains vital.

Osthole (7-methoxy-8-isopentenoxy-coumarin, chemical structure shown in Fig. 1), is a natural coumarin derivative, which is extracted from many medicinal plants, such as *Angelica pubescens*, *Cnidium monnieri* and *Peucedanum ostruthium* (Teng et al., 1994; Chen et al., 2000; Chou et al., 2007). It has been clinically used in the treatment of skin disease and gynecopathy for many years and has taken considerable attention recently because of its diverse pharmacological functions, including anti-oxidant, anti-inflammatory, anti-tumor, anti-platelet, estrogen-like, and anti-nociceptive effects and so on (Ko et al., 1989; Zhou et al., 2002; Kuo et al., 2005; Nakamura et al., 2009; Wang et al., 2008; Sun et al., 2009). We have previously also shown that osthole had a neuroprotective effect on MPP+-induced cytotoxicity in PC12 cells via inhibition of ROS production (Liu et al., 2010). Recently, Ji et al. (2010) found that osthole can improve chronic cerebral hypoperfusion induced cognitive deficits and neuronal damage in hippocampus. The aim of our present study was to examine the neuroprotective effect of osthole on MCAO in rats and its potential mechanisms.

2. Results

2.1. *Physiological parameters*

There were no significant differences for these variables (arterial blood pressure, $PaCO_2$, PaO_2, pH, rectal temperature and blood glucose) at baseline, during MCAO and at reperfusion. These variables remained in the normal range during the experimental period observed (data not shown).

2.2. *Effects of pretreatment with osthole on neurological deficit and infarction*

To determine the effect of osthole against cerebral ischemic injury, the neurological deficit and infarct volume in response to ischemic injury were examined 24 h after MCAO. Rats in the sham-operated group had no infarct and neurological deficit (Fig. 2), in contrast, cerebral infarct volume and neurological deficit scores in the ischemia–reperfusion rats were significantly higher (Fig. 2). As shown in Fig. 2 pretreatment with 20 mg/kg or 40 mg/kg of osthole decreased cerebral infarct volume and ameliorated neurological deficit induced by ischemia–reperfusion. However, no significant difference in infarct volume was found between the MCAO group and the osthole 10 mg/kg group. Furthermore, the dose of 40 mg/kg of osthole was more protective than the dose of 20 mg/kg on the cerebral injury after MCAO (Fig. 2).

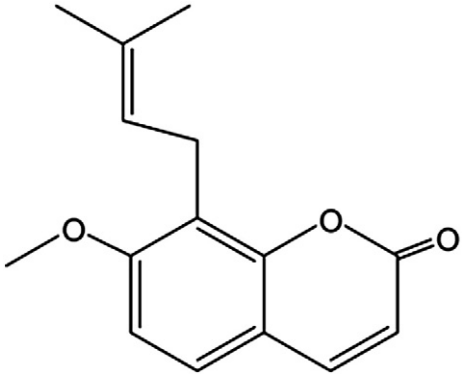

Fig. 1 – Chemical structure of osthole.

2.3. *Effect of pretreatment with osthole on brain edema formation*

Twenty-four hours after MCAO, the brain water content in the ischemic area of the MCAO group was significantly higher than that of the sham-operated group (Fig. 3). Pretreatment with osthole 20 mg/kg or 40 mg/kg significantly reduced brain edema versus MCAO rats. However, no significant difference in WBC was found between the MCAO group and the osthole 10 mg/kg group (Fig. 3). Furthermore, the dose of 40 mg/kg of osthole was also more protective than the dose of 20 mg/kg on the brain injury after MCAO. So we chose the dosage of 40 mg/kg for our subsequent experiment.

2.4. *Biochemical observations*

Table 1 shows the significantly increased content of MDA and activity of MPO and the significantly decreased level of GSH in the MCAO group compared to the sham group. Pretreatment with osthole significantly brought down the increased content of MDA and the activity of MPO, and raised the GSH level of rats 24 h after MCAO.

As shown in Fig. 4, the expression of interleukin (IL)-1β and IL-8 in the MCAO group were much higher than that in the sham-operated group. Pretreatment with osthole significantly also brought down increased expression contents of IL-1β and IL-8 of rats 24 h after MCAO.

3. Discussion

In the present study, we first investigated the neuroprotective effect of osthole in a model for MCAO. Recent studies have confirmed the pivotal role of oxidative stress in the pathogenesis of acute ischemic stroke (Khan et al., 2009). Therefore, there had been considerable efforts to explore natural substances for their neuroprotective potential. More attention had been focused on a wide array of natural anti-oxidants that can scavenge free radicals and protect cells from oxidative damage, such as resveratrol and protocatechuic acid (Parnham and Sies, 2000). Already in previous studies it was suggested that osthole showed a protective effect against chronic cerebral hypoperfusion induced cognitive deficit (Ji et al., 2010) and brain memory impairment of mice in an acute senile model induced by $AlCl_3$. Ischemia–reperfusion injury may cause acute ischemic stroke, local cerebral cell injury or