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Effect of the flavonoid, oroxylin A, on transient cerebral hypoperfusion-induced memory impairment in mice

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Abstract

Oroxylin A is a flavonoid compound that is found in the root of *Scutellaria baicalensis* Georgi. The aim of this study was to determine the effects of oroxylin A on memory impairment induced by transient bilateral common carotid artery occlusion (2VO) in mice. The ameliorating effect of oroxylin A on memory impairment was investigated using a passive avoidance task, the Y-maze task, and the Morris water maze task in mice. Oroxylin A was found to significantly reverse 2VO-induced cognitive impairments in the passive avoidance and Y-maze tasks in a dose dependant manner ($P<0.05$). Moreover, oroxylin A (5 mg/kg, p.o.) shortened the escape-latency and prolonged swimming times in the target quadrant during the probe trial in the Morris water maze task ($P<0.05$). Histochemical and immunohistochemical studies showed that the number of Nissl bodies and OX-42 positive cells in the hippocampal CA1 and dentate gyrus regions were attenuated by oroxylin A. Moreover, phosphorylated cAMP response element-binding protein (CREB) and brain derived neurotrophic factor (BDNF) positive cell numbers were markedly increased in animals treated with oroxylin A than in untreated 2VO controls. These results suggest that oroxylin A dramatically attenuates the memory impairment induced by 2VO, and that this effect may be mediated by the neuroprotective effects of oroxylin A as supported oroxylin A induced reductions in activated microglia and increases in BDNF expression and CREB phosphorylation.

Keywords: Oroxylin A; Memory; Passive avoidance task; Y-maze task; Morris water maze task; Carotid artery occlusion

1. Introduction

The hippocampus is highly vulnerable to transient cerebral ischemia (Pulsinelli et al., 1982), and much interest has been focused on the effects of global forebrain ischemia on the hippocampal formation, and in particular on CA1 pyramidal cells, which are extremely vulnerable to ischemic insults and die in response to only a few minutes of blood flow reduction (Pulsinelli et al., 1982; Smith et al., 1984). Ischemic cell death in the hippocampus is selective. Most CA1 pyramidal neurons die after 15 min of ischemia, with maximal cell death occurring one week after reperfusion. Ischemia-induced neuronal degeneration is also observed in other structures, such as, the striatum, cerebral cortex, and thalamus (Pulsinelli et al., 1982; Smith et al., 1984; Johansen and O'Hare, 1989; Freund et al., 1990). Many reports have shown learning and memory deficits in rat models of permanent or transient bilateral common carotid artery occlusion (2VO), an accepted model of vascular dementia (Masada et al., 1997; Pazos et al., 1999; Hartman et al., 2005).

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In addition, several studies have shown mild neuronal damage in mice administered transient 2VO (Sheng et al., 1999; Wellons et al., 2000; Urayama et al., 2002). However, it is unclear whether memory dysfunction is caused by transient hypoperfusion in mice administered 2VO.

Oroxylin A (5, 7-dihydroxy-6-methoxyflavone) is a flavonoid isolated from the root of *Scutellaria baicalensis* Georgi that is widely used in traditional Chinese medicine (Tomimori et al., 1982). Several previous reports have revealed that oroxylin A is anti-oxidative and anti-inflammatory. For example, it suppresses superoxide and nitric oxide generation (Jiwajinda et al., 2002) and inhibits lipopolysaccharide-induced iNOS and COX-2 gene expression by suppressing nuclear factor-kappaB activation (Chen et al., 2000). Polyphenolic fractions from *S. baicalensis* attenuate the memory dysfunctions caused by chronic global ischemia in rats, however, they did not identify the compound involved (Shang et al., 2005). *S. baicalensis* contains many flavonoid compounds, such as, baicalein, wogonin, and oroxylin A. We have found that baicalein and wogonin do not affect or worsen memory functions (unpublished data). Recently, it was reported that oroxylin A possesses antagonistic properties at the $GABA_A$ receptor (Huen et al., 2003). $GABA_A$ antagonists have been reported to ameliorate the memory dysfunction induced by scopolamine (Lal et al., 1988; Sharma and Kulkarni, 1990; Diez-Ariza et al., 2003). Thus, we reasoned that oroxylin A, a $GABA_A$ antagonist, may ameliorate memory dysfunction induced by hypoperfusion in mouse 2VO model.

The purpose of this study was to investigate whether mice that have experienced cerebral hypoperfusion induced by transient 2VO show learning and memory impairments, and if so, whether these impairments can be attenuated by oroxylin A. Cognitive function was evaluated using the passive avoidance task, the Y-maze task, and the Morris water maze task. In addition, morphological changes in the 2VO mouse hippocampus with or without oroxylin A treatment were investigated using histochemical and immunohistochemical methods.

2. Materials and methods

2.1. Animals

Male ICR mice (25–30 g) were purchased from the Orient Co., Ltd, a branch of Charles River Laboratories (Seoul). Animals were housed 5 or 6 per cage, allowed access to water and food ad libitum, and maintained under a constant temperature (23 ± 1 °C) and humidity (60 ± 10%) under a 12-h light/dark cycle (light on 07.30–19.30 h). Animal treatment and maintenance were carried out in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Kyung Hee University, Korea.

2.2. Materials

Oroxylin A was donated by one of the author (K.H. Son), and its purity was 99.9%. Oroxylin A was suspended in a 10% Tween 80 solution. Anti-CD11b antibody (OX-42), anti-phosphorylated cAMP response element-binding protein (pCREB) antibody, and anti-brain derived neurotrophic factor (BDNF) antibody were purchased from Serotec Ltd. (UK), Upstate Lake Placid (USA), or Santa Cruz Biotech (USA), respectively. All other materials were of the highest grade available and were obtained from normal commercial sources.

2.3. Surgeries and drug administration

ICR mice were anesthetized with 1.0% isoflurane and 70% nitrous oxide in oxygen. Mice were subjected to a transient cerebral hypoperfusion as described by Zhao et al. (2005), with minor modifications. Transient cerebral hypoperfusion was induced by occluding both common carotid arteries with aneurysm clips for 7 min. Circulation was restored by removing clips. Mice that received the same surgical operation without clipping of the carotid arteries served as sham-operated controls.

Mice were treated orally with 1.25, 2.5, or 5 mg/kg of oroxylin A 60 min after reperfusion, and then once a day for a week. The sham operated vehicle treatment group and the untreated 2VO control group received 10% Tween 80 solution at the same volumes and times. The last treatment was completed 1 h prior to each test.

2.4. The passive avoidance task

Testing was carried out in identical illuminated and non-illuminated boxes (20 × 20 × 20 cm), separated by a guillotine door (5 × 5 cm) (Gemini Avoidance System, San Diego). The illuminated compartment contained a 50 W bulb, and the floor of the non-illuminated compartment (20 × 20 × 20 cm) was composed of 2 mm stainless steel rods spaced 1 cm apart. For the acquisition trial, mice were initially placed in the illuminated compartment and the door between the two compartments was opened 10 s later. When mice entered the dark compartment, the door automatically closed and an electrical foot shock (0.5 mA) of 3 s duration was delivered through the stainless steel rods. One hour before the acquisition trial, mice were administered vehicle or oroxylin A (1.25, 2.5, or 5 mg/kg). Twenty-four hours after the acquisition trial, mice were replaced in the illuminated compartment for the retention trial. The time taken for a mouse to enter the dark compartment after door opening was defined as latency for both acquisition and retention trials. Latency to enter the dark compartment was recorded up to 300 s.

2.5. The Y-maze task

The Y-maze was a horizontal maze (40 cm long and 3 cm wide with walls 12 cm high) with three arms (labeled A, B and C) at 120° angles from each other (Sarter et al., 1988). The maze floor and walls were constructed from dark opaque polyvinyl plastic. Mice were initially placed within one arm, and the sequence (i.e., ABCCAB) and number of arm entries were recorded manually for each mouse over an 8 min period. An alternation was defined as entry into all three arms consecutively (i.e., ABC, CAB, or BCA but not BAB). One hour before this test, mice were treated with vehicle or oroxylin A (1.25, 2.5, or 5 mg/kg). Maze arms