Neuroscience Letters 623 (2016) 13–21

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Neuroprotective and memory enhancing effects of auraptene in a rat model of vascular dementia: Experimental study and histopathological evaluation

Mustafa Ghanbarabadi[a], Mehrdad Iranshahi[b], Sakineh Amoueian[c], Soghra Mehri[d], Vahideh Sadat Motamedshariaty[d], Seyed Ahmad Mohajeri[e,*]

[a] Student Research Committee (SRC), Mashhad University of Medical Sciences, Mashhad, Iran
[b] Biotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Pathology department, Imam Reza hospital, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[e] Targeted Drug Delivery Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

HIGHLIGHTS

- A rat model of vascular dementia was applied in present work.
- Neuroprotective and memory enhancing effects of auraptene was studied.
- Biochemical and histopathological experiments were also applied.
- Auraptene significantly improved spatial learning memory performance.
- Biochemical and histopathological improvement was also observed by auraptene.

ARTICLE INFO

Article history:
Received 7 March 2016
Received in revised form 20 April 2016
Accepted 21 April 2016
Available online 27 April 2016

Keywords:
Auraptene
Vascular dementia
Memory enhancing effect
Neuroprotection
Brain ischemia

ABSTRACT

Vascular dementia and Alzheimer disease are most common type of dementia. These diseases have been associated with cognitive decline and affected personal behavioral activities. Moreover, the pattern of cerebral blood flow in mild cognitive disorder has appeared as a predictive indication for the development into Alzheimer's disease. Permanent, bilateral occlusion of the common carotid arteries (2VO) is a standard animal model to study vascular dementia and chronic cerebral hypoperfusion. In present study neuroprotective and memory enhancing effects of auraptene (AUR), a citrus coumarin, were studied in 2VO rats. Different doses (25, 8 & 4 mg/kg) of AUR were administered orally. The spatial memory performance was tested with Morris water maze after 2VO induction. Biochemical experiments and histopathological evaluations were also applied to investigate the neuroprotective effect of AUR in brain tissue. In comparison with 2VO group, AUR could significantly decrease the scape latency time in treated rats. Also AUR increased the percentage of time spent and traveled pathway in target quadrant on final trial test day. All behavioral results were confirmed by biochemical and histopathological data. Biochemical data indicated that AUR could decrease malondialdehyde (MDA), as lipid peroxidation indicator, and increase glutathione (GSH) content in cortex and hippocampus tissues. Histopathological data showed that AUR could protect cerebrocortical and hippocampus neurons against ischemia. This study demonstrated the memory enhancing effect and neuroprotective activity of AUR after induction of brain ischemia in a rat model of vascular dementia.

1. Introduction

Dementia is one of the most important cerebral disorders especially after the age of 65. This disease affects person's memory and cognition and decreases daily functions. Other symptoms e.g. emotional and language problems and decrease in motivation are

* Corresponding author.
E-mail addresses: mohajeria@mums.ac.ir, seyedahmad_mohajeri@yahoo.com (S.A. Mohajeri).

http://dx.doi.org/10.1016/j.neulet.2016.04.047
0304-3940/

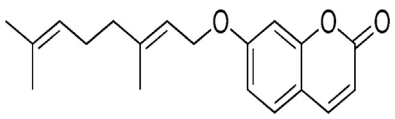

Fig. 1. Chemical structure of auraptene.

common in this disorder. Vascular dementia, Alzheimer's disease, lewy body and frontotemporal dementia has been reported as different types of dementia. Infarction or stroke, damaged and narrowed brain's blood vessels due to sediment of cholesterol and other substances are from the most common reasons of the vascular dementia. Although the pathology reasons of vascular dementia and Alzheimer's disease are different, similar symptoms are often presented by patients [19]. Recently some studies have reported that vascular dementia may play a role in progression of Alzheimer's disease [10,31]. Bilateral permanent common carotid ligation or two vessel occlusion (2VO) is a standard animal model for study of vascular dementia and chronic cerebral hypoperfusion and its effects on spatial learning and cognition [13]. Previous studies indicated that chronic cerebral hypoperfusion causes ischemia, hypoxia, pathological damages, inflammatory injuries and biochemical changes in different regions of the brain such as hippocampus and cerebral cortex [7]. Other researchers have reported that formation of free radicals and depletion of antioxidant content in the plasma and brain tissue play important role in neuronal damage during vascular dementia [38]. Inflammatory cells such as microglia and leukocytes are usually activated in ischemic condition. Accumulation of these inflammatory cells in the brain leads to the neuronal damage. The molecular signaling generated by ischemic cells activates the immunity system components during the ischemic/stroke processes. Promotion of inflammatory signaling contributes to the tissue damage [18]. Therefore, anti-inflammatory and antioxidant agents can be applied to reduce brain histopathological injuries and restore cognitive and memory skills [52]. Recently, natural products and herbal medicines have been widely used for management and treatment of central nervous system disorders [3]. The safety and less side effects, cultural acceptability and suitable efficacy are the main characteristics of the herbal medicines. Coumarins are phenolic compounds which found in different plants, bacteria and fungi. More than 1300 coumarins have been detected in natural products. Auraptene (AUR) is 7-geranyloxycoumarin (Fig. 1) which is identified in citrus fruits [14]. Previous works have reported that AUR has anti-inflammatory and anti-carcinogenesis activity [27]. As a citrus coumarin, it can effectively inhibit activation of microglia and expression of cyclooxynase-2 (COX-2) made by astrocytes and thereby attenuates the cell death in hippocampus and brain. A study by Okuyama et al. demonstrated that AUR ameliorates lipopolysaccharide-induced inflammatory in the brain [34]. In the present work neuroprotective and memory enhancing effects of AUR were evaluated in a rat model of vascular dementia. Spatial learning memory, histopathological damages and biochemical changes were evaluated after induction of permanent cerebral hypoperfusion. The results were promising and indicated the efficacy of AUR in management of neuronal damages and memory enhancement after cerebral ischemia.

2. Materials and methods

2.1. Chemicals

Auraptene (7-geranyloxycoumarin) was synthesized in our institute based on a previously described method [2]. Briefly, 7-hydroxycoumarin and *trans*-geranyl bromide were reacted in acetone at room temperature, in the presence of DBU (1, 8-diazabicyclo [5.4.0] undec-7-ene). Auraptene was purified from the concentrated reaction mixture as white crystals using column chromatography (petroleum ether/ethyl acetate, 9:1 v/v) and its structure was confirmed by 1H- and 13C NMR. The purity of auraptene was calculated using HPLC as 95%. DTNB) 5, 5′-dithiobis 2-nitrobenzoic acid) (Sigma, USA), GSH (Glutathione) (Sigma, Germany), KCl (Merck, Germany), MDA (malondialdehyde) (Merck, Germany), *N*-butanol (Merck, Germany), Phosphoric acid (Merck, Germany), TBA (Thiobarbituric acid) (Merck, Germany) and TCA (Trichloro acetic acid) (Sigma, Germany) were used in this study.

2.2. Animals

Male Wistar rats (200–250 g) were obtained from animal house in pharmaceutical research center, BuAli research institute, Mashhad University of Medical Sciences. They were maintained in a temperature-controlled room ($21 \pm 2\,^{\circ}C$) on a 12/12-h light/dark cycle and had free access to food and water. Handling and experimental procedures for all animals were in accordance with the Mashhad University of Medical Sciences Ethics Committee Acts.

2.3. Surgery

Permanent cerebral hypoperfusion was induced using bilateral common carotid occlusion as an animal model for vascular dementia. The surgical procedure was according to the method described in our previous studies [17]. Ketamine (60 mg/kg) and xylazine (6 mg/kg) (i.p.) were mixed and applied for anesthesia. Then a midline ventral neck incision was made, the right common carotid artery was exposed, separated gently from vague nerve and double ligated with 5–0 type surgical silk thread (day 1). In the next step, the left common carotid artery was ligated, as described above after one week (day 8). The sham-operated rats (non-ischemic) underwent the same two-step surgical procedure without carotid occlusion. The body temperature was monitored and maintained at $37 \pm 0.5\,^{\circ}C$ during the surgery.

2.4. Experimental design

Animals were randomly divided into six groups: (1) sham-operated animals (non-ischemic group) underwent the surgical procedure without ligation of the common carotid arteries (n = 10); (2) 2VO group (ischemic rats) received vehicle (n = 10); treatment groups (group 3–5) received auraptene (25, 8 & 4 mg/kg per day, n = 10, n = 10, n = 10) and (6) positive control group received piracetam (600 mg/kg per day, n = 10) [15]. Auraptene and piracetam were suspended in the vehicle (arabic gum 5% w/v) and administered orally from day 1 to day 20 (end of final trial test day).

2.5. Behavioral experiments

One week after the second surgery (day 15), the Morris water maze test was applied to evaluate the performance of spatial memory. The Morris water maze consists of a black circular pool with 60 cm height and 136 cm diameter, filled with water (temperature about $20 \pm 1\,^{\circ}C$, 35 cm in depth) and situated in a dark room with visual cues on the room walls. A black hidden platform (diameter: 10 cm) was submerged 2 cm below the water surface in the target quadrant. The pool was divided into four geographical quadrants: Southwest (SW), southeast (SE), northwest (NE) and northeast (NE). Four points [east (E), west (w), north (N) and south (S)] were designed as a swimming starting position. A video camera was located up the pool to record the animals swimming path. The swimming speed of each animal, traveled distance, escape latency time to reach the hidden platform, swimming time and traveled