Journal of Ethnopharmacology 214 (2018) 190–196

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

The Japanese Angelica acutiloba root and yokukansan increase hippocampal acetylcholine level, prevent apoptosis and improve memory in a rat model of repeated cerebral ischemia

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ARTICLE INFO

Keywords:
Acetylcholine
Apoptosis
Japanese Angelica root
Memory
Yokukansan

ABSTRACT

Ethnopharmacological relevance: Japanese Angelica acutiloba root (Angelica root) is included in several Kampo medicines including Yokukansan (YKS). Angelica root and YKS are used for the treatment of a variety of psychological and neurodegenerative disorders. Development of safe and effective therapeutic agents against cerebrovascular disorders will improve the treatment of patients with dementia.

Aim of the study: The effect of Angelica root and YKS on ischemia-impaired memory has not yet been fully investigated. The present study investigated whether Angelica root is also involved in memory improving and neuroprotective effect of YKS in a model of cerebrovascular ischemia.

Materials and methods: Male Wistar rats grouped into sham rats received saline, and other three groups subjected to repeated cerebral ischemia induced by 4-vessel occlusion (4-VO), received a 7-day oral administration of either saline, Angelica root or YKS. Memory was evaluated by eight-arm radial maze task. Acetylcholine release (ACh) in the dorsal hippocampus was investigated by microdialysis-HPLC. Apoptosis was determined by terminal deoxynucleotidyl transferase (TdT)-mediated fluorescein-deoxyuridine triphosphate (dUTP) nick-end labeling.

Results: Ischemia induced apoptosis, reduced release of ACh, and impaired the memory (increased error choices and decreased correct choices). Angelica root and YKS improved the memory deficits, upregulated the release of ACh and prevented 4-VO-induced hippocampal apoptosis.

Conclusion: The dual ACh-increasing and neuroprotective effect of Angelica root could make it a promising therapeutic agent useful for the treatment of symptoms of cerebrovascular dementia. Angelica root could be one of the components contributing to the memory-improving and neuroprotective effects of YKS.

1. Introduction

Traditional herbal medicine has a long history of human use. The use of herbs for improvement of memory and cognitive function in the mental disorders has been an active field of research.

Memory impairment is one of the cardinal clinical pictures of dementia. Several herbs are used to improve memory such as Ginkgo biloba, Huperzia serrata, Curcuma longa, Panax ginseng, Panax notoginseng, Bacopa monnieri, Salvia miltiorrhiza, Crocus sativus, Camellia sinensis (Chang et al., 2016), Rhodiola rosea, Periwinkle-Vinca minor, Periwinkle-Vinca minor, Centella asiatica, Melissa officinalis, Polygala tenuifolia willd, Salvia miltiorrhiza bung, and Withania somnifera (Sun et al., 2013). Moreover, a variety of herbs are compounded in multicomponent formulations to treat disorders that have multiple arrays of pathological causes such as dementia/vascular dementia. Yokukansan (YKS), chotosan, kososan and kamishoyosan are

Abbreviations: ACh, Acetylcholine; AChE, acetylcholinesterase; Angelica root, Japanese Angelica acutiloba root; ANOVA, Analysis of variance; ChAT, choline acetyltransferase; CA1, cornu ammonis area 1; FITC, Fluoresein-4-isothiocyanate; HPLC, high-performance liquid chromatography; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated fluorescein-deoxyuridinetriphosphate (dUTP) nick-end labeling; RAM, radial maze task; RI, Repeated ischemia; 4-VO, 4-vessel occlusion; YKS, Yokukansan

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https://doi.org/10.1016/j.jep.2017.12.025
Received 9 September 2017; Received in revised form 16 December 2017; Accepted 16 December 2017
Available online 19 December 2017

examples of such formulations (Arumugam and Watanabe, 2017; Matsumoto et al., 2013). The active ingredients in thes herbs and formulations had also been determined (Su et al., 2014). There is suggestive evidence that three of the herbs that constitute YKS, namely Japanese Angelica acutiloba root (Angelica root), Poria cocas and Glycerrhiza uralensis, have memory improving property (Lin et al., 2012) and take place in many memory-improving kampo formulations.

YKS is a Kampo medicine (Japanese variant of Chinese traditional medicine that involves the use of herbs) originated from the traditional Chinese medicine *Yi-Gan-San*. YKS displays an improving effect in a variety of psychological and neurodegenerative disorders, both clinically and experimentally. It has been reported that YKS improves behavioral and psychological symptoms of dementia, such as hallucination, delusion, agitation and aggression in the dementia (Iwasaki et al., 2005; Shinno et al., 2008), Alzheimer's disease (Hayashi et al., 2010) and Parkinsonian patients with dementia without worsening their cognitive function or ability to perform activities of daily living (Kawanabe et al., 2010). Moreover, YKS improves involuntary movement disorders in Huntington's disease (Satoh et al., 2009) and neuroleptic-induced tardive dyskinesia (Miyaoka et al., 2008). YKS alleviates the emotional abnormality under conditions of excessive stress (Tsuji et al., 2014), anxiety-like behavior in an animal model of cerebrovascular dementia (Nogami et al., 2011), inhibits head-twitches (Egashira et al., 2008), the aggressive behavior induced by amyloid-β peptide (Sekiguchi et al., 2011) and social isolation (Uchida et al., 2009). Moreover, YKS ameliorates olfactory bulbectomy- (Yamada et al., 2011), thiamine deficiency- (Ikarashi et al., 2009), ischemia- (Liu et al., 2014), amyloid-β- (Fujiwara et al., 2011) and age-related memory deficits (Mizoguchi et al., 2011). However, the quiddity and extent of the contribution of its components to the improving effect of YKS in the neurodegenerative disorders have not yet been completely understood. Angelica root is one of the components of YKS. Angelica root displays an anxiolytic effect by activities involving antagonism of serotonin 2 A receptors (Nogami et al., 2011), and mimetic effect on GABA (A)-benzodiazepine receptor complex (Egashira et al., 2011). We had previously determined the effectiveness of Angelica root in improving the scopolamine-induced impairment of memory (Hatip-Al-Khatib et al., 2004). Accordingly, the present study was conducted to investigate the effect of Angelica root, in comparison with YKS, on memory impairment, hippocampal neuronal damage and acetylcholine (ACh) level in rats subjected to repeated cerebral ischemia (RI) in 4-vessel occlusion (4-VO) model.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 300–350 g (KYUDO Co., LTD, Saga, Japan) were housed under controlled conditions of temperature (23 ± 2 °C) and relative humidity (60 ± 5%) with a 12-h light/dark cycle. Food and water were available ad libitum, except that the rats used in the maze task were subjected to a restricted feeding schedule started at the beginning and continued throughout the experiment. The schedule was achieved by reducing the daily consumption of ration (10–12 g/day; CE-2, Clea Japan, Tokyo, Japan) so that the body weight of each rat was maintained at 80–90% of the free-feeding level. Animal care and the experimental procedures were based on the ethical regulations of the Animal Care and Use Committee of Fukuoka University.

2.2. Authentication of plant extracts

The dried water-soluble extracts powder of YKS (TJ-54, voucher No. 29101690) and the Angelica root (voucher No. 2081002010) were supplied by Tsumura & Co., Japan. YKS contains the following seven herbs including Japanese Angelicae acutiloba-Kitagaw, root (Apiaceae), Angelica root (3.0 g); Atractylodis lancea- De Candolle, rhizome (Asteraceae), Sojutsu (4.0 g); Bupleurum falcatum Linné, root (Umbelliferae; bupleurum root), Saiko (2.0 g); Cnidium officinale-Makino, rhizome (Umbelliferae), Senkyu (3.0 g); Glycyrrhiza uralensis-Fisher, root (Fabaceae), Kanzo (1.5 g); Poria Cocos-Wolf, sclerotium (Polyporaceae), Bukuryo (4.0 g), and Uncaria rhynchophylla-Miquel, Thorn (Rubiaceae), Chotoko (3.0 g).

The identification and authentication of each plant material had been conducted according to the Japanese Pharmacopoeia and the company's standard. The extracts were manufactured according to Good Manufacturing Practice (GMP), and subjected to factory release test. The quality control was based on defined by the Ministry of Health, Labour and Welfare of Japan. Samples of the extracts retained in Tsumura & Co.

The following active components had been identified by three-dimensional HPLC: Six ingredients had been identified in Angelica root: umbelliferon, xanthotoxin, bergapten, senkyunolide, ligustilid and ferulic acid. On the other hand, 24 ingredients had been detected in YKS: ferulic acid; liquirtin apioside; liquirtin; 4E,6E,12E-tetradecatrien-8,10-diyne-1,3,14-triol; formononetin-7-O-glucoside; liquiritigenin; glycyrrhizin; geissoschizine methyl ether; hirsutein; xanthotoxin; hirsutin; saikosaponin b2,saikosaponin b1; 12-isovaleroyl-2E,8E,10E-triene-4,6-diyne-1,14-diol;14-isovaleroyl-2E,8E,10E-triene-4,6-diyne-1,12-diol; atractylodinol; ligustilide; atractylodin; acetylatractylodinol; glycycoumarin; formononetin; isoliquiritigenin; isoliquiritin; isoliquiritin apioside, and glycycroside (Ikarashi and Mizoguchi, 2016).

The doses of Angelica root and YKS were calculated on the basis of dry extracts, and administered in aqueous solutions for seven consecutive days commencing after ischemia. The experiments were conducted one hour after the last dose of Angelica root and YKS. The dose of YKS was selected on the basis of the maximum pharmacological and neuroprotective effect produced by 1000 mg/kg YKS. On the other hand, Angelica root constitutes 15% of YKS. Accordingly, the selected dose of Angelica root (150 mg/kg) represents the equivalent amount in the administered dose of YKS (1000 mg/kg).

2.3. Experimental schedule

The experimental timetable is given in Fig. 1. After training sessions, the selected rats (n = 40) were divided into four equally sized groups. Sham group, the rats were subjected only to cauterization of the vertebral arteries and received distilled water. The common carotid arteries in this group were fitted with occluders but not occluded. The other three groups were RI rats received a 7-day oral treatment of either distilled water as the vehicle, Angelica root 150 mg/kg or YKS 1000 mg/kg orally. Two rats from each group were assigned to the apoptosis study.

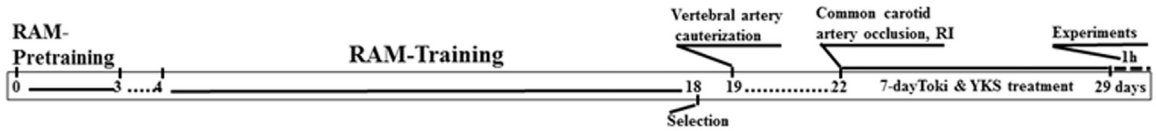

Fig. 1. The experimental timeline scheme showing the schedule of eight-arm radial maze (RAM) tests, induction of repeated ischemia (RI), and the conduction of experiments one hour after the last administration of Angelica root and YKS. Other details are given in the relevant parts of the text.