Journal of Ethnopharmacology 146 (2013) 294–299

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Ginsenosides Rg5 and Rh3 protect scopolamine-induced memory deficits in mice

Eun-Jin Kim [a,b], Il-Hoon Jung [a], Thi Kim Van Le [a], Jin-Ju Jeong [a], Nam-Jae Kim [b], Dong-Hyun Kim [a,b,*]

[a] Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, 1, Hoegi, Dongdaemun-gu, Seoul 130-701, Republic of Korea
[b] East-West Medical Research Institute, Kyung Hee University, 1, Hoegi, Dongdaemun-gu, Seoul 130-701, Republic of Korea

ARTICLE INFO

Article history:
Received 2 November 2012
Received in revised form
27 December 2012
Accepted 29 December 2012
Available online 9 January 2013

Keywords:
Panax ginseng
Araliaceae
Ginsenoside Rg5
Ginsenoside Rh3
Memory deficit-protecting effect

ABSTRACT

Ethnopharmacological relevance: *Panax ginseng* (family Araliaceae) is traditionally used as a remedy for cancer, inflammation, stress and aging.
Aim of study: To explore whether ginsenosides Rg5 and Rh3, the main constituents of heat-processed ginseng (the root of *Panax ginseng*), could protect memory deficit.
Materials and methods: We isolated ginsenosides Rh3 and Rg5 from heated-processed ginseng treated with and without human feces, respectively. Then we investigated their protective effects on memory impairment using the passive avoidance, Y-maze and Morris water maze tasks in mice. Memory deficit was induced in mice by the intraperitoneal injection of scopolamine.
Results: Ginsenosides Rg5 or Rh3 increased the latency time reduced by scopolamine in passive avoidance test. Treatment with ginsenoside Rg5 or Rh3 significantly reversed the lowered spontaneous alteration induced by scopolamine in Y-maze task. Ginsenoisde Rg5 or Rh3 (10 mg/kg) significantly shortened the escape latencies prolonged by treatment with scopolamine on the last day of training trial sessions in Morris water maze task. Furthermore, ginsenosides Rg5 and Rh3 inhibited acetylcholinesterase activity in a dose-dependent manner, with IC_{50} values of 18.4 and 10.2 μM, respectively. The inhibitory potency of ginsenoside Rh3 is comparable with that of donepezil (IC_{50}=9.9 μM). These ginsenosides also reversed hippocampal brain-derived neurotrophic factor (BDNF) expression and cAMP response element-binding protein (CREB) phosphorylation reduced by scopolamine. Of them, ginsenoside Rh3 more potently protected memory deficit.
Conclusions: Ginsenoside Rg5 and its metabolite ginsenoside Rh3 may protect memory deficit by inhibiting AChE activity and increasing BDNF expression and CREB activation.

1. Introduction

Alzheimer's disease (AD) is a progressive degenerative disease of the brain that is characterized by deterioration of memory and cognitive function (Whitehouse et al., 1981; Eikelenboom et al., 1994). A decrease in cholinergic function in the central nervous system can result in a decline in memory and cognitive function with advanced age (Terry and Buccafusco, 2003; Sarter and Bruno, 2004). Scopolamine, an anti-cholinergic drug, causes memory impairments in healthy young humans that parallel the memory impairments seen in non-demented drug-free elderly subjects (Tariot et al., 1996; Araujo et al., 2005). Scopolamine is increasingly disruptive with increasing age and declining cognitive status. Many attempts have been made to improve cognitive deficits by increasing brain cholinergic activity with acetylcholinesterase (AChE) inhibitors such as donepezil. However, number of drugs approved for the use of treatment of patients with memory impairment is limited due to their side effects such as pain, nausea and vomiting (Cambell et al., 1978; Doody, 1999).

Ginseng (the root of *Panax ginseng* C.A. Meyer, Family Araliaceae) is frequently used for diabetes, cancer, stress and allergic diseases as a traditional Chinese medicine in Asian countries. Heat processes have been adopted for enhancing the pharmacological activities of ginseng, and these processes alter its chemical compositions (Kwon et al., 2001; Bae et al., 2006). In particular, the heat-processed ginsengs, which have been frequently used in Asian countries for cancer, inflammation, stress and aging, uniquely contains ginsenoside Rg5 as a main constituent (Fig. 1) as main constituents (Kim et al., 2000; Kwon et al., 2001; Bae et al., 2006). Ginsenoside Rg5 exhibits anticancer, antidermatitic, anti-allergic, platelet anti-aggregating, and radical scavenging activities (Lee et al., 1997; Shin et al., 2006; Kim et al., 2012). Related to the memory deficit-protecting effects of ginsenosides,

* Correspondence to: Department of Pharmaceutical Science, Kyung Hee University, 1, Hoegi, Dongdaemun-gu, Seoul 130-701, Republic of Korea. Tel.: +82 2 961 0374; fax: +82 2 957 5030.
E-mail address: dhkim@khu.ac.kr (D.-H. Kim).

0378-8741/$ - see front matter © 2013 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.jep.2012.12.047

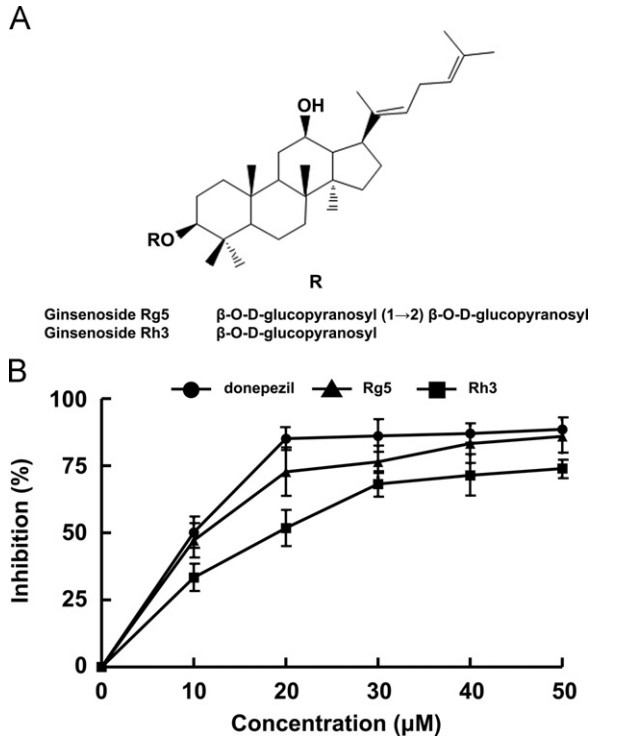

Fig. 1. The structures of ginsenosides Rg5 and Rh3 (A) and their enhibitory effects on acetylcholinesterase activity (B). Inhibition is expressed as percent inhibition of enzyme activity in comparison with the medium control, and presented as mean ± SEM (n=3).

ginsenoside Rg3 and ginsenoside Rg5/Rk1 mixture (1:1, w/w) isolated from heat-processed ginseng show memory enhancing effect in scopolamine-treated mice (Bao et al., 2005). Ginsenoside Rg3 isolated from red ginseng, and its metabolite ginsenoside Rh2, as well as ginsenoside Rg1 also protected scopolamine-induced memory deficit in mice (Yang et al., 2009; Fang et al., 2012). However, when ginsenoside Rg5 was incubated with human feces, it was metabolized to ginsenoside Rh3 (Bao et al., 2005; Shin et al., 2006). Nevertheless, the neuroprotective effect of ginsenoside Rh3 has not been studied.

During a screening program for Chinese traditional medicines to protect memory impairment, heat-processed ginseng and its main constituent ginsenoside Rg5 potently protected scopolamine-induced memory deficit in mice. Therefore, we investigated the protective effect of ginsenoside Rg5 or its metabolite ginsenoside Rh3 against memory deficit in scopolamine-treated mice.

2. Materials and methods

2.1. Materials

(−) Scopolamine hydrobromide, acetylthiocholine (ATCh), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylcholine esterase (AChE, electric eel type VI-S) and dimethylsulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA.). Antibodies against brain-derived neurotrophic factor (BDNF), cAMP response element-binding protein (CREB), p-CREB and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, L.A. U.S.A.). The protease inhibitor cocktail was purchased from Roche Applied Science (Mannheim, Germany). Immobilon-P nylon membrane was from Millipore Co. (Billerica, MA, U.S.A.). Polyvinylidene difluoride (PVDF) membrane and enhanced chemiluminescence (ECL) detection kit were purchased from Millipore (Billerica, MA, USA). Donepezil hydrochloride monohydrate (DNZ; purity, >99%) was kindly provided by WhanIn Pharmaceutical Co., Ltd. (Seoul, Korea). Ginsenosides Rg5 (purity, >95%) and Rh3 (purity, >95%) (Fig. 1A) were isolated by our previously reported method (Shin et al., 2006).

2.2. Animals

Male ICR mice (28–30 g) were purchased from Samtako Biokorea (Seoul, Korea). All animals were fed on standard laboratory chow (Samyang Co., Seoul, South Korea), housed in wire cages at 24 ± 2 °C and 50 ± 10% humidity and allowed to water ad libitum. All experiments were performed in accordance with the National Institutes of Health and Kyung Hee University guides for Laboratory Animals Care and Use, and approved by the Committee for the Care and Use of Laboratory Animals in the Kyung Hee Medical Center, Kyung Hee University.

2.3. AChE activity assay

AChE activity was measured using Ellman's coupled enzyme assay (Ellman et al., 1961). The reaction mixture consisted of 125 μL of 3 mM DTNB, 25 μL of 15 mM ATCh, 50 μL of 50 mM Tris-HCl (pH 8.0), and 25 μL of test agents in a microplate. The mixture was pre-incubated for 10 min, and then 25 μL AChE (0.226 U/mL) was added before scanning at 405 nm for 10 min in a microplate reader, Model Biotek μQuant MQX200 (Winooski, VT, U.S.A.). Enzyme activity was calculated as a percentage compared to buffer without any inhibitor.

2.4. Passive avoidance task

Passive avoidance task was performed according to previously described (Yang et al., 2009). The apparatus consists of a two-compartment acrylic box in which a lighted compartment (20 × 20 × 20 cm) is connected to a dark compartment (20 × 20 × 20 cm) by an entrance hole (5 × 5 cm). Briefly, in the acquisition trial a mouse was placed in the lighted chamber, and when the mouse entered the dark chamber, a 0.3 mA electrical shock of 2 s durations was delivered through floor grids. Ginsenoside Rg5, Rh3 (5, 10 and 20 mg/kg, *p.o.*) or donepezil (5 mg/kg, *p.o.*) as a positive control were orally given 1 h before treatment with scopolamine. Memory impairment was induced by treatment with scopolamine (1 mg/kg, *i.p.*) and the maze task was performed 30 min after treatment with scopolamine. A retention trial was performed 24 h after the acquisition trial, and latency times to reenter the dark chamber were measured. The success rate was then calculated as the number of mice that did not enter the dark compartment divided by the total number of mice and expressed as a percentage (%). The maximum entry latency time allowed in the acquisition session and retention session was 180 and 300 s, respectively.

2.5. Y-maze task

Y-maze is a three-arm horizontal maze (40 cm-long and 3 cm-wide with 12 cm-high walls) in which the arms are symmetrically disposed at 120° angles from each other. The maze floor and walls were constructed from dark opaque polyvinyl plastic as has been described previously (Joh et al., 2012). Mice were initially placed within one arm, and the sequence (i.e., ACABC, etc) and number of arm entries were recorded manually for each mouse over 8 min period. An actual alternation was defined as entries into all three arms on consecutive choices (i.e., ABC, CAB or BAC but not ABA). Maze arms were thoroughly cleaned between tasks to remove