Effect of Black Ginseng on Memory Improvement in the Amnesic Mice Induced by Scopolamine

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Abstract: This study compared the effects of black, white, and red ginseng extracts (WGE, RGE, BGE, 200 mg/kg, p.o.) on learning and memory deficits associated with scopolamine treatment (SCOP, 2 mg/kg, i.p.). Tacrine (THA, 10 mg/kg, p.o.) was used as a positive control. Ginseng significantly reversed SCOP-induced memory impairment in the passive-avoidance test and also reduced escape latency in training trials of the Morris water maze test. The increased acetylcholinesterase (AChE) activity produced by SCOP was significantly inhibited by WGE and RGE (p<0.001). SCOP administration had no effect on choline acetyltransferase (ChAT) activity, but RGE and BGE significantly increased ChAT activity (p<0.05). SCOP administration increased oxidative damage in the brain. Treatment of amnesic mice with ginseng extracts decreased malondialdehyde (MDA) levels and restored superoxide dismutase (SOD) and catalase (CAT) activity to control levels. These results suggest that black ginseng enhances cognitive activity by regulation of cholinergic enzymes and antioxidant systems.

Key words: back ginseng, scopolamine, acetylcholinesterase, choline acetyltransferase

INTRODUCTION

Alzheimer’s disease produces significant cognitive impairment that arises from dysfunction in numerous neurotransmitter systems, particularly from damage to cholinergic neurons known to play an important role in learning and memory [1]. Cholinergic depletion is used as a marker of neurological pathology and is associated with memory loss and the severity of Alzheimer’s disease symptoms [2]. Scopolamine (SCOP) is a muscarinic cholinergic receptor antagonist that causes learning and memory impairments in humans and animals similar to those observed in Alzheimer’s patients. It is also widely used in animal models to evaluate the effects of potential anti-dementia drugs [3, 4].

Ginsenosides, the main active constituents in ginseng, are reported to have pharmacological effects on the central nervous system (CNS), as well as possessing anti-cancer, anti-diabetic, anti-oxidative, anti-ageing, and immune-strengthening effects [5, 6]. The discovery of the effects of specific ginsenosides has led attempt structural conversion of the specific ginsenoside. For example, nine repeated cycles of steaming, commonly used for Rehmannia root preparation, have been applied to ginseng to make black ginseng, which can increase ginsenoside Rg3 in red ginseng. Ginsenoside Rg3 is known for its neuroprotective, anti-anemic, and analgesic effects [7, 8] and is being mass-produced in China, where has been successfully commercialized as anti-cancer treatment agent [9].

We compared the effects of black ginseng, white ginseng, and red ginseng on SCOP-induced memory impairment in mice using the passive-avoidance test and Morris water maze. We also investigated ginseng’s anti-oxidant activity, effects on acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) activity, enzymes responsible for acetylcholine (ACh) degradation and synthesis, and effects on SCOP-induce oxidative brain damage.

MATERIALS AND METHODS

Ginseng extract preparation

White ginseng and red ginseng made from 4-year-old
ginseng were purchased from a local ginseng center (Geumsan, Korea). To prepare black ginseng, ginseng was subjected to nine cycles of steaming at 98°C for 3 hr followed by drying at 65°C for 18 hr. To prepare the extract, ginseng was crushed into powder and ultrasonicated three times in 10 volumes of 20% ethanol at 50°C for 1 hr, then was filtered, lyophilized. The extraction yield of white ginseng, red ginseng, and black ginseng was 36.02%, 48.44%, and 54.50%, respectively.

Saponin analysis
Saponin determination was analyzed as described by Shi et al. [10], with the following modifications. To extract saponin, 1 g of white ginseng, red ginseng, or black ginseng was added to 20 ml of 20% ethanol and ultrasonicated three times, after which the water-saturated butanol layer was concentrated in vacuo. For saponin analysis, the sample was dissolved in 20 ml of distilled water and transferred to a separatory funnel containing the same volume of ethyl ether. Lipid components in the sample were removed by extracting with ethyl ether three times. The sample was further extracted with 20 ml of water-saturated butanol three times, after which the water-saturated butanol layer was concentrated in vacuo. The samples were then dissolved in 10 ml of 80% methanol and filtered through a 0.45-µm membrane filter. Saponin levels were quantified by HPLC analysis (SPD 20A, SIMADZU, Kyoto, Japan) using an ACE 5 C18 column (250 × 0.4 mm, 5 µm) and UV detector (203 nm). The mobile phase was a gradient of water and acetonitrile. To elute saponin, the acetonitrile concentration was adjusted as follows: 0–30 min, 20%; 30–60 min, 20–45%; 60–78 min, 45–75%; 78–80 min, 75–80%; 80–100 min, 80–100%. After injecting 10 µL of sample, the mobile-phase flow rate was adjusted to 1 mL/min. As controls, ginsenoside standards (Rg1, Re, Rf, Rb1, Rc, Rb2, Rd, 20(S)-Rg3, 20(R)-Rg3) with > 98% purity were purchased from Hongjiu Biotech Co., Ltd. (Jilin, China).

Animals
ICR mice, 8 weeks old and weighing 25–30 g, were purchased from DaeHan Biolink Co. Ltd. (Eumseong-gun, Korea) and acclimated for 1 week at 23 ± 2°C, 55 ± 5% humidity, with a 12:12-hr dark:light cycle. Mice were fed water and food ad libitum. Animals were cared for under the guidelines of the United States National Institutes of Health (No. 85-23, revised 1985), and all experiments were approved by Chungnam National University animal experiments ethics committee.

Administration of drugs
Scopolamine (Sigma, St. Louis, MO, USA) was injected to produce learning and memory deficits (2 mg/kg, i.p.), and tacrine, a cholinesterase inhibitor was used as a positive control (10 mg/kg, p.o.). Ginseng extract (200 mg/kg, p.o.) was administered as described by Qiao et al. [11] and Lee et al. [12]. Ginseng extract and THA were administered 1 hr before behavioral experiments, and SCOP was administered 30 min before to induce memory impairments. All drugs were dissolved in saline, and the control group received equal volumes of saline.

Passive-avoidance test
Passive avoidance test was carried out in identical illuminated and non-illuminated boxes (Jungdo Bio & Plant Co. Ltd, Seoul, Korea). The illuminated compartment (20 × 20 × 20 cm) contained a 100 W bulb. The non-illuminated compartment had a floor (20 × 20 × 20 cm) composed of 2 mm stainless steel rods spaced 1 cm apart. These two compartments were separated by a guillotine door (5 × 5 cm). For the acquisition trial, mice were initially placed in the illuminated compartment and the door between the two compartments was opened 10 sec later. When mice entered the dark compartment, the door automatically closed and an electrical foot shock (0.5 mA) of 3 sec duration was delivered through the stainless steel rods (acquisition trial). Twenty-four hours after the acquisition trial, mice were again placed in the illuminated compartment to test retention (retention trial). The time taken for a mouse to enter the dark compartment after the door opened was defined as latency. If a mouse did not enter the dark compartment within 300 sec, it was assumed that the mouse had remembered the single ‘acquisition’ trial experience.

Morris water maze test
To evaluate spatial learning, the Morris water maze test was used, as described by Morris [13]. A round tub (diameter 90 cm, height 50 cm) was filled with water (23 ± 2°C) to a height of 30 cm, and a clear round platform (10 cm in diameter) was placed in one location 1 cm below the water level. Before the experiment, white dye was dispersed evenly throughout the water so that platform was not visible. On the first test day, the mouse was allowed to swim freely for 60 sec with no platform in the tub. For the next 4 days, the mouse was trained three times per day with different sites in the tub. Once the mouse located the platform, it was allowed to stay on it for 10 sec. If the mouse did not find the platform within 120 sec, it was placed on the platform for 10 sec. The time interval between