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Research article

Administration of red ginseng ameliorates memory decline in aged mice

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ABSTRACT

Background: It has been known that ginseng can be applied as a potential nutraceutical for memory impairment; however, experiments with animals of old age are few.
Methods: To determine the memory enhancing effect of red ginseng, C57BL/6 mice (21 mo old) were given experimental diet pellets containing 0.12% red ginseng extract (approximately 200 mg/kg/d) for 3 mo. Young and old mice (4 mo and 21 mo old, respectively) were used as the control group. The effect of red ginseng, which ameliorated memory impairment in aged mice, was quantified using Y-maze test, novel objective test, and Morris water maze. Red ginseng ameliorated age-related declines in learning and memory in older mice. In addition, red ginseng's effect on the induction of inducible nitric oxide synthase and proinflammatory cytokines was investigated in the hippocampus of aged mice.
Results: Red ginseng treatment suppressed the production of age-processed inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-α, and interleukin-1β expressions. Moreover, it was observed that red ginseng had an antioxidative effect on aged mice. The suppressed glutathione level in aged mice was restored with red ginseng treatment. The antioxidative-related enzymes Nrf2 and HO-1 were increased with red ginseng treatment.
Conclusion: The results revealed that when red ginseng is administered over long periods, age-related decline of learning and memory is ameliorated through anti-inflammatory activity.

1. Introduction

Memory impairment is considered one of the most predominant outcomes of aging. Thus, it is very important to prevent memory decline for healthy aging [1–4]. Much of the research on aging and age-related diseases focuses on the role of oxidative stress and inflammation, or the damage accompanying these processes. Also, several resources have suggested that the hippocampal complex is a major region for memory deficits in older animals [1–3]. Alzheimer's disease, senile dementia, and other age-related diseases have exhibited age-related pathological changes specifically in the hippocampal region [5–9]. The imbalance between reactive oxygen species (ROS) and antioxidant scavenging during aging can lead to an oxidative environment that is explained by the free radical theory of aging. Therefore, aging is thought to be related to an exacerbation of oxidative damage associated with accelerated inflammation [10–13].

In traditional Asian medicine, several different herbs have been utilized to treat brain injury-related neurological disorders. Ginseng or ginseng extract containing prescriptions have had significant ameliorating effects on treating neurological symptoms in older humans [14]. The root of *Panax ginseng* has been a widely used herbal medicine for many centuries as a general tonic for human healthcare [15,16]. Their amounts and composition can vary depending on the types of *P. ginseng* (i.e., red and white ginsengs) and are taken in various commercial forms [17]. Red ginseng is a steamed form, and through heat-induced chemical transformation, it possesses enhanced and newly formed pharmacological properties [18,19]. It has been known that some red ginseng-containing traditional medicines have had significant

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therapeutic effects in treating stroke damage [20–22]. Further evidence has shown the amelioration of learning and memory deficits in both old and young rats after red ginseng administration [4]. Some previous studies have suggested that the non-saponin fraction of red ginseng may stimulate and improve learning and memory functions. The objective of this study is to investigate the use of red ginseng to ameliorate the decline of learning and memory in older mice.

2. Materials and methods

2.1. Animals and red ginseng administration

Male C57BL/6 mice (28–30 g) were purchased from Orient Lab Animal (Seoul, Korea). In each cage, five to six mice were given *ad libitum* access to food and water, and maintained at an ambient temperature (23°C) with a 12-h diurnal light cycle (lights on from 07:00 AM to 07:00 PM). Mice were housed in transparent polycarbonate cages at the laboratory and raised until they were 4 mo (young group) or 20–21 mo (aged group) old. Mice were fed experimental diet pellets containing 0.12% red ginseng extract (approximately 200 mg/kg/d) for 3 mo. Korean Red Ginseng (KRG) extract was produced by the Korea Ginseng Corporation (Seoul, Korea) from the roots of 6-yr-old red ginseng, *P. ginseng* Meyer, which was harvested in the Republic of Korea. KRG was refined by steaming fresh ginseng at 90–100°C for 3 h, and was then dried at 50–80°C. KRG was derived from red ginseng water extract at the temperature range of 85–90°C for 8 h while circulating hot water three times. The water content of the collected extract was 36% of the total weight. Korea Ginseng Corporation has declared the general composition of KRG as follows: ash, 2.5%; total fat, 0.05%; total crude saponin, 70 mg/g; and total ginsenosides, 20 mg/g.

All behavioral experiments were performed in the room adjacent to where the animals were housed. Mice were maintained under the same temperature and lighting conditions. All experiments using male C57BL/6 mice followed the Animal Care and Use Guidelines of School of Medicine, Ewha Womans University, Korea. The Y-maze test was conducted 2 d after the last red ginseng treatment, followed by the novel objective test and the water maze test, one test at a time at 2-d intervals.

2.2. Y-maze task

The Y-maze tested spontaneous spatial recognition as a hippocampus-dependent memory test. The Y-maze, a horizontal maze consisting of three arms (40 cm × 3 cm × 12 cm), has arms symmetrically disposed at a 120° angle. The floor and walls of the maze were made with a dark-colored opaque polyvinyl plastic. Mice were placed in one arm. The sequence (e.g., ABCAB) and number of arm entry were manually recorded for each mouse for an 8-min period. Entry into all three arms on consecutive choices was defined as an actual alteration (i.e., ABC, CAB, or BCA, but not BAB). Between tests, maze arms were cleaned to remove residual odors. Memory enhancement was tested 1 h after the final administration of red ginseng or saline. The alternation percentage was defined as the following equation: % alternation = [(number of alternations)/(total arm entries − 2)] × 100. The arm entry numbers served as a locomotor activity indicator.

2.3. Novel object recognition

This test was used to measure objective recognition. The arena consisted of a cage bottom and black walls (30 cm × 40 cm × 20 cm). Objects were of the same size but differed in shape, color, and surface texture. On Day 1, mice were individually habituated to the arena for 8-min sessions, wherein the animals were able to freely examine the open field box. Six hours later, two identical objects were deposited in each corner, and each animal was allocated 8 min to examine the objects. Each pair was used the same number of times. On Day 2, a novel object replaced one object in a counterbalanced fashion dependent on the object, side, and genotype. Each mouse was given 8 min to examine the familiar and novel objects while being video-recorded. Exploration was specified as either sniffing or touching the object with one or both forepaws. The exploration index was derived from the absolute exploration duration (T): $[(T_{\text{Novel}} - T_{\text{Familiar}})/(T_{\text{Novel}} + T_{\text{Familiar}})] \times 100$. This value is defined as an index of recognition memory while considering the individual differences compared to the total object exploration time.

2.4. Morris water maze test

We studied the spatial cognition of mice of both ages using standardized testing prior to behavioral studies in accordance with the Morris water maze protocol described in detail elsewhere [23]. The Morris water maze consists of a circular pool [90 cm (diameter) × 45 cm (height)] including an inner surface. Water mixed with 3,000 mL of milk filled the pool to a depth of 30 cm (20°C). The room in which the pool was housed was dimly lit and soundproof with various visual cues. Conceptually, the pool was divided into quadrants. In one of the pool quadrants, a white platform [6 cm (diameter) × 29 cm (height)] was submerged 1 cm beneath the water surface to prevent it from being seen at water level. Day 1 was committed to swimming training in 60-s periods with no platform. During the next 4 d, mice had four trials each day with the platform submerged in place. The mouse was permitted to rest on the platform for 10 s after locating it. In the case where the mouse did not locate the platform within the given 60 s, researchers placed it on the platform for 10 s. After each trial, the animal was returned to its home cage to dry under a heat lamp. The time prior to the start of the next trial was 30 s. Latency, or the time taken to locate the hidden platform, was recorded using a video camera-based Ethovision System (Nodulus, Wageningen, the Netherlands) during each trial. At the start of each training trial, mice were deposited facing the pool wall in the water in a different order of pool quadrants each day. One day after the final training trial session, mice underwent a probe trial session where the platform was removed, requiring the mice to swim for the full 60 s to search for the platform. Swimming time in the pool quadrant where the platform was previously based was recorded.

2.5. Immunoblot analysis

Using homogenized buffer (0.25M sucrose, 10mM Tris–HCl, pH 7.4, 0.5mM EDTA, 1mM phenylmethylsulfonyl fluoride, 1mM Na_3VO_4), brain tissue was homogenized and centrifuged twice at 16,300 × g for 15 min at 4°C. A protein assay kit (Pierce Chemical, Rockford, IL, USA) was used to assay samples for protein concentration. Proteins were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline/Tween 20 solution. The blots were incubated with inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2; Millipore Technology Inc., Danvers, MA, USA), tumor necrosis factor (TNF)-α, and interleukin (IL)-1β (both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). As an internal control, GAPDH (glyceraldehyde 3-phosphate dehydrogenase) (Santa Cruz Biotechnology, Inc.) was performed. Tris-buffered saline/Tween 20 was used to wash the blots, and then horseradish peroxidase-conjugated secondary antibodies (Cell Signaling