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 nonsaponin 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 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.

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 allowed 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): (TNovel − T familiar)/(TNovel + T familiar) × 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 was 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 Na3VO4), 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). 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
Technology Inc., Beverly, MA, USA) were applied. Blots were then developed using the enhanced chemiluminescence detection kit (GE Healthcare, Buckinghamshire, UK).

2.6. Polymerase chain reaction

TRIzol (Invitrogen, Carlsbad, CA, USA) was used according to the manufacturer’s instructions to isolate total RNA from the hippocampus. cDNA synthesis was accomplished by reverse-transcribing 2 μg of total RNA using the SuperScript First-Strand Synthesis System (Invitrogen). cDNA was then amplified with polymerase chain reaction using primers for nuclear factor E2-related factor 2 (Nrf2) (F: TCTCCTGCTGGAAAAAGAA, R: AATGTGGCTGCTGTGCTTTA) and hemeoxygenase-1 (HO-1) (F: ATACCGCTACTGGGTGAC, R: TGTCACCGTGTGCTGACCT). Polymerase chain reaction products were separated using 1% agarose gel electrophoresis and visualized using ethidium bromide staining.

2.7. Statistical analysis

All values were expressed as mean ± standard deviation. Results were subjected to one-way analysis of variance (ANOVA) using the Newman–Keuls multiple comparison test. Differences with \( p < 0.05 \) were considered statistically significant. All analyses were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Amelioration of memory decline in aged mice in Y-maze task

To determine if red ginseng has the potential to modulate memory function, older mice (C57BL/6 male, 20–21 mo old) were tested using the Y-maze task. Mice were administered with pellets containing 0.12% red ginseng extract (200 mg/kg/d) for 3 mo. Aged mice demonstrated spatial memory function impairment as they exhibited less spontaneous alteration than that of young mice (male, 4 mo old) in the Y-maze task. Red ginseng was determined to have enhanced spatial memory as red ginseng-treated mice displayed higher spontaneous alteration in the novel arm of the maze than that of the saline group \( [F(2,21) = 8.388, p = 0.0021, \text{one-way ANOVA}] \). Red ginseng resist spatial memory retention as there was a significant effect on the percentage of alternation (Fig. 1).

3.2. Amelioration of memory decline in aged mice in novel object task

The time spent with the novel object compared to the total time spent exploring both objects represents a long-term memory index. Mice were administered with pellets containing 0.12% red ginseng extract (200 mg/kg/d) for 3 mo. Treatment with red ginseng enhanced memory function during the novel object recognition task (Fig. 2). During the training session (Fig. 2A), no significant difference in time was observed among the older mice groups when investigating each object pair, and there were no significant differences in total exploration times among the test groups. During training, mice exhibited no preference for any single object over another. There was also no difference between aged mice and the remaining mice in terms of exploration time, implying that the experimental groups were equally motivated on average to explore objects (Fig. 2B). However, after presenting the novel object, mice treated with red ginseng demonstrated a preference for the unfamiliar object after 24 h of retention \( [F(2,21) = 11.41, p = 0.004, \text{one-way ANOVA}] \).

3.3. Amelioration of memory decline in aged mice in Morris water maze test

We used the Morris water maze test to evaluate red ginseng’s effect on learning and spatial memory. Mice were administered with pellets containing 0.12% red ginseng extract (200 mg/kg/d) for 3 mo. The aged group demonstrated longer escape latencies during the training sessions than those of the younger group. Mice treated with red ginseng had significantly shorter escape latencies, which were prolonged in the aged mice group \( (p < 0.05) \). After the final day of training trials, swimming times in the platform quadrant for the red ginseng-treated mice were significantly less than those of the saline vehicle-treated younger group \( (p < 0.05) \) (Fig. 3). The red ginseng-treated older mice had significantly more shortened swimming times within the platform quadrant \( [F(2,21) = 17.69, p = 0.0001, \text{one-way ANOVA}] \). However, there were no significant differences observed between groups in swimming speeds. During the training sessions in this experiment, older mice had increased

Fig. 1. Effect of red ginseng on memory in Y-maze task. For 3 mo, mice were administered experimental diet pellets containing 0.12% red ginseng extract. Spontaneous alteration behavior and number of entries during 8-min sessions were measured. Data analyses were completed using one-way ANOVA for multiple comparison and Student–Newman–Keuls test as post hoc test. All values are expressed as mean ± SD \( \overline{\text{SD}}(n = 6) \). * \( p < 0.05 \) in comparison with young group. ** \( p < 0.05 \) in comparison with aged control group. ANOVA, analysis of variance; SD, standard deviation.
Fig. 2. Effect of red ginseng on memory in novel object recognition test. (A) The training \( (\text{Novel} - \text{Familiar})/\left(\text{Novel} + \text{Familiar}\right) \) C2100 session. (B) The test session was conducted 24 h after the training session. During the testing session of the novel object recognition memory task, mice were treated with saline or red ginseng extract. Results are expressed as mean ± SD (\( n = 6 \)). * \( p < 0.05 \) in comparison with each group. SD, standard deviation.

Fig. 3. Effect of red ginseng on memory in Morris water maze test. Red ginseng extract was administered to mice for 3 mo. The training trial and the probe trial sessions were performed as described in Materials and methods. Results are expressed as mean ± SD (\( n = 6 \)). * \( p < 0.05 \) in comparison with young group. * \( p < 0.05 \) in comparison with aged control group. SD, standard deviation.
escape latency time; however, the escape latency time shortened on Day 4 after red ginseng treatment. At the probe trial session, treatment with red ginseng increased the swimming time in the platform quadrant. Escape latency decreased from day to day since the first trial, which represents long-term or reference memory, whereas short-term or working memory is represented by the difference between the first and second trials of sessions [24]. The time in the platform quadrant reflects spatial memory change [25]. The results of this experiment suggest that treatment with red ginseng improves long-term memory in the older mouse group.

3.4. Downregulation of iNOS, COX-2, TNF-α, and IL-1β protein expression by long-term red ginseng administration in aged mice

Red ginseng demonstrated an anti-inflammatory effect on age-related responses along with iNOS, COX-2, TNF-α, and IL-1β expressions. Mice were administered pellets containing 0.12% red ginseng extract (200 mg/kg/d) for 3 mo. The elevated levels of iNOS, COX-2, TNF-α, and IL-1β in the hippocampus of the brain were suppressed after the administration of red ginseng (Fig. 4). These results revealed that red ginseng has an anti-inflammatory effect on age-related proinflammatory signal expression in aged mice.

3.5. Elevation of Nrf2 and HO-1 expression by long-term red ginseng administration in aged mice

Older mice were administered with 0.12% red ginseng extract (200 mg/kg/d) for 3 mo. As a result, the expression levels of the nucleus Nrf2 and hippocampus HO-1 of the brain increased after the administration of red ginseng (Fig. 5). These results revealed that red ginseng has an antioxidative effect in older mice.

4. Discussion

This study has demonstrated that red ginseng ameliorated the decline of both learning potential and memory retention in aged mice. These findings indicate that red ginseng affects cognitive function in aged mice. Ginseng extract administration has been shown in previous animal studies to influence learning and memory functions [4,26–28]. Ginsenosides Rb1 and Rg1, the main elements of the saponin fraction of ginseng, have been reported as being responsible for improving learning and memory functions in animals [29,30]. Studies have reported that ginseng saponin fraction modulates neuronal responses to neurotransmitted excitation or stress [23,31,32]. Treatment with the nonsaponin fraction ameliorated a decline in memory of older rats [4]. Results of this study suggested that red ginseng contains substances capable of improving learning and memory in aged mice models. Furthermore, ginseng extracts have demonstrated memory-enhancing effects in patients with Alzheimer's disease [33]. Ginseng fraction decreased the release of Aβ1–42 and prevented Aβ1–40-induced memory dysfunction in mice. In addition to this, amyloid plaque deposition in a transgenic Alzheimer's disease mice model was attenuated by long-term oral administration of ginseng fraction [34].

Many studies on P. ginseng suggest that ginseng possesses properties with beneficial effects such as anti-inflammation and antioxidation activities [1]. For a long time, red ginseng has been used as a preventive medicine to promote immune function as well as prevent inflammation for many diseases in Korea. Ginsenosides have been shown to possess an anti-inflammatory effect of suppressing the nitric oxide (NO) level and nuclear factor-kappa B signaling in lipopolysaccharide-induced microglial cells [35]. Several studies have also reported other effects such as neuroprotectiveness of Rg1 or its metabolites.

It has been known that inflammation in the central nervous system increases in conjunction with aging [36]. Elevated levels of proinflammatory cytokines IL-1β and TNF-α in the brain of normal aged mice or rats are also present [37–39]. Furthermore, chronic inflammation is related to the progression of age-related diseases including Alzheimer's disease and Parkinson disease [40,41]. Our studies demonstrated that proinflammatory cytokines expression is upregulated during aging, contributing to neuroinflammation in the brains of aged mice. We have also determined that ginseng has an anti-inflammatory effect by the downregulation of the molecules iNOS, COX2, TNF-α, and IL-1β in aged mice.

Aging is also typically associated with increased oxidative stress. An imbalance between ROS generation and removal, and oxidative stress development plays a significant role in age-relative diseases [12,42]. Therefore, dietary antioxidative supplementation could defend against the effects of ROS that encourage the development of many chronic diseases [43]. It has been reported that

![Fig. 4. Effect of red ginseng on expressions of inflammation-related enzyme and cytokine in the hippocampus of mice. Mice were administered red ginseng extract for 3 mo. Expression of iNOS, COX-2, TNF-α, and IL-1β in the hippocampus of aged mice was measured using immunoblot analysis. Long-term treatment of red ginseng decreased the expression of both iNOS and COX-2 in aged mice. β-Actin was used as an internal control. All values are expressed as mean ± SD (n = 6). * p < 0.05, significant difference between the control group and red ginseng treated group in aged mice. COX-2, cyclooxygenase-2; IL-1, interleukin-1; iNOS, inducible nitric oxide synthase; SD, standard deviation; TNF, tumor necrosis factor.](image-url)
downregulation of GSH (glutathione, reduced form) is apparent in the liver, kidneys, heart, and lungs of older rats compared to those of younger rats [44–46]. In the preliminary investigation, GSH level was raised to normal levels after aged mice were administered with red ginseng (data not shown). These results indicated that red ginseng improved free radical scavenging activity in aged mice, which may be attributable to the active principles of red ginseng to scavenge free radicals and restore the GSH level. More evidence presented has also revealed that ginsenosides increased cellular GSH levels by diminishing the free radicals [47]. This result strongly implies that the antioxidant effect of red ginseng is due to the reduction of oxidative damage in older mice. Several studies have reported that Nrf2, a transcription factor, is essential for the regulation of antioxidative gene expressions. Antioxidant enzymes play a crucial role against oxidative stress. After oxidative stress exposure, Nrf2 is translocated to the nuclei. Elevation of Nrf2 after relocating to the nucleus accelerates antioxidant enzyme expression such as NAD(P)H dehydrogenase (quinone 1) (NQO1) and HO-1 [48–52]. The evidence attesting to Nrf2’s protective role against pathological conditions or oxidative stress, including neurodegenerative diseases and liver injuries, has been reported in previous studies [50,51,53]. Therefore, expression levels of Nrf2 and HO-1 in the hippocampus of older mice were measured. In this study, administration of red ginseng extract has enabled significant improvements in Nrf2 and HO-1 oxidative enzyme activity in aged mice. These results indicated that red ginseng has an anti-oxidative effect and improves the memory of aged mice. In conclusion, red ginseng extract administration ameliorated inflammatory and oxidative stress via the reduction of proinflammatory cytokines while enhancing the memory by modulation of antioxidant enzyme activity.

Conflicts of interests

The authors declare they have no conflict of interests.

Acknowledgments

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