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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

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: TCTCCTCGCTGGAAAAAGAA, R: AATGTGCTGGCTGTGCTTTA) and hemeoxygenase-1 (HO-1) (F: ATACCCGCTACCTGGGTGAC, R: TGTCACCCTGTGCTTGACCT). 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 [$F(2,21) = 8.388$, $p = 0.0021$, one-way ANOVA]. Red ginseng resists 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 [$F(2,21) = 11.41$, $p = 0.004$, 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 [$F(2,21) = 17.69$, $p = 0.0001$, 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

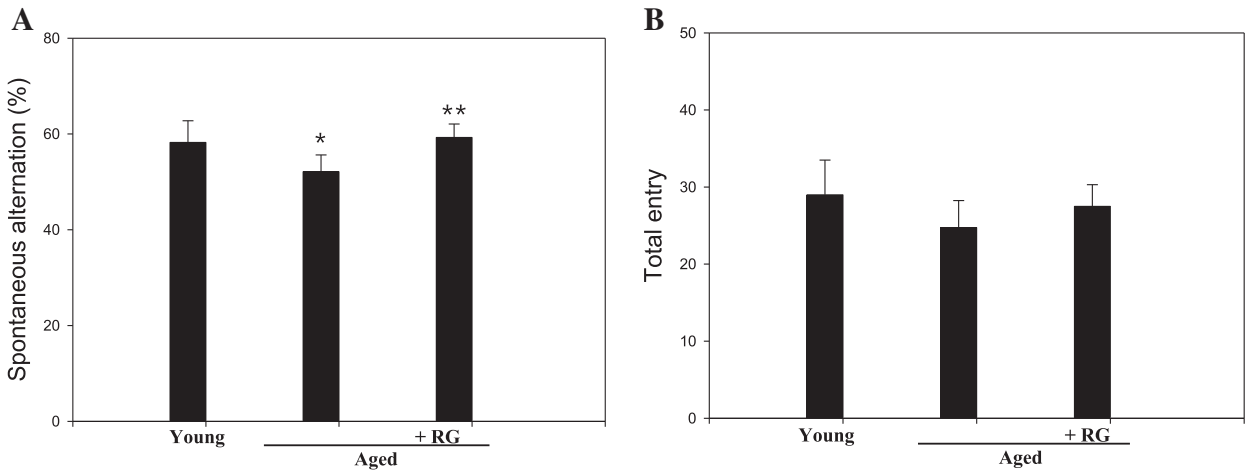

Fig. 1. Effect of red ginseng on memory in Y-maze task. For 3 mo, mice were administered experimental diet pellets containing 0.12% red ginseng extract. Spontaneous alteration behavior and number of entries during 8-min sessions were measured. Data analyses were completed using one-way ANOVA for multiple comparison and Student–Newman–Keuls test as *post hoc* test. All values are expressed as mean ± SD ($n = 6$). * $p < 0.05$ in comparison with young group. ** $p < 0.05$ in comparison with aged control group. ANOVA, analysis of variance; SD, standard deviation.

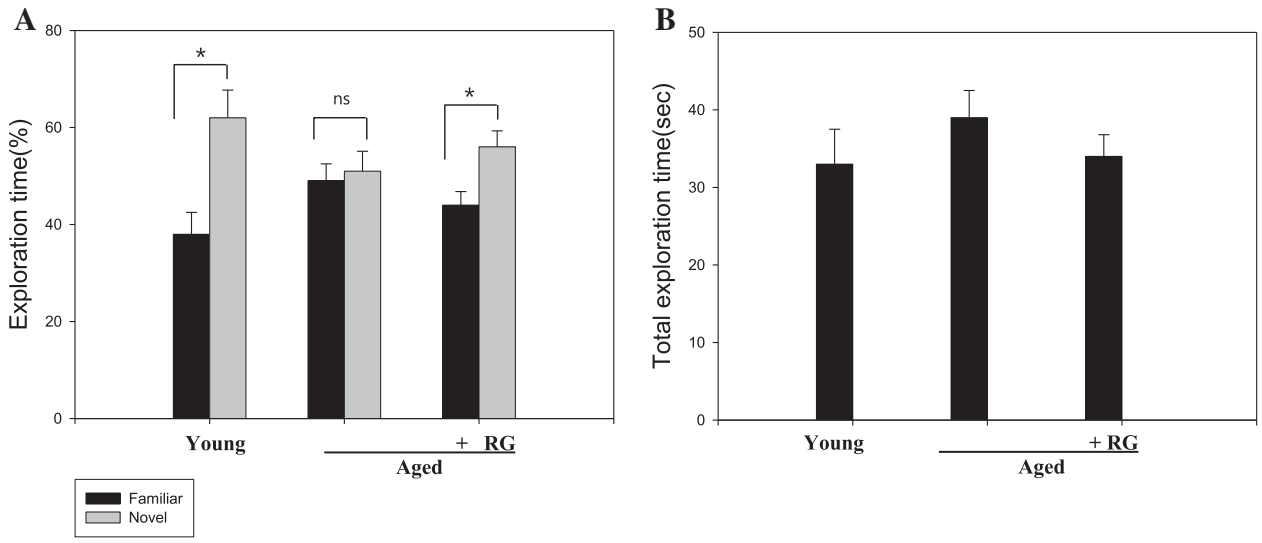

Fig. 2. Effect of red ginseng on memory in novel objective recognition test. (A) The training $[(T_{Novel} - T_{Familiar})/(T_{Novel} + T_{Familiar})] \times 100$ session. (B) The test session was conducted 24 h after the training session. During the testing session of the novel object recognition memory 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.

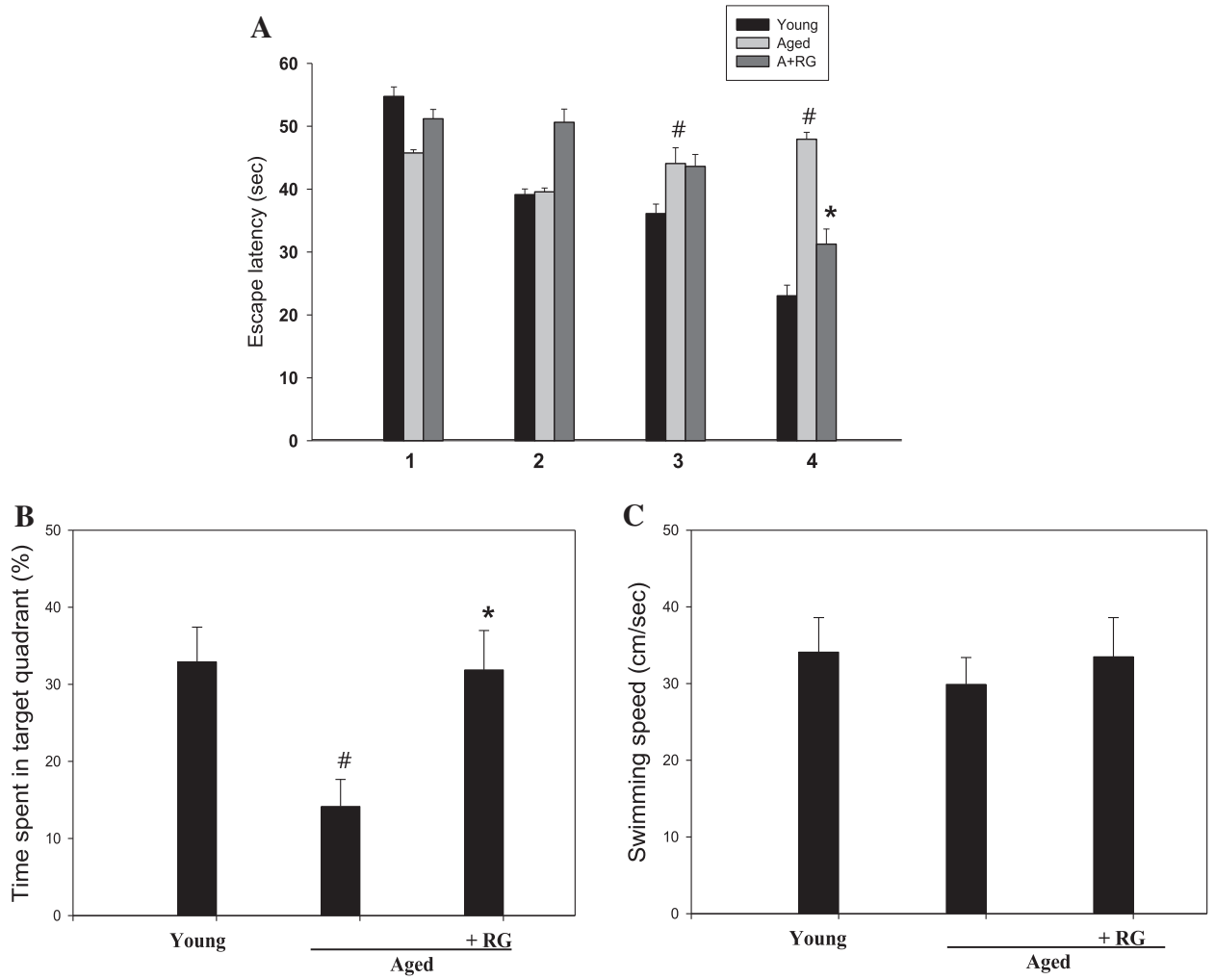

Fig. 3. Effect of red ginseng on memory in Morris water maze test. Red ginseng extract was administered to mice for 3 mo. The training trial and the probe trial sessions were performed as described in *Materials and methods*. Results are expressed as mean ± SD ($n = 6$). # $p < 0.05$ in comparison with 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 mice 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 $A\beta_{1-42}$ and prevented $A\beta_{1-40}$-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. Ginsenoside has 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

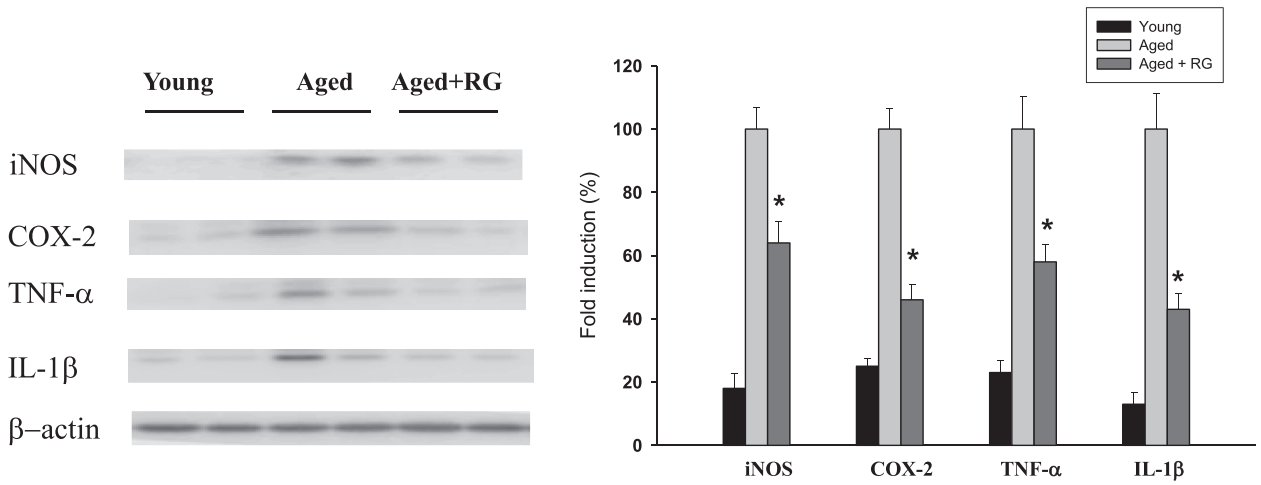

Fig. 4. Effect of red ginseng on expressions of inflammation-related enzyme and cytokine in the hippocampus of mice. Mice were administered red ginseng extract for 3 mo. Expression of iNOS, COX-2, TNF-α, and IL-1β in the hippocampus of aged mice was measured using immunoblot analysis. Long-term treatment 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.

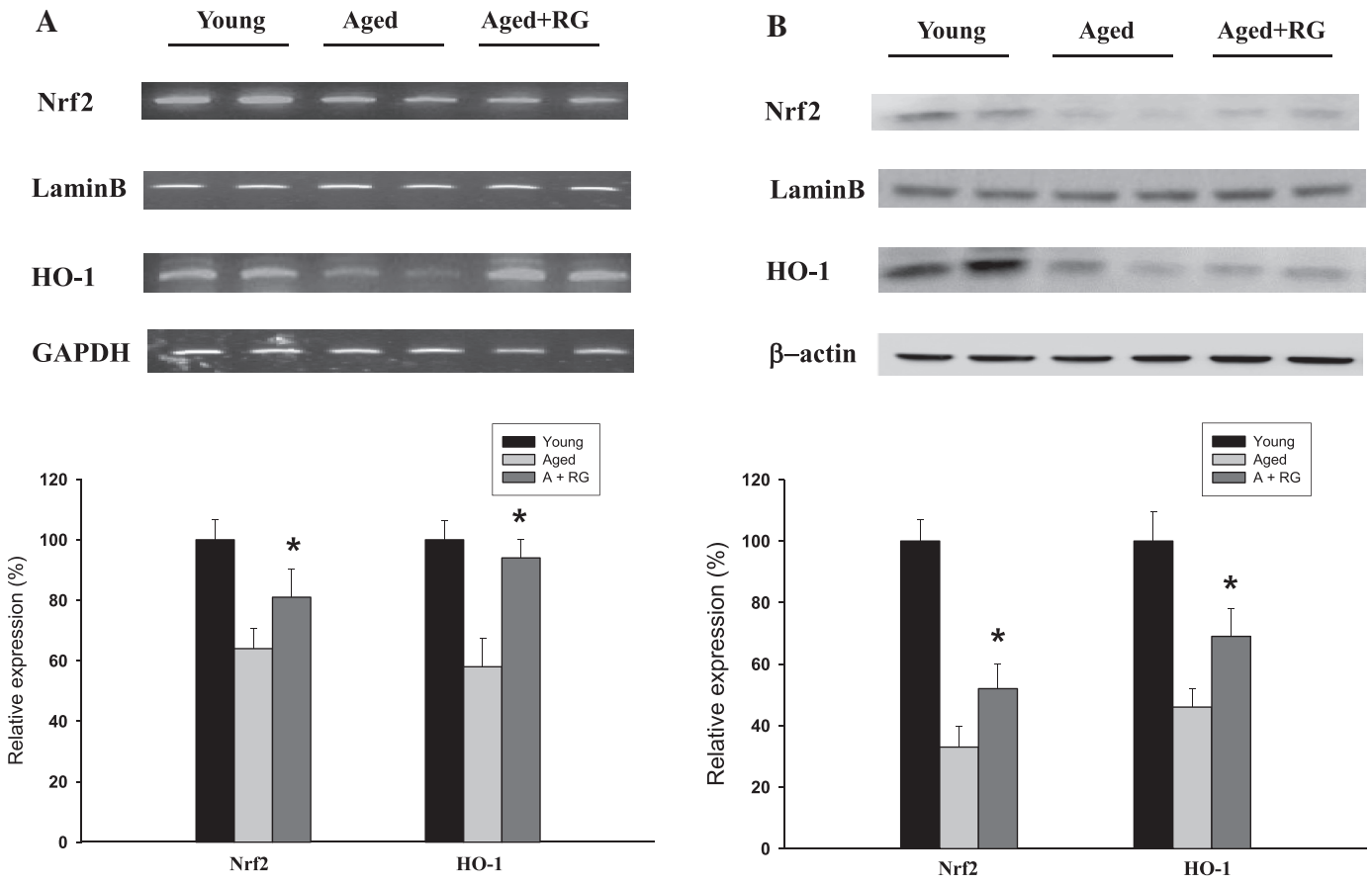

Fig. 5. Change in Nrf2 and HO-1 expression by red ginseng administration. Mice were administered red ginseng extract for 3mo. Expression of Nrf2 (nucleus faction) and HO-1(total fraction) in the hippocampus of the brain was measured using (A) PCR and (B) immunoblot analysis. Red ginseng treatment increased the expression of Nrf2 and HO-1 in red ginseng-treated aged mice. All values are expressed as mean ± SD from three independent experiments. * $p < 0.05$, significant difference between the control group and red ginseng treated group in aged mice. HO-1, hemeoxygenase-1; Nfr2, nuclear factor E2-related factor 2; PCR, polymerase chain reaction; SD, standard deviation.

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 antioxidant enzyme activity in aged mice. These results indicated that red ginseng has an antioxidative 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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References

[1] Barnes CA, McNaughton BL. An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses. Behav Neurosci 1985;99:1040–8.
[2] Gallagher M, Pelleymounter MA. An age-related spatial learning deficit: choline uptake distinguishes "impaired" and "unimpaired" rats. Neurobiol Aging 1988;9:363–9.
[3] Rapp PR, Gallagher M. Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. Proc Natl Acad Sci U S A 1996;93:9926–30.
[4] Zhong YM, Nishijo H, Uwano T, Tamura R, Kawanishi K, Ono T. Red ginseng ameliorated place navigation deficits in young rats with hippocampal lesions and aged rats. Physiol Behav 2000;69:511–25.
[5] Ott BR, Saver JL. Unilateral amnesic stroke. Six new cases and a review of the literature. Stroke 1993;24:1033–42.

[6] Heinonen O, Soininen H, Sorvari H, Kosunen O, Paljarvi L, Koivisto E, Riekkinen Sr PJ. Loss of synaptophysin-like immunoreactivity in the hippocampal formation is an early phenomenon in Alzheimer's disease. Neurosci 1995;64:375–84.
[7] Mizutani T, Kashara M. Degeneration of the intrahippocampal routes of the perforant and alvear pathways in senile dementia of Alzheimer type. Neurosci Lett 1995;184:141–4.
[8] Carr DB, Goate A, Phil D, Morris JC. Current concepts in the pathogenesis of Alzheimer's disease. Am J Med 1997;103:3S–10S.
[9] Corey-Bloom J, Sabbagh MN, Bondi MW, Hansen L, Alford MF, Masliah E, Thal LJ. Hippocampal sclerosis contributes to dementia in the elderly. Neurology 1997;48:154–60.
[10] Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 1993;90:7915–22.
[11] Goto S, Takahashi R, Kumiyama AA, Radak Z, Hayashi T, Takenouchi M, Abe R. Implications of protein degradation in aging. Ann N Y Acad Sci 2001;928:54–64.
[12] Johnson FB, Sinclair DA, Guarente L. Molecular biology of aging. Cell 1999;96:291–302.
[13] Chung HY, Kim HJ, Kim KW, Choi JS, Yu BP. Molecular inflammation hypothesis of aging based on the anti-aging mechanism of calorie restriction. Microsc Res Tech 2002;59:264–72.
[14] Terasawa K, Shimada Y, Kita T, Yamamoto T, Tosa H, Tanaka N, Saito Y, Kanaki E, Goto S, Mizushima N, et al. Choto-san in the treatment of vascular dementia: a double-blind, placebo-controlled study. Phytomedicine 1997;4:15–22.
[15] Tang W, Eisenbrand G. Chinese drugs of plant origin: chemistry, pharmacology, and use in raditional and modern medicine. 1st ed. Berlin: Springer-Verlag; 1992. p. 711.
[16] Oliynyk S, Oh S. Actoprotective effect of ginseng: improving mental and physical performance. J Ginseng Res 2013;37:144–66.
[17] Kang KS, Kim HY, Pyo JS, Yokozawa T. Increase in the free radical scavenging activity of ginseng by heat-processing. Biol Pharm Bull 2006;29:750–4.
[18] Konoshima T, Takasaki M, Tokuda H, Nishino H, Duc NM, Kasai R, Yamasaki K. Anti-tumor-promoting activity of majonoside-R2 from Vietnamese ginseng, *Panax vietnamensis* Ha et Grushv. (I). Biol Pharm Bull 1998;21:834–8.
[19] Park JD. Recent studies on the chemical constituents of Korean ginseng (*Panax ginseng* C. A. Meyer). Korean J Ginseng Sci 1996;20:389–415.
[20] Gong X, Sucher NJ. Stroke therapy in traditional Chinese medicine (TCM): prospects for drug discovery and development. Trends Pharmacol Sci 1999;20:191–6.
[21] He B, Chen P, Yang J, Yun Y, Zhang X, Yang R, Shen Z. Neuroprotective effect of 20(R)-ginsenoside Rg(3) against transient focal cerebral ischemia in rats. Neurosci Lett 2012;526:106–11.
[22] Cheon SY, Cho KJ, Lee JE, Kim HW, Lee SK, Kim HJ, Kim GW. Cerebroprotective effects of red ginseng extract pretreatment against ischemia-induced oxidative stress and apoptosis. Int J Neurosci 2013;123:269–77.
[23] Tachikawa E, Kudo K, Kashimoto T, Takahashi E. Effects of ginseng saponins on receptor stimulation-responses. Nihon Yakurigaku Zasshi 1997;110:126–31 [In Japanese].
[24] Kim HS, Hwang SL, Nah SY, Oh S. Changes of [^{3}H]MK-801, [3H]muscimol and [^{3}H]flunitrazepam binding in rat brain by the prolonged ventricular infusion of ginsenoside Rc and Rg1. Pharmacol Res 2001;43:473–9.
[25] Park HJ, Rhee MH, Park KM, Nam KY, Park KH. Effect of non-saponin fraction from *Panax ginseng* on cGMP and thromboxane A2 in human platelet aggregation. J Ethnopharmacol 1995;49:157–62.
[26] Nitta H, Matsumoto K, Shimizu M, Ni XH, Watanabe H. *Panax ginseng* extract improves the scopolamine-induced disruption of 8-arm radial maze performance in rats. Biol Pharm Bull 1995;18:1439–42.
[27] Nitta H, Matsumoto K, Shimizu M, Ni XH, Watanabe H. *Panax ginseng* extract improves the performance of aged Fischer 344 rats in radial maze task but not in operant brightness discrimination task. Biol Pharm Bull 1995;18:1286–8.
[28] Zhao R, McDaniel WF. Ginseng improves strategic learning by normal and brain-damaged rats. Neuroreport 1998;9:1619–24.
[29] Yamaguchi Y, Haruta K, Kobayashi H. Effects of ginsenosides on impaired performance induced in the rat by scopolamine in a radial-arm maze. Psychoneuroendocrinology 1995;20:645–53.
[30] Mook-Jung I, Hong HS, Boo JH, Lee KH, Yun SH, Cheong MY, Joo I, Huh K, Jung MW. Ginsenoside Rb1 and Rg1 improve spatial learning and increase hippocampal synaptophysin level in mice. J Neurosci Res 2001;63:509–15.
[31] Kim DH, Jung JS, Suh HW, Huh SO, Min SK, Son BK, Park JH, Kim ND, Kim YH, Song DK. Inhibition of stress-induced plasma corticosterone levels by ginsenosides in mice: involvement of nitric oxide. Neuroreport 1998;9:2261–4.
[32] Dou DQ, Zhang YW, Zhang L, Chen YJ, Yao XS. The inhibitory effects of ginsenosides on protein tyrosine kinase activated by hypoxia/reoxygenation in cultured human umbilical vein endothelial cells. Planta Med 2001;67:19–23.
[33] Lee ST, Chu K, Sim JY, Heo JH, Kim M. *Panax ginseng* enhances cognitive performance in Alzheimer disease. Alzheimer Dis Assoc Disord 2008;22:222–6.
[34] Hwang SH, Shin EJ, Shin TJ, Lee BH, Choi SH, Kang J, Kim HJ, Kwon SH, Jang CG, Lee JH, et al. Gintonin, a ginseng-derived lysophosphatidic acid receptor ligand, attenuates Alzheimer's disease-related neuropathies: involvement of non-amyloidogenic processing. J Alzheimers Dis 2012;31:207–23.
[35] Wu CF, Bi XL, Yang JY, Zhan JY, Dong YX, Wang JH, Wang JM, Zhang R, Li X. Differential effects of ginsenosides on NO and TNF-alpha production by LPS-activated N9 microglia. Int Immunopharmacol 2007;7:313–20.
[36] Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 2000;908:244–54.
[37] Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, Johnson RW. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. FASEB J 2005;19:1329–31.
[38] Maher FO, Nolan Y, Lynch MA. Downregulation of IL-4-induced signalling in hippocampus contributes to deficits in LTP in the aged rat. Neurobiol Aging 2005;26:717–28.
[39] Chen J, Buchanan JB, Sparkman NL, Godbout JP, Freund GG, Johnson RW. Neuroinflammation and disruption in working memory in aged mice after acute stimulation of the peripheral innate immune system. Brain Behav Immun 2008;22:301–11.
[40] Mrak RE, Griffin WS. Glia and their cytokines in progression of neurodegeneration. Neurobiol Aging 2005;26:349–54.
[41] Hirsch EC, Hunot S. Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol 2009;8:382–97.
[42] Rikans LE, Hornbrook KR. Lipid peroxidation, antioxidant protection and aging. Biochim Biophys Acta 1997;1362:116–27.
[43] Lai LS, Chou ST, Chao WW. Studies on the antioxidative activities of Hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. J Agric Food Chem 2001;49:963–8.
[44] Liu R, Choi J. Age-associated decline in gamma-glutamylcysteine synthetase gene expression in rats. Free Radic Biol Med 2000;28:566–74.
[45] Wu JL, Wu QP, Yang XF, Wei MK, Zhang JM, Huang Q, Zhou XY. l-Malate reverses oxidative stress and antioxidative defenses in liver and heart of aged rats. Physiol Res 2008;57:261–8.
[46] Aydin AF, Kucukgergin C, Ozdemirler-Erata G, Kocak-Toker N, Uysal M. The effect of carnosine treatment on prooxidant–antioxidant balance in liver, heart and brain tissues of male aged rats. Biogerontology 2010;11:103–9.
[47] Zhu D, Wu L, Li CR, Wang XW, Ma YJ, Zhong ZY, Zhao HB, Cui J, Xun SF, Huang XL, et al. Ginsenoside Rg1 protects rat cardiomyocyte from hypoxia/reoxygenation oxidative injury via antioxidant and intracellular calcium homeostasis. J Cell Biochem 2009;108:117–24.
[48] Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 1997;236:313–22.
[49] Kwak MK, Itoh K, Yamamoto M, Sutter TR, Kensler TW. Role of transcription factor Nrf2 in the induction of hepatic phase 2 and antioxidative enzymes in vivo by the cancer chemoprotective agent, 3H-1, 2-dimethiole-3-thione. Mol Med 2001;7:135–45.
[50] Lee JM, Calkins MJ, Chan K, Kan YW, Johnson JA. Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. J Biol Chem 2003;278:12029–38.
[51] Shih AY, Johnson DA, Wong G, Kraft AD, Jiang L, Erb H, Johnson JA, Murphy TH. Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. J Neurosci 2003;23:3394–406.
[52] Lee JM, Johnson JA. An important role of Nrf2-ARE pathway in the cellular defense mechanism. J Biochem Mol Biol 2004;37:139–43.
[53] Vargas MR, Johnson DA, Sirkis DW, Messing A, Johnson JA. Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. J Neurosci 2008;28:13574–81.