Long-term administration of ginsenoside Rh_1 enhances learning and memory by promoting cell survival in the mouse hippocampus

JINGANG HOU, JIANJIE XUE, MIRA LEE, JIAOJIAO YU and CHANGKEUN SUNG

Department of Food Science and Technology, College of Agriculture and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea

Received August 5, 2013; Accepted November 6, 2013

DOI: 10.3892/ijmm.2013.1552

Abstract. Ginsenosides, the secondary plant metabolites produced by *Panax ginseng* are responsible for the enhancing effects on learning observed following treatment with *Panax ginseng*. A number of studies have provided correlational evidence that cell proliferation and survival are closely associated with hippocampal-dependent learning tasks. In this study, to investigate the beneficial effects of ginsenoside Rh_1 on hippocampal cells and learning, mice (6 months old) were administered ginsenoside Rh_1 at a dose of 5 and 10 mg/kg/day for a period of 3 months. Saline-treated mice were used as controls. The enhancement of memory and learning in the mice was evaluated by hippocampal-dependent tasks (passive avoidance tests and Morris water maze tests) and the immunohistochemical marker of cell proliferation, bromodeoxyuridine (BrdU). In addition, the levels of brain-derived neurotrophic factor (BDNF) were measured following treatment. Based on our data, the Rh_1-treated group (5 and 10 mg/kg) showed a significantly improved learning and memory ability in the passive avoidance tests compared with the control group; however, only treatment with 10 mg/kg ginsenoside Rh_1 significantly promoted spatial learning ability in the Morris water maze test. Ginsenoside Rh_1 significantly enhanced cell survival in the dentate gyrus of mice, although it did not enhance hippocampal cell proliferation. In addition, ginsenoside Rh_1 upregulated the expression of BDNF. These findings address the potential therapeutic significance of ginsenoside Rh_1 as a nutritional supplement in memory loss and neurodegenerative diseases.

Correspondence to: Professor Changkeun Sung, Department of Food Science and Technology, College of Agriculture and Biotechnology, Chungnam National University, 220 Gong-dong, Yuseong-gu, Daejeon 305-764, Republic of Korea
E-mail: sungchangkeun@126.com

Key words: ginsenoside Rh_1, hippocampus, cell survival, nutrition, mouse

Introduction

A wide range of food or dietary supplements that are derived from plants have been shown to be able to modify the functions of the central nervous system. Ginsenosides, the secondary plant metabolites produced by *Panax ginseng*, are classified into two major groups in terms of the number and position of sugar moieties: 20 (S)-protopanaxadiol (PPD) and 20 (S)-protopanaxatriol (PPT) saponins. The diversity of individual ginsenosides may be responsible for their specific pharmacological effects (1). Increasing evidence has indicated the beneficial effects of ginsenosides on the central nervous system (2-6). However, the majority of these studies have focused on the beneficial effects of ginsenosides Rg_1 and Rb_1.

Over the past three decades, an increasing number of studies have focused on the correlation between neurogenesis and memory formation (7-9). The vast majority of these studies describe neurogenesis in the subregions of the hippocampus [the subventricular zone and subgranular zone (SGZ)] (10,11), whereas only a few studies have investigated neuronal survival (12). Several factors that affect hippocampal neurogenesis cause corresponding changes in cognitive performance. For example, voluntary running improves performance in the Morris water maze task by increasing cell proliferation in the SGZ (13). Aged animals and animals under stress display impaired memory and learning in hippocampal-dependent tasks (14-16). Therefore, the putative function of neurogenesis in the SGZ in learning and memory is considered an index for the evaluation of substrates that exert beneficial effects. On the other hand, brain-derived neurotrophic factor (BDNF) has been shown to promote the differentiation and survival of neurons in the adult brain (17).

In this regard, only a few studies have reported the effects of the oral administration of metabolites (18,19) of the two ginsenosides, Rg_1 and Rb_1, such as ginsenosides Rh_1, PPT, compound K and PPD. Thus, the question remains of whether ginsenoside Rh_1 can affect learning and memory ability. This issue is not only of academic interest but also has a number of practical implications for future research and product development. Therefore, in the present study, we investigated the effects of the long-term administration of ginsenoside Rh_1 on memory and learning in the adult mouse brain.

Materials and methods

Animals and housing conditions. Male ICR mice, 6 months of age were housed in a temperature-controlled animal room with a 12-h light-dark cycle and allowed access to food and water *ad libitum*. All experiments were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health (USA), and were approved and monitored by the Ethics Committee of Animal Experiments at Chungnam National University, Daejeon, Korea. Prior to the experiments, the mice were left undisturbed for 7 days and were randomly assigned to 1 of 4 experimental groups: i) the saline (0.9% NaCl)-treated group (n=16); ii) the group treated with 5 mg/kg Rh_1 (n=16); iii) the group treated with 10 mg/kg Rh_1 (n=16); and iv) the behavioral test group (n=30, passive avoidance test; n=30, water maze test). The behavioral test group was subdivided into 3 groups (control, 5 mg/kg Rh_1 and 10 mg/kg Rh_1; 10 mice per group) and the other 3 groups were subdivided into 2 groups (n=8 per group) for the evaluation of neurogenesis and cell survival in the hippocampus.

Bromodeoxyuridine (BrdU) and ginsenosides protocol. Mice in the saline-treated group (0.9% NaCl) and the ginsenoside (FuSong County Natural Biotechnology, Co., Ltd., Fusong, China)-treated groups (Rh_1, 5 and 10 mg/kg body weight) were orally administrated saline and ginsenoside, respectively for a period of 3 months. Rh_1 doses were converted between adult human (60 kg) and mouse (20 g) body weights, using the body surface area normalization method, as previously described (20). The selected doses corresponded to 2-3 and 5-6 g of ginseng per day in an adult human (60 kg body weight). Considering that the long-term intravenous administration of ginsenosides would cause inflammation or anxiety, we selected oral administration, even though this would be associated with less bioactivity.

The thymidine analogue, BrdU, is incorporated into the DNA of dividing cells and can be detected immunohistochemically in their progeny. The behavioral tests were performed at the end of the drug administration. To determine the effects of ginsenoside Rh_1 on neurogenesis, BrdU (100 mg/kg body weight) was administered to the mice twice per day for 3 consecutive days prior to sacrifice; to determine the effects of ginsenoside Rh_1 on cell survival, BrdU was administered to the mice twice per day for 3 consecutive days (20 days prior to sacrifice) during the the 3rd month of the treatment period. Following the evaluation of neurogenesis and cell survival, the animals (8 per group) were sacrificed, the brains were excised and the brain tissue was then subjected to immunohistochemical and protein expression analysis. The overall experimental protocol is presented in Fig. 1.

Morris water maze test. Mice from the different groups were subjected to a Morris water maze test for 5 consecutive days in the terminal phase of the administration process. The escape platform (diameter, 10 cm; height, 24 cm) was hidden 1 cm below the surface of the water, which had been made opaque by the addition of non-toxic black paint. Each animal was subjected to 4 experimental trails per day, each lasting 60 sec and each time commencing from 4 different starting points that randomly varied each day. If an animal was not able to find the platform it was manually place on it at the end of the trail. The animals were allowed to rest on the platform for 15 sec. A probe test was performed on day 6.

Passive avoidance test. The passive avoidance test was performed in identical compartments. The illuminated compartment (20x20x20 cm) contained a 100 W bulb, and the floor of the non-illuminated compartment (20x20x20 cm) was composed of 2 mm stainless steel rods at 1 cm intervals. These 2 compartments were separated by a guillotine door (5x5 cm). For the acquisition trials, the mice were initially placed in the illuminated compartment and the door was opened 15 sec later. When the mouse entered the non-illuminated compartment, the door was closed and an electrical foot shock (0.5 mA) of 3 sec in duration was delivered through the stainless steel rods. Twenty-four hours after the acquisition trial, the mice were again placed in the illuminated compartment for the retention trials. The time taken for a mouse to enter the non-illuminated compartment after the opening of the door was termed as the step-through latency time in the retention trials. If a mouse did not enter the non-illuminated compartment within 180 sec, it was assumed that the mouse had remembered the single training trial.

Immunohistochemistry. The mice were sacrificed and the brains were removed after the final BrdU injection. The brains were fixed in 4% phosphate-buffered paraformaldehyde for 12 h. The brain tissues were then embedded in paraffin and cut into sections. The sections were mounted on glass slides and stored overnight at 42˚C. Following deparaffinization with xylene and rehydration in a graded series of ethanol, the sections were rinsed in 0.01 M phosphate-buffered saline (PBS). BrdU is a widely used S-phase marker of neurogenesis.

For BrdU-immunostaining, the sections were hydrolyzed with 2 N hydrochloride (HCl) in PBS (pH 7.4) at 37˚C for 15 min, and then stained using the Invitrogen BrdU staining kit (Invitrogen, Carlsbad, CA, USA). The sections were incubated in serum blocking solution, in a 1:50 dilution of a mouse monoclonal antibody against BrdU (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and incubated overnight at 4˚C in biotinylated secondary antibody at room temperature for 30 min, and finally in streptavidin-peroxidase conjugate at room temperature for 20 min. After each step, the sections were rinsed with PBS. The sections were then incubated in 3,3'-diaminobenzidine (DAB) solution. Subsequently, the sections were incubated in 1% ferric chloride solution at room temperature for 5 min. BrdU-positive nuclei exhibited deposits of dark brown or black-colored precipitates. The sections were counterstained with hematoxylin and cover-slipped with histomount.

Microscopy and cell counting. Every 10th section throughout the hippocampus was processed for BrdU immunohistochemistry. Using this spacing ensures that the same neuron will not be counted in two sections. All BrdU-labeled cells in the dentate gyrus (granule cell layer) and hilus were counted in each section. To distinguish single cells within clusters, all counts were performed at x400 magnification under a light microscope (Olympus BX-41; Olympus, Tokyo, Japan), omitting cells in the outermost focal plane. A cell was counted as being in the SGZ of the dentate gyrus if it was touching or in

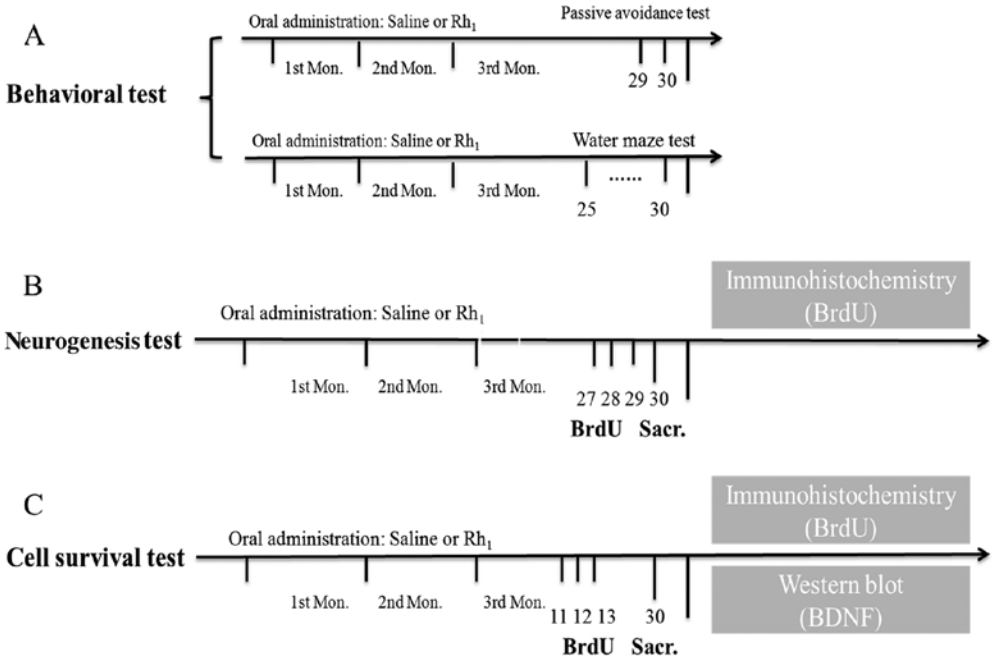

Figure 1. Overview of the experimental protocol. (A) Scheme for behavioral tests, including the passive avoidance and Morris water maze test. (B) For the analysis of neurogenesis, the animals were sacrificed 24 h after the final bromodeoxyuridine (BrdU) injection. (C) For cell survival analysis, BrdU was administered to the animals during the 3rd month and the animals were then sacrificed 20 days after the final injection. Mon., months; BDNF, brain-brain-derived neurotrophic factor.

the SGZ. Cells that were located more than two cells away from the SGZ were classified as hilar. The total number of BrdU-labeled cells per section was determined and multiplied by 6 to obtain the total number of cells per dentate gyrus.

Western blot analysis. The mice were anesthetized and decapitated after 3 months of treatment. The hippocampus dissected from each animal was homogenized ultrasonically in protein extraction buffer (PRO-PREP™ 17081; iNtRON Biotechnology, Seongnam, Korea). The supernatant was collected after centrifugation at 15,000 rpm for 30 min at 4˚C. Following quantification, the samples (20 μg protein per lane) were subjected to preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 15% gel and electrophoretically transferred onto PVDF membranes (Millipore, Billerica, MA, USA) using a trans-blot device (Bio-Rad, Hercules, CA, USA) at a 15 V constant current overnight at 4˚C. The PVDF membranes were soaked in 5% skim milk in PBS solution for 2 h at room temperature to block non-specific binding, rinsed in PBST, and incubated with a rabbit polyclonal anti-SNAP-25 antibody (diluted 1:300 in 5% skim milk in TBST; Santa Cruz Biotechnology, Inc.) overnight at 4˚C. The membranes were then washed 3 times for 10 min each in PBST and incubated for 2 h with a secondary antibody, goat anti-rabbit IgG (1:10,000; Santa Cruz Biotechnology, Inc.). After washing twice for 15 min in PBST, the signal was detected using an ECL system. Western blot analysis for β-actin was performed using the same procedure using a goat polyclonal anti-actin antibody (1:1,000; Santa Cruz Biotechnology, Inc.) as the primary antibody. The blots were quantified using image analysis software (ImageJ). Bond intensity values were expressed as a percentage of the control average.

Statistical analysis. Data are expressed as the means ± SEM. Statistical differences were assessed by one-way analysis of variance (ANOVA) using repeated measures where appropriate. The post-hoc Duncan's test was carried out where appropriate. The level for a statistically significant difference was set at $P<0.05$.

Results

Behavioral test. For the Morris water maze, repeated ANOVA (time x group) revealed a significant effect decrease in escape latency (in days) following treatment with Rh_1 [5 mg/kg, $F(2,24)=3.72$, $P=0.067$; 10 mg/kg, $F(3,42)=11.65$, $P<0.0001$].

In fact, all groups, including the control group showed a general decrease in overall latency throughout the acquisition phase (Fig. 2A).

In the probe tests, the group treated with 10 mg/kg ginsenoside Rh_1 showed a significant increase in the number of crosses (number of times mouse crosses the platform location) in the target quadrant ($P<0.05$), whereas the group treated with 5 mg/kg ginsenoside Rh_1, did not show statistically significant results ($P>0.48$) compared with the control group (Fig. 2B).

ANOVA for the time spent in the platform quadrant yielded significant results for the groups treated with Rh_1 [5 mg/kg, $F(1,14)=13.62$, $P=0.071$; 10 mg/kg, $F(1,14)=17.85$, $P<0.002$]. Post-hoc comparisons revealed that a general increase in the time spent in the target quadrant throughout the acquisition phase (Fig. 2C).

For the passive avoidance tests, no differences were observed among all the groups in the step-through latency during the acquisition trials (Fig. 3). ANOVA for the step-through latency during the retention trials revealed significant differences

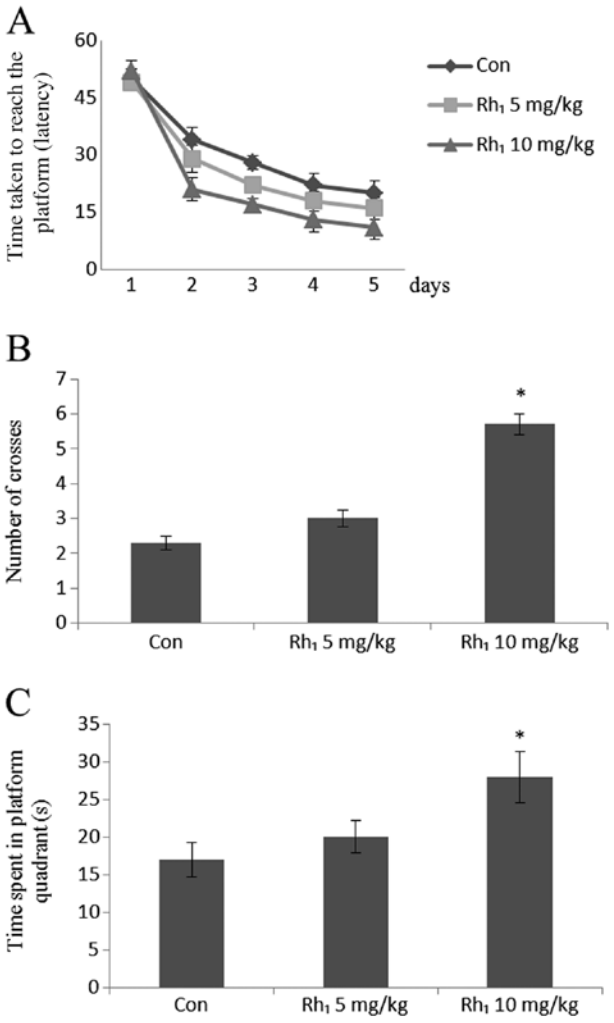

Figure 2. Three parameters in which the ginsenoside Rh_1-treated mice differed from the control group in the Morris water maze test. (A) Escape latency. (B) Number of crosses (number of times mouse crosses the platform location). (C) Time spent in platform quadrant. On day 1, there was no significant difference between the ginsenoside treated groups and the control group, indicating an equal baseline. At the other time points, significant differences were observed between the control group and the group treated with 10 mg/kg ginsenoside Rh_1($P<0.05$) but with the group treated with 5 mg/kg Rh_1 ($P>0.05$), and similar statistical results were observed in the number of crosses and the time spent in the platform quadrant. Data are expressed as the means ± SEM ($^*P<0.05$ vs. control group). Con, control.

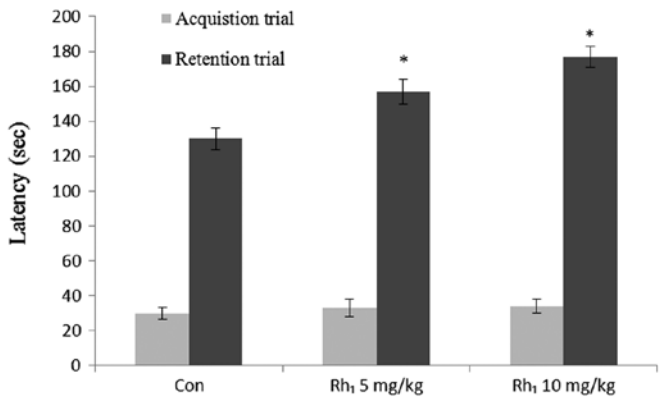

Figure 3. Differences were observed between the ginsenoside Rh_1-treated mice and the control group in the passive avoidance test. Data are expressed as the means ± SEM ($^*P<0.05$ vs. normal control group). Con, control.

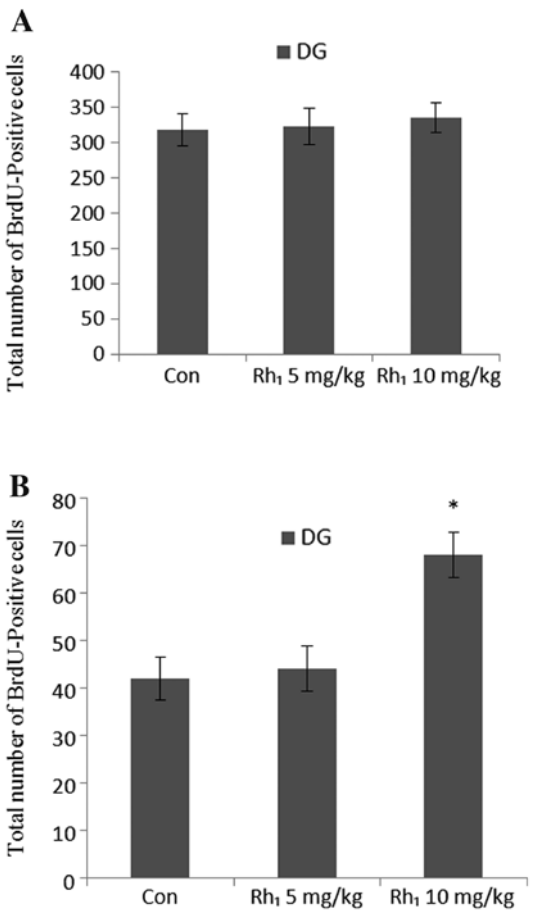

Figure 4. Effects of long-term administration of ginsenoside Rh_1 on neurogenesis. (A) Cell proliferation. Mice were sacrificed 2 h after the final bromodeoxyuridine (BrdU) injection. The number of BrdU-positive cells was determined. There was no statistical difference in observed in the proliferation of cells in the dentate gyrus (DG) of the hippocampus. (B) Cell survival. Mice were sacrificed 20 days after the final BrdU injection. Significant differences were observed in cell survival in the DG of the hippocampus following treatment with ginsenoside Rh_1. $^*P<0.05$ vs. control group. Con, control.

among the groups treated with Rh_1 [5 mg/kg, $F(1,16)=0.59$, $P<0.031$; 10 mg/kg, $F(1,16)=0.40$, $P<0.01$].

Effects of long-term ginsenoside Rh_1 administration on cell proliferation in the mouse hippocampus. The animals were administered ginsenoside Rh_1 for 3 months and sacrificed after the final BrdU injection. Analysis of the number of BrdU-labeled cells demonstrated that the long-term administration of ginsenoside Rh_1 had no statistically significant effect on the number of BrdU-positive cells in the dentate gyrus (5 mg/kg, $P>0.30$; 10 mg/kg, $P>0.47$) (Fig. 4A).

Effects of long-term ginsenoside Rh_1 administration on cell survival in the mouse hippocampus. To specially determine the effects of long-term ginsenoside Rh_1 administration on cell survival, a BrdU injection was administered on the 1st day of the 3rd month. ANOVA revealed an overall significant effect in the number of BrdU-positive cells [$F(2,18)=51.87$, $P<0.0003$], and post-hoc tests revealed that treatment with 10 mg/kg Rh_1 yielded significant results ($P<0.05$) compared with the control group (Fig. 4B). The results of BrdU immunohistochemistry

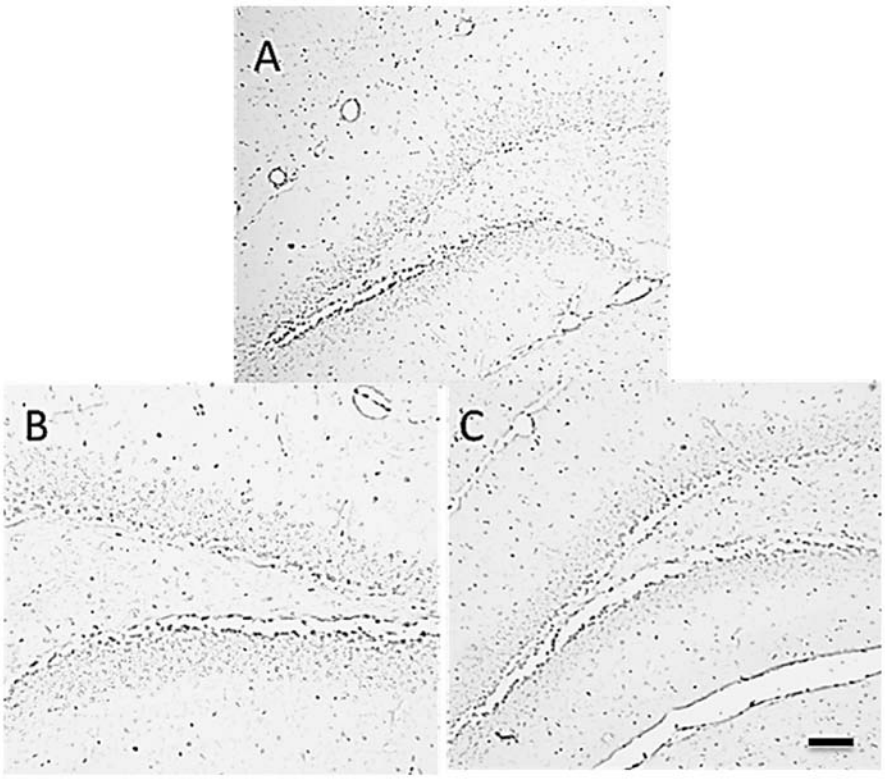

Figure 5. Bromodeoxyuridine (BrdU) immunohistochemistry for cell survival. BrdU-positive cells in the dentate gyrus (DG).(A) control; (B) Rh_1 5 mg/kg; (C) Rh_1 10 mg/kg. Scale bar, 100 μm.

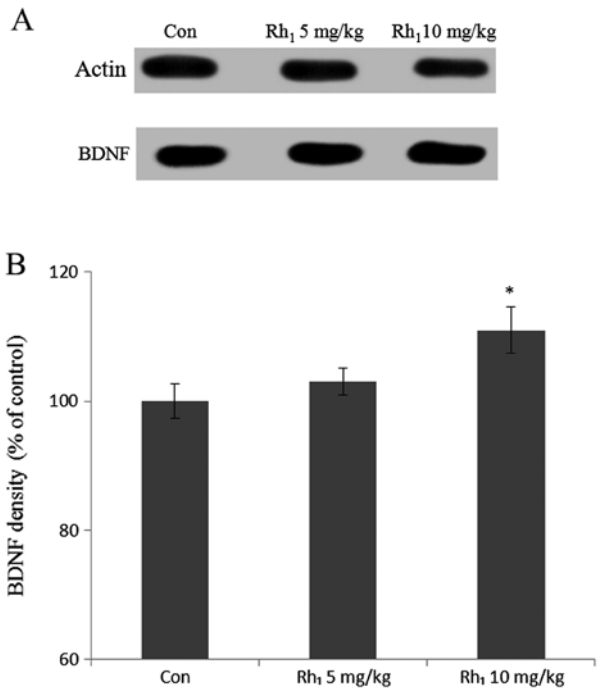

Figure 6. Effects of ginsenoside Rh_1 on brain-derived neurotrophic factor (BDNF) expression levels. (A) Western blot analysis of hippocampal tissue using polyclonal antibody against BDNF. (B) Data showing BDNF densities in the hippocampus (% of the control). Bars represent the means ± SEM. *P<0.05 vs. normal control group. Con, control.

are presented in Fig. 5. These results suggest that the long-term administration of Rh_1 increased cell survival in the hippocampus.

Effects of long-term ginsenoside Rh_1 administration on BDNF expression levels. The mice were sacrificed and BDNF protein expression was quantified by western blot analysis. BDNF density was measured in the hippocampus (Fig. 6). BDNF density in the control group was 100±2.8% and in the groups treated with 5 mg/kg and 10 mg/kg Rh_1 was 103.2±2.4 and 112±3.7% of the control, respectively. Treatment with 10 mg/kg Rh_1 yielded statistically significant results compared with the control group (P<0.05).

Discussion

In this study, we report that the long-term administration of ginsenoside Rh_1 enhances spatial recognition memory, as shown by a Morris water maze test and a passive avoidance test. Both of these tasks require the involvement of the hippocampus; thus, we observed a significant increase in hippocampal cell survival in the treated animals, as shown by the increase in the number of BrdU-labeled cells. These findings are consistent with those from a previous study, demonstraring that the long-term administration of ginsenoside Rb1 enhanced spatial learning and memory (21). To the best of our knowledge, only a few studies have reported the pharmacological functions of ginsenoside Rh_1 and PPT (22) in learning and memory. In the present study, we invesgigated the potential pharmacological effects of ginsenoside Rh_1 (Fig. 7).

In fact, a relatively higher dose of Rh_1 is required for optimal memory and learning in a water maze task. However, we observed a trend for the enhancing effects on memory to be more pronounced in the group administered the higher dose vs. the group administered the lower dose, although there was

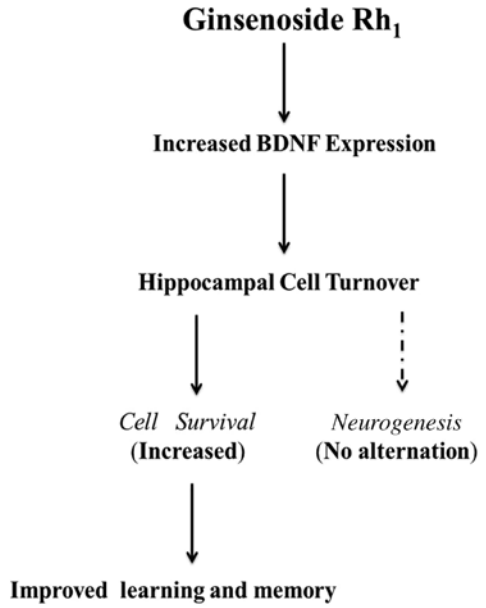

Figure 7. Schematic representation of Rh_1-regulated potential mechanisms of action leading to improved learning and memory. Rh_1 promotes brain-derived neurotrophic factor (BDNF) expression in the hippocampus, resulting in hippocampal cell turnover in terms of increased cell survival.

no significant difference observed between the 2 Rh_1 treatment groups in the passive avoidance test.

The regulation of neurogenesis can occur at different stages, including cell proliferation, differentiation and survival. Several experimental methods have been conducted to investigate the role of adult hippocampal neurogenesis in learning and memory, such as low-dose brain irradiation (23,24), various types of stress (25) and methylazoxymethanol acetate treatment (8). These methods have been shown to significantly reduce neurogenesis, as well as hippocampal-dependent tasks, whereas these studies suggest some potential roles for neurogenesis in learning and memory. In addition, the decreased survival of proliferating cells in the hippocampus is associated with a decline in spatial memory, as observed in a previous study (26). Hence, to elucidate the memory enhancing effects of treatment with ginsenoside Rh_1, we hypothesized that this treatment would have an impact on hippocampal neurogenesis. A significant increase in cell survival in the hippocampus was observed in the treated groups compared with the control group. By contrast, the number of BrdU-positive cells in the hippocampus did not differ between the treated groups and the control group, suggesting that cell proliferation at the time of BrdU injection was unaffected. Nonetheless, further studies are required to determine whether the treatment-induced increase in cell survival underlies the enhancement of memory and learning which was observed.

It is also possible that this increase was consistent with the mechanisms observed in previous studies, as in the central nervous system, BDNF regulates neuronal activity and is important for the positive selection and survival of functionally active neurons (27) and protects newborn neurons from death during the differentation process from immature to mature neurons (28). In the present study, ginsenoside Rh_1 enhanced the survival of cells in the dentate gyrus following the increase in BDNF expression. In this perspective, this result may partly explain the mechanisms by which ginsenoside Rh_1 improves the learning and memory process.

Apart from the neurogenesis factor, there are still other factors affecting the learning and memory process. A critical role for T cell-derived interleukin (IL)-4 in the regulation of learning and memory through the meningeal myeloid cell phenotype and BDNF expression has been indicated (29). Stress and corticosteroid hormones are known to affect learning and memory processes (30). Plasticity levels in the gray and white matter of the brain change during the learning process (31). The activation of cAMP-response element binding protein (CREB)-gene expression has a significant impact on memory (32).

On the other hand, Alzheimer's disease is a progressive neurologic disease that results in the irreversible loss of neurons, particularly in the cortex and hippocampus. Parkinson's disease is the second most common neurodegenerative disorder, after Alzheimer's disease. It is characterized pathologically by the loss of neurons. According to a previous study (33), ginsenoside Rg_1 significantly inhibits β-secretase activity *in vitro* and protects against Aβ-induced cytotoxicity in PC12 cells. In our study, ginsenoside Rh_1, a metabolite of the major ginsenoside Rg_1, demonstrated great potential as a therapeutic agent by promoting cell survival.

In conclusion, the long-term administration of ginsenoside Rh_1 resulted in improved behavioral performance in hippocampal-dependent tasks. Although ginsenoside Rh_1 is able to promote cell survival in the dentate gyrus of the mouse hippocampus, it is likely that a combination of increased cell survival, as well as unknown factors, contribute to the enhanced performance induced by the long-term administration of ginsenoside Rh_1. Further studies are required for the analysis of the differentiation of survived cells and other possible factors.

References

1. Christensen LP: Ginsenosides chemistry, biosynthesis, analysis, and potential health effects. In: Advances in Food and Nutrition Research. Taylor S (ed). Vol. 55. Academic Press, New Yotk, pp1-99, 2008.
2. Van Kampen J, Robertson H, Hagg T and Drobitch R: Neuroprotective actions of the ginseng extract G115 in two rodent models of Parkinson's disease. Exp Neurol 184: 521-529, 2003.
3. Kennedy DO and Scholey AB: Ginseng: potential for the enhancement of cognitive performance and mood. Pharmacol Biochem Behav 75: 687-700, 2003.
4. Reay JL, Scholey AB and Kennedy DO: Panax ginseng (G115) improves aspects of working memory performance and subjective ratings of calmness in healthy young adults. Hum Psychopharmacol 25: 462-471, 2010.
5. Kim EJ, Jung IH, Van Le TK, Jeong JJ, Kim NJ and Kim DH: Ginsenosides Rg5 and Rh3 protect scopolamine-induced memory deficits in mice. J Ethnopharmacol 146: 294-299, 2013.
6. Quan Q, Wang J, Li X and Wang Y: Ginsenoside Rg1 decreases Aβ(1-42) level by upregulating PPARγ and IDE expression in the hippocampus of a rat model of Alzheimer's disease. PloS One 8: e59155, 2013.
7. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD and Gage FH: Functional neurogenesis in the adult hippocampus. Nature 415: 1030-1034, 2002.
8. Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T and Gould E: Neurogenesis in the adult is involved in the formation of trace memories. Nature 410: 372-376, 2001.
9. Aimone JB, Wiles J and Gage FH: Potential role for adult neurogenesis in the encoding of time in new memories. Nat Neurosci 9: 723-727, 2006.

10. Johansson CB, Svensson M, Wallstedt L, Janson AM and Frisén J: Neural stem cells in the adult human brain. Exp Cell Res 253: 733-736, 1999.
11. Leuner B, Gould E and Shors TJ: Is there a link between adult neurogenesis and learning? Hippocampus 16: 216-224, 2006.
12. Drapeau E, Montaron MF, Aguerre S and Abrous DN: Learning-induced survival of new neurons depends on the cognitive status of aged rats. J Neurosci 27: 6037-6044, 2007.
13. Olson AK, Eadie BD, Ernst C and Christie BR: Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. Hippocampus 16: 250-260, 2006.
14. Klempin F and Kempermann G: Adult hippocampal neurogenesis and aging. Eur Arch Psychiatry Clin Neurosci 257: 271-280, 2007.
15. Mirescu C and Gould E: Stress and adult neurogenesis. Hippocampus 16: 233-238, 2006.
16. Rinwa P and Kumar A: Piperine potentiates the protective effects of curcumin against chronic unpredictable stress-induced cognitive impairment and oxidative damage in mice. Brain Res 1488: 38-50, 2012.
17. Quadrato G, Benevento M, Alber S, *et al*: Nuclear factor of activated T cells (NFATc4) is required for BDNF-dependent survival of adult-born neurons and spatial memory formation in the hippocampus. Proc Natl Acad Sci 109: E1499–E1508, 2012.
18. Lai L, Hao H, Liu Y, *et al*: Characterization of pharmacokinetic profiles and metabolic pathways of 20(S)-ginsenoside Rh1 in vivo and in vitro. Planta Med 75: 797-802, 2009.
19. Joh EH, Lee IA, Jung IH and Kim DH: Ginsenoside Rb1 and its metabolite compound K inhibit IRAK-1 activation-the key step of inflammation. Biochem Pharmacol 82: 278-286, 2011.
20. Yang L, Zhang J, Zheng K, Shen H and Chen X: Long-term ginsenoside Rg1 supplementation improves age-related cognitive decline by promoting synaptic plasticity associated protein expression in C57BL/6J mice. J Gerontol A Biol Sci Med Sci: July 5, 2013 (Epub ahead of print).
21. Liu L, Hoang-Gia T, Wu H, *et al*: Ginsenoside Rb1 improves spatial learning and memory by regulation of cell genesis in the hippocampal subregions of rats. Brain Res 1382: 147-154, 2011.
22. Wang YZ, Chen J, Chu SF, *et al*: Improvement of memory in mice and increase of hippocampal excitability in rats by ginsenoside Rg1's metabolites ginsenoside Rh_1 and protopanaxatriol. J Pharmacol Sci 109: 504-510, 2009.
23. Santarelli L, Saxe M, Gross C, *et al*: Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301: 805-809, 2003.
24. Snyder JS, Hong NS, McDonald RJ and Wojtowicz JM: A role for adult neurogenesis in spatial long-term memory. Neuroscience 130: 843-852, 2005.
25. Shors TJ, Mathew J, Sisti HM, Edgecomb C, Beckoff S and Dalla C: Neurogenesis and helplessness are mediated by controllability in males but not in females. Biol Psychiatry 62: 487-495, 2007.
26. Wati H, Kudo K, Qiao C, Kuroki T and Kanba S: A decreased survival of proliferated cells in the hippocampus is associated with a decline in spatial memory in aged rats. Neurosci Lett 399: 171-174, 2006.
27. Thoenen H: Neurotrophins and neuronal plasticity. Science 270: 593-598, 1995.
28. Bergami M, Rimondini R, Santi S, Blum R, Götz M and Canossa M: Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. Proc Natl Acad Sci USA 105: 15570-15575, 2008.
29. Derecki NC, Cardani AN, Yang CH, *et al*: Regulation of learning and memory by meningeal immunity: a key role for IL-4. J Exp Med 207: 1067-1080, 2010.
30. Schwabe L, Schächinger H, de Kloet ER and Oitzl MS: Corticosteroids operate as a switch between memory systems. J Cogn Neurosci 22: 1362-1372, 2010.
31. Zatorre RJ, Fields RD and Johansen-Berg H: Plasticity in gray and white: neuroimaging changes in brain structure during learning. Nat Neurosci 15: 528-536, 2012.
32. Benito E and Barco A: CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. Trends Neurosci 33: 230-240, 2010.
33. Wang YH and Du GH: Ginsenoside Rg1 inhibits β-secretase activity in vitro and protects against Abeta-induced cytotoxicity in PC12 cells. J Asian Nat Prod Res 11: 604-612, 2009.