



Research article

Effects of ginsenoside k-g3, an Rg3-enriched fraction, on scopolamine-induced memory impairment and learning deficit in mice

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... promise as potential nutraceuticals for cognitive impairment, their use has been limited due to high production cost and low potency. In particular, the process of extracting pure Rg3 from ginseng is laborious and expensive.

Methods: We described the methods in preparing ginsenoside k-g3, an Rg3-enriched fraction, and evaluated its effects on scopolamine-induced memory impairment in mice.

Results: Ginsenoside k-g3 (25–200 mg/kg) significantly reversed scopolamine-induced cognitive impairment in the passive avoidance, but not in Y-maze testing. Ginsenoside k-g3 (50 and 200 mg/kg) improved escape latency in training trials and increased swimming times within the target zone of the Morris water maze. The effect of ginsenoside k-g3 on the water maze task was more potent than that of Rg3 or Red ginseng. Acute or subchronic (6 d) treatment of ginsenoside k-g3 did not alter normal locomotor activity of mice in an open field. Ginsenoside k-g3 did not inhibit acetylcholinesterase activity, unlike donepezil, an acetylcholinesterase inhibitor. Rg3 enrichment through the ginsenoside k-g3 fraction enhanced the efficacy of Rg3 in scopolamine-induced memory impairment in mice as demonstrated in the Morris water maze task.

Conclusion: The effects of ginsenoside k-g3 in ameliorating scopolamine-induced memory impairment in the passive avoidance and Morris water maze tests indicate its specific influence on reference or long-term memory. The mechanism underlying the reversal of scopolamine-induced amnesia by ginsenoside k-g3 is not yet known, but is not related to anticholinesterase-like activity.

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1. Introduction

Alzheimer's disease (AD), the most common age-related neurodegenerative disorder [1], is characterized by the formation of neurofibrillary tangles in the medial temporal lobe and cortical areas of the brain [2] and senile plaques [3]. The brains of patients with AD show losses of choline acetyltransferase activity or basal forebrain cholinergic neurons, which are correlated with cognitive impairments [4–6]. The current mainstay of treatment for cognitive loss associated with AD has been muscarinic or nicotinic receptor ligands and acetylcholinesterase (AChE) inhibitors [7], drugs

which also show unwanted side effects such as diarrhea, nausea, vomiting, muscle cramps, sedation and bradycardia [8].

Ginseng (the root of *Panax ginseng* Meyer) is frequently used in Asian countries as a traditional medicine. The major components of ginseng are ginsenosides; a diverse group of steroidal saponins [9,10] capable of exerting many beneficial effects including enhancement of memory and cognitive functions. Acceleration of memory acquisition and improved cognition has been reported with treatment of ginsenosides Rb1 and Rg1 in animal models [11,12]. For instance, Rg1 exerted ameliorative effects on scopolamine-induced memory impairment in rats in a radial arm maze

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task [13], while Rb1 improved A β (25–35) induced memory dysfunction, axonal hypertrophy, and synaptic loss in a mouse model of AD [14]. Both ginsenosides enhanced cholinergic function [15], conferred neuroprotection [16], and promoted neurite outgrowth in cultured neurons [17]. These mechanisms are thought to explain the memory-enhancing activities of these ginsenosides.

Rg3, another type of ginsenoside, has also been shown to protect against scopolamine-induced memory deficit in mice [18–20]. Scopolamine is an antimuscarinic agent that decreases central cholinergic activity and causes impairment of learning and memory [21]. Moreover, the neuroprotective effects of Rg3 have also been demonstrated in many studies [15,22–25]. In fact, Rg3 was the most effective ginsenoside in inhibiting N-methyl-D-aspartic-acid-induced neurotoxicity in hippocampal neurons [26]. Rg3 was also observed to produce the most significant reduction of accumulation of the Alzheimer's amyloid β peptide in a cell-based model system, as well as in a mouse model of AD [27]. Altogether, these studies indicate the potentiality of Rg3 in the treatment of AD.

Despite the attractive features of ginsenosides as potential nutraceuticals for AD, their use has been limited for several reasons, including high production cost and poor bioavailability. In particular, the process of extracting pure Rg3 from ginseng is laborious and expensive [28]. Furthermore, conventional manufacturing processes produce only minimal amounts of Rg3. Thus, Rg3 enrichment methods are required to ensure higher yields, which in turn could increase bioavailability and efficacy of the ginsenoside. Moreover, although enriched with Rg3, these fractions may also contain other beneficial ginsenosides or phytochemicals that may exert other important biological activities. For these reasons, Rg3-enriched preparations may be more attractive formulations than preparations containing purified Rg3 alone, from a drug development standpoint.

In this study, we investigated the production of ginsenoside k-g3; an Rg3-enriched fraction. Furthermore, we evaluated the efficacy of this preparation in ameliorating scopolamine-induced memory impairment in mice. In addition, we examined whether the effects of ginsenoside k-g3 were mediated via cholinergic signaling by measuring *in vitro* its capacity to inhibit AChE activity.

2. Materials and methods

2.1. Animals

Male ICR mice (20–25 g), obtained from Hanlim Laboratory Animals Co. (Hwasung, Korea), were used in this study. They were maintained on a standard light–dark cycle, at ambient temperature (22 \pm 2°C) and humidity (55 \pm 5%), with free access to chow pellets and water. Prior to behavioral assays, mice were acclimated to their home cages for at least 6 d. The experimental groups, consisting of eight to 10 animals per drug and dose, were chosen by means of a randomized schedule. All mice were used only once. Animal treatment and maintenance were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23 revised 1985) and the Animal Care and Use Guidelines of Sahmyook University, Korea.

2.2. Materials

The water extract of red ginseng (RG) was obtained from the Korea Ginseng Corporation (Seoul, Korea). RG was given orally (p.o.) at a dose of 100 mg/kg. Meanwhile, Rg3, obtained from VitroSys Inc. (Yeongju, Korea), was prepared in 10% Tween 80 solution and given at doses of 20 and 40 mg/kg (p.o.). Ginsenoside k-g3, prepared using the methods stated below, was obtained from

Cheiljedang Corp. (Seoul, Korea), dissolved in saline, and given to mice (p.o.) at doses of 12.5 mg/kg, 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg. Selection of doses was based on results from our preliminary studies (unpublished findings). The control group was given saline solution. Donepezil, an AChE inhibitor used as positive control, was purchased from Sigma (St. Louis, MO, USA). The drug was given at a dose of 5 mg/kg (p.o.). Scopolamine hydrochloride was obtained from Sigma.

2.3. Preparation of ginsenoside Rg3-enriched fraction from Ginseng

Dried Korean ginseng (*Panax ginseng*) root was purchased from Ginseng Nonghyup (Punggi, Korea). Korean ginseng was extracted three times with 10 volumes of 70% fermentation ethanol at 80°C for 4 h, and then concentrated twice under vacuum at 50°C. The crude extract was suspended in distilled water and then subjected to DIAION HP20 column chromatography (Mitsubishi Chemical Industries, Tokyo, Japan), with successive elution by distilled water and 50–100% v/v fermentation ethanol at room temperature. The eluted saponin fraction was converted with acidified water (citric acid, pH 2.5) at 121°C for 2 h. The ginsenoside Rg3-enriched fractions were concentrated by vacuum, evaporated at 50°C, and then lyophilized by freezing dryer (Ilshin BioBase, Gyeonggi, Korea). The fraction powder was also dissolved in methanol, and ginsenoside Rg3 was analyzed by HPLC. HPLC was carried out on an LC system equipped with an autosampler and a binary gradient pump (Capillary HP 1100; Agilent Technologies, Santa Clara, CA, USA). A reversed-phase column (Venusil XBP C18, 250 mm \times 4.6 mm, internal diameter 5 μ m; Agela Technology, Newark, DE, USA) was used for quantitative determination of ginsenoside Rg3 (20 mg/g). The mobile phase consisted of acetonitrile (A) and water (B) with a flow rate at 1.6 mL/min, and the column was kept constant at 30°C. The detection wavelength was set at 203 nm.

2.4. Locomotor activity test

We measured the effects of ginsenoside k-g3 on general locomotor activity. Thirty minutes after drug or saline (control group) administration, separate groups of mice were placed individually in the center of an activity box (measuring 47 cm \times 47 cm), bordered by 42-cm high side walls. Spontaneous activity was measured for 10 min using automated systems (Ethovision System; Noldus Information Technology, Wageningen, Netherlands). The following indices of locomotor activity were recorded by the computer program: moved distance, movement duration, and frequency of rearing. In separate groups of mice, the effects of the repeated (6 d) administration of ginsenoside k-g3 on locomotion were also investigated. Locomotor activity tests were conducted during the 1st, 3rd and the final day of drug treatment.

2.5. Scopolamine-induced memory impairment in animal models

2.5.1. Y-maze test

Y-maze tests were conducted as described previously [29]. One hour before the tests, mice were administered with the test compounds or with saline or donepezil (positive control). After 30 min, scopolamine [1 mg/kg, intraperitoneally (i.p.)] was injected to induce memory impairment. The effects of the drugs on spontaneous alternation behavior of mice were measured for 8 min. An arm entry was defined as the entry of all four paws and the tail into one arm. The sequence of arm entries was recorded using automated systems (Ethovision System). The alternation behavior (actual alternations) was defined as the consecutive entry into three arms, that is, the combination of three different arms, with stepwise combinations in the sequence. The maximum number of

alternations was considered as the total number of arms entered minus 2, and the percentage of alternation behavior was calculated as (actual alternations/maximum alternations) \times 100. The number of arm entries was used as an indicator of locomotor activity.

2.5.2. Passive avoidance test

The passive avoidance task was conducted in identical illuminated and nonilluminated boxes (Gemini Avoidance System, San Diego Instruments, San Diego, CA, USA), as described previously [29,30]. Mice underwent an acquisition trial and a retention trial that followed 24 h later. One hour before the acquisition trial, mice were given the test drugs, saline (control group) or donepezil. Thirty minutes later, they were treated with scopolamine (1 mg/kg, i.p.) or vehicle.

2.5.3. Morris water maze test

Tests were conducted in a water maze as described previously [29]. A white platform (6 cm in diameter and 29 cm high) was placed in one of the quadrants of the pool and submerged 1 cm below the water surface so that it was not visible. The methods used in a previous study [29] were also followed in this work but with some modifications. During the first experimental day, mice were trained to swim in the maze (in the absence of the platform) for 60 s. Five subsequent days after training, mice were given two trial sessions per day with the white platform in place. The interval between each trial sessions was 30 min [31]. During each trial session, the time taken to find the hidden platform (escape latency) was recorded using the Ethovision System. A probe trial was conducted 1 d after the last training trial sessions using the methods described previously [29]. The swimming time in the pool quadrant where the platform had previously been placed was recorded. Test drug or donepezil was given 1 h before the first trial session at every consecutive day. Thirty minutes after drug or donepezil administration, mice were injected with scopolamine (1 mg/kg, i.p.).

2.6. AChE inhibition assay

AChE activity assays were carried out using an acetylthiocholine iodide substrate based on the colorimetric method [32]. The methods used have been described in detail in a previous study [33]. Absorbance was measured at 410 nm immediately after adding the enzyme source (400 μ L) to the reaction mixtures (OPTIZEN 2120UV, Mecasys Co. Ltd., Daejeon, Korea). Readings were taken at 30-s intervals for 5 min. The drug concentrations required to inhibit AChE activity by 50% (IC_{50}) were calculated using enzyme inhibition dose response curves. Donepezil was used as a positive control.

2.7. Statistical analysis

All data are expressed as mean \pm standard error of the mean. Results from the Y-maze and Morris water maze and open field tests were analyzed using one-way analysis of variance (ANOVA). When significant values were obtained, Dunnett's test was used for *post hoc* analysis. Student's *t* test was also used when comparing means of two groups (e.g., control vs. scopolamine-treated animals). Results from the passive avoidance task were analyzed using Kruskal–Wallis nonparametric ANOVA. If significant results were found, each treatment group was compared using the Dunn's *post hoc* test. Statistical significance was set at $p < 0.05$. Nonlinear regression was used to analyze results from the AChE inhibition assay. The IC_{50} values were obtained using this statistical tool. All statistical analyses were conducted using GraphPad Prism 5 (San Diego, CA, USA).

3. Results and discussion

3.1. Chemical analysis of ginseol k-g3

As shown in Fig. 1A, crude ginseng extracts contained 11.02 mg/g ginsenoside Rg1, 14.63 mg/g of Rb1, 11.11 mg/g of Rc, and 0.75 mg/g of Rg2. Notably, ginsenoside Rg3 was not detected in the crude ginseng extracts. Meanwhile, ginseol k-g3 (an Rg3-enriched fraction) contained 50.71 mg/g and 37.22 mg/g of the Rg3 s and r forms, respectively (Fig. 1B). Therefore, the ginsenoside Rg3-enriched fraction obtained from DIAION HP20 column of the crude ginseng extract contained 80–90 mg/g freeze-dried powder. This yield corresponded to an \sim 80 times greater concentration of ginsenoside Rg3 determined in the crude ginseng extract. Nevertheless, as shown in Fig. 1B, ginseol k-g3 also contained other ginsenosides such as Rk1 and Rg5 in the following compositions: 41.68 mg/g and 75.04 mg/g, respectively.

3.2. Effects of ginseol k-g3 on locomotor activity of mice

The effects of single and repeated treatment of ginseol k-g3 at various doses (12.5 mg/kg, 25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg) on locomotor activity of mice were examined. The effects of RG (100 mg/kg), Rg3 (20 mg/kg and 40 mg/kg) and donepezil (5 mg/kg) were also evaluated for comparison. As shown in Fig. 2A, single treatment with the four doses of ginseol k-g3 did not affect locomotor activity of mice ($p > 0.05$). Furthermore, ginseol k-g3 did not affect rearing frequency of mice ($p > 0.05$, Fig. 2B). It was also notable that RG, the two doses of Rg3, as well as the single dose of donepezil did not alter ambulatory and stereotypic behaviors of mice (Fig. 2A and B). Meanwhile, no differential locomotor activities were observed in both saline- and ginseol k-g3-treated mice during Day3 and Day 6 of drug administration. As shown in Fig. 2C, the total moved distance and rearing frequency (Fig. 2D) were similar between control and ginseol k-g3-treated mice, and also in mice treated with RG and Rg3. Altogether, these results indicate that ginseol k-g3 does not cause sedation upon single or repeated administration. These findings also demonstrate that ginseol k-g3 does not impair motor function or exploratory activity.

3.3. Effects of ginseol k-g3 on the Y-maze task

Spontaneous alternation behavior determined using the Y-maze test has been viewed as an indicator of spatial short-term memory [34]. In this test, mice must remember the arm most recently entered in order to alternate arm choice. Furthermore, treatment with scopolamine has been demonstrated to impair spontaneous alternation behavior in animal models [21]. As shown in Fig. 3A, spontaneous alternation behavior in scopolamine-treated mice was significantly lower than in mice treated with vehicle ($p < 0.01$). One-way ANOVA showed lack of effect of all doses of ginseol k-g3 in improving scopolamine-induced reduction of spontaneous alternation in mice ($p > 0.05$). RG and the two doses of Rg3 also failed to enhance spontaneous alternation behavior in scopolamine-treated mice. In contrast, donepezil significantly reversed the cognitive deficit induced by scopolamine in the Y-maze task [$t(18) = 4.71, p < 0.001$]. Together, these results suggest that that ginseol k-g3, RG and Rg3 do not influence short-term or working memory. Meanwhile, as shown in Fig. 3B, no significant differences were observed among experimental groups in the number of arm entries. This result corroborates the observation that ginseol k-g3 does not affect general locomotor activity of mice.

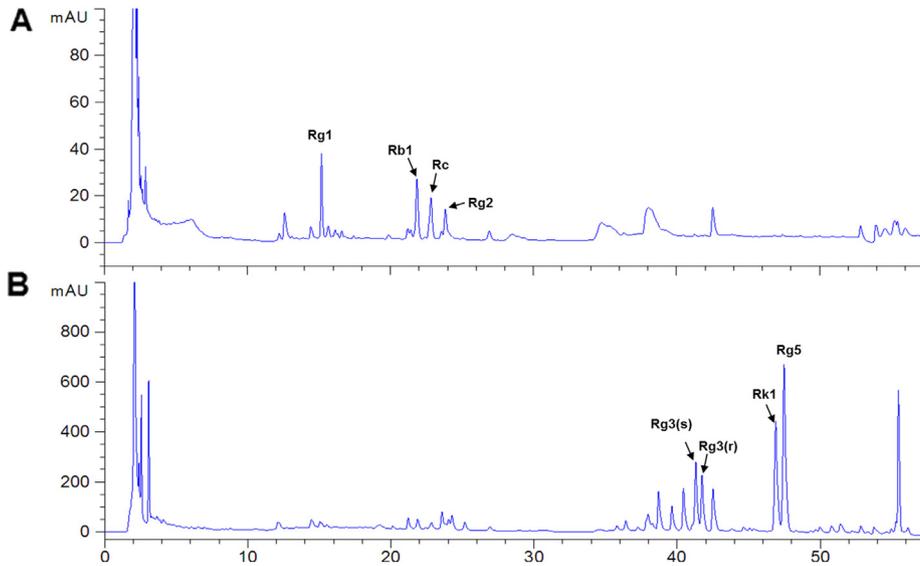


Fig. 1. A representative HPLC chromatogram of ginsenosides at 203 nm. (A) Ginsenosides Rg1, Rb1, Rc, Rg2, but not Rg3, were detected in the crude ginseng extracts. (B) Ginsenosides k-g3 (an Rg3-enriched fraction) contained 50.71 mg/g and 37.22 mg/g of the Rg3 s and r forms, respectively. It also contained other ginsenosides such as Rk1 and Rg5.

3.4. Effects of ginsenosides k-g3 on the passive avoidance task

The passive avoidance task has long been used to evaluate learning and memory in rodent models [35]. Moreover, acquisition of a passive avoidance response has been used to measure long-

term memory of an aversive experience. In Fig. 4, a significant group effect was found on step-through latency in retention trial with scopolamine [$H(9) = 32.69, p < 0.001$]. The step-through latency time of the scopolamine-treated group was significantly shorter than that of the control group ($p < 0.001$). In contrast, the

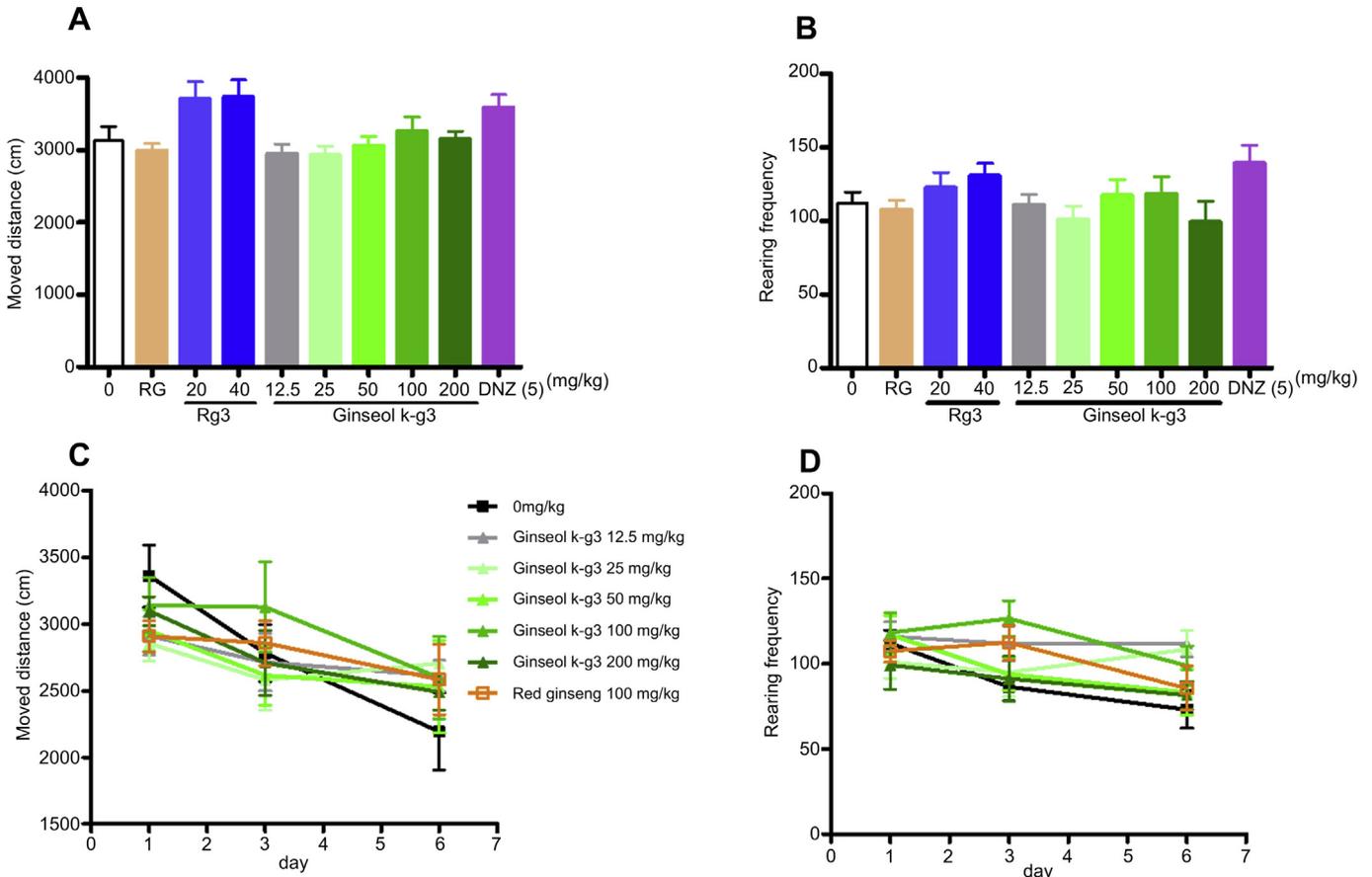


Fig. 2. Effects of ginsenosides k-g3 on locomotor activity of mice. The effects of single (A, B) and repeated treatment (C, D) of ginsenosides k-g3 at various dosages (12.5, 25, 50, 100, and 200 mg/kg) on locomotor activity of mice were examined. The following parameters were measured: moved distance and rearing frequency. The effects of Red ginseng (100 mg/kg), Rg3 (20 and 40 mg/kg) and donepezil (DNZ, 5 mg/kg) were also evaluated for comparison. Data represent mean \pm SEM ($n = 8-10$ animals).

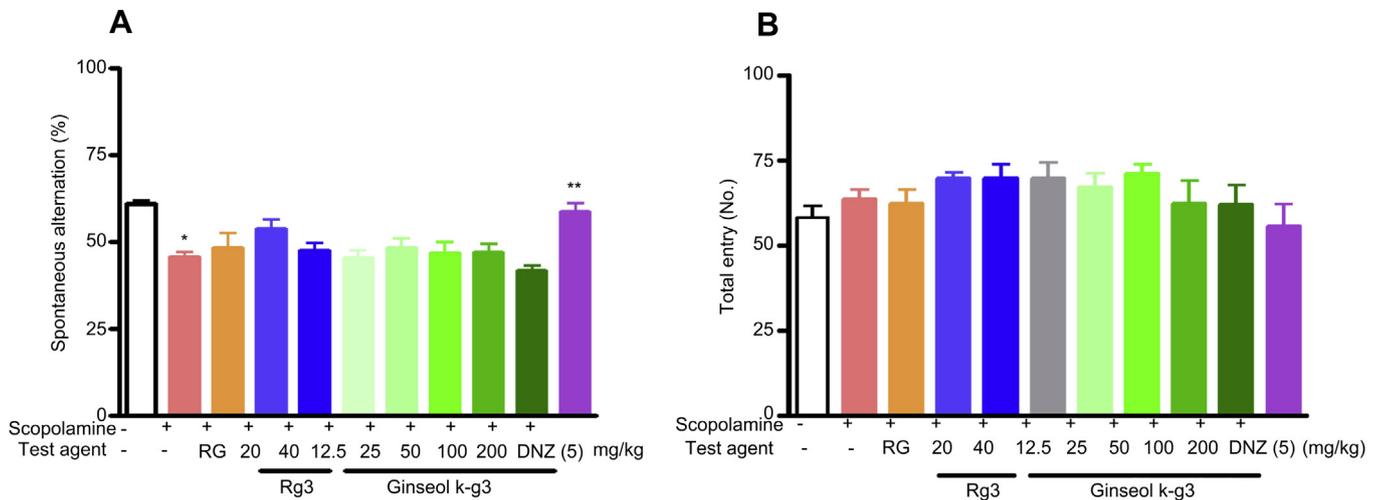


Fig. 3. Effects of ginseol k-g3 on the Y-maze task. The effects of single treatment of ginseol k-g3 at various dosages (12.5, 25, 50, 100, and 200 mg/kg), Red ginseng (100 mg/kg), Rg3 (20 and 40 mg/kg) and donepezil (DNZ, 5 mg/kg), or the same volume saline (Control) on spontaneous alternation behavior (A) and total arm entries (B) of mice treated with scopolamine were examined. Data represent mean \pm SEM ($n = 8-10$ animals). * $p < 0.01$, significant vs. control group, ** $p < 0.01$ significant vs. scopolamine-treated group.

step-through latency time for the donepezil-treated group was higher than that of the scopolamine-treated group ($p < 0.01$). The shorter step-through latency time induced by scopolamine was improved by RG, Rg3 (20 mg/kg and 40 mg/kg, $p < 0.05$). A previous study has documented the memory enhancing effects Rg3 on scopolamine-induced cognitive deficit in the passive avoidance task [18]. Importantly, ginseol k-g3 (25 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg) also recovered scopolamine-induced amnesia. Altogether, these findings indicate that RG, Rg3 and the Rg3-enriched fraction, ginseol k-g3, affect conditioning and/or associative memory. Considering that ginseol k-g3, and also Rg3 and RG, significantly improved scopolamine-induced memory impairment in mice in the passive avoidance but not in the Y-maze task, it could be hypothesized that these substances modulate long-term but not short-term or working memory.

3.5. Effects of ginseol k-g3 on the Morris water maze task

To verify the selective memory (i.e., long-term) enhancement capacity of ginseol k-g3 in mice, we measured the effects of ginseol k-g3 on scopolamine-induced memory deficits in the Morris

water maze task. The water maze test is another widely used behavioral assay to measure hippocampus-dependent long-term and spatial memory [36,37]. In this test, decrease in escape latency observed from day to day in the first trial represents long-term memory, while that from the first trial to the second trial represents working or short-term memory [37]. Moreover, the time in the quadrant with the platform indicates changes in spatial memory [37,38]. The escape latencies of mice during the second trial sessions across the training days were tabulated. Fig. 5A shows that escape latencies in groups given vehicle (control) or scopolamine, with or without the test drugs, varied significantly with respect to day [$F(4,448) = 33.10$, $p < 0.001$] and treatment [$F(9,448) = 8.91$, $p < 0.001$]. Two-way ANOVA, however, did not show significant interaction between day and treatment. In contrast to the vehicle-treated groups (Control), scopolamine-treated mice consistently exhibited longer escape latency across the training days consistent with our previous observations [29]. Furthermore, treatment of ginseol k-g3 at a dose of 50 mg/kg significantly attenuated scopolamine-induced delay in escape latency during Day 4 and Day 5 of training ($p < 0.05$). The 200 mg/kg dose of ginseol k-g3 also shortened escape latency during Day 5 of training ($p < 0.05$). Treatment of donepezil also produced shorter mean escape latencies than the scopolamine-treated group Day 4 and Day 5 of training ($p < 0.01$). Notably, however, we did not observe any beneficial effect of RG and Rg3 treatment on scopolamine-induced lengthening of escape latencies of mice. Only the ginseol k-g3-treated groups showed amelioration of scopolamine-induced memory impairment in the Morris water maze task, therefore, we can assume that the significant effects of ginseol k-g3 have been brought by Rg3 enrichment. Furthermore, it was observed during the probe trial session that the treatment groups were significantly different in terms of swimming times within the quadrant that normally contained the platform (target quadrant) [$F(9, 95) = 37.93$, $p < 0.01$] (Fig. 5B). The mean swimming time within the platform quadrant in scopolamine-treated mice was significantly reduced compared to vehicle-treated controls ($p < 0.05$). Treatment of ginseol k-g3-enriched fraction (50 mg/kg and 200 mg/kg) and donepezil (5 mg/kg) significantly ameliorated the shortened swimming time within the platform quadrant induced by scopolamine. Interestingly, Rg3 also improved swimming time within the target quadrant. Together, these results demonstrate that Rg3 exerts beneficial effects in modulating long-term memory

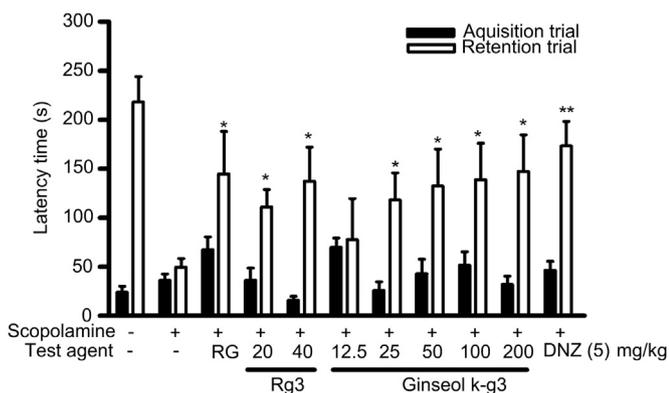


Fig. 4. Effects of ginseol k-g3 on the passive avoidance task. The effects of single treatment of ginseol k-g3 at various dosages (12.5, 25, 50, 100, and 200 mg/kg), Red ginseng (100 mg/kg), Rg3 (20 and 40 mg/kg) and donepezil (DNZ, 5 mg/kg), or the same volume of saline (Control) on passive avoidance behavior of scopolamine-treated rats were examined. Data represent mean \pm SEM ($n = 8-10$ animals). # $p < 0.05$, significant vs. Control group, * $p < 0.05$; ** $p < 0.01$ significant vs. scopolamine-treated group.

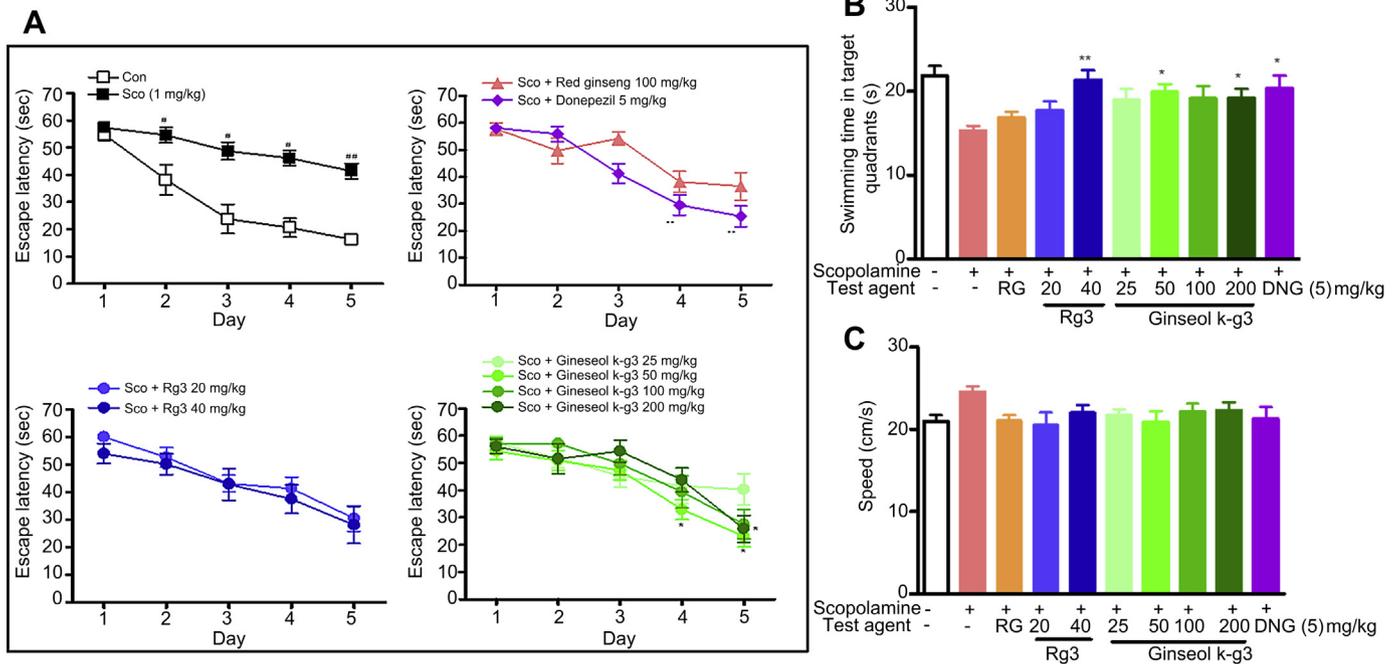


Fig. 5. Effects of ginseng k-g3 on the Morris water maze task. The effects of ginseng k-g3 at various dosages (25, 50, 100, and 200 mg/kg), Red ginseng (100 mg/kg), Rg3 (20 and 40 mg/kg) and donepezil (DNZ, 5 mg/kg), or the same volume saline solution (Control) on escape latency (A) during water maze training session of mice treated with scopolamine were examined. (B) Effects of test drugs on swimming time and (C) speed during the probe trial session of the water maze test. Probe trials were performed over 2 min as described in *Methods*. Data represent mean \pm SEM ($n = 8-10$ animals). $\#p < 0.05$; $\#\#p < 0.01$, significant vs. Control group, $*p < 0.05$; $**p < 0.01$, significant vs. scopolamine-treated group.

in scopolamine-treated mice. Furthermore, enrichment of Rg3 through the ginseng k-g3 preparation further increased the efficacy of Rg3. As shown in Fig. 5C, there were no differences in the swimming speeds among the groups during the probe trial (Fig. 5C). This finding corroborates the observation that ginseng k-g3 does not affect locomotor and exploratory behaviors of mice. This is another attractive feature of ginseng k-g3 when used as a drug for AD, given the observation that muscle weakness or sedation has been associated with the use of recent AD therapies [8].

3.6. Effects of ginseng k-g3 on AChE activity

In light of the positive effects of ginseng k-g3 on scopolamine-induced memory impairment in mice, we hypothesized that the

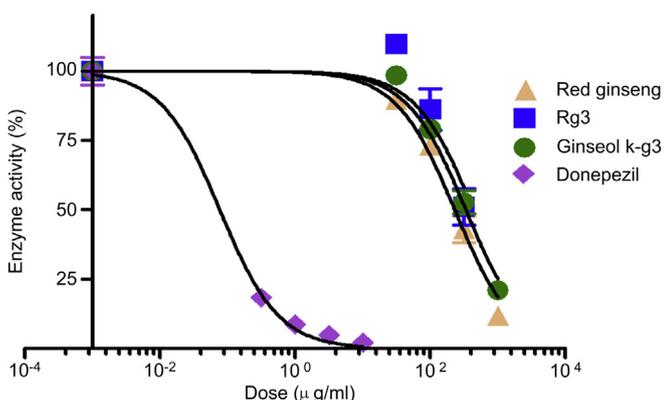


Fig. 6. Effects of ginseng k-g3 on acetylcholinesterase (AChE) activity in vitro. The effects of ginseng k-g3, Red ginseng, Rg3 and donepezil on acetylcholinesterase (AChE) activity inhibition were examined. The % change of AChE activities were determined as described in *Methods*. Error bars show mean \pm SEM from 3 independent experiments.

Rg3-enriched preparation enhanced long-term memory through the cholinergic nervous system. As shown in Fig. 6, donepezil, a widely used drug for AD, significantly inhibited AChE activity in a dose-dependent manner, with an IC_{50} value of 0.0769 μ g/mL. RG, Rg3 and ginseng k-g3 also inhibited AChE activity but were not as potent as donepezil. However, the IC_{50} values of RG, Rg3 and ginseng k-g3 were found to be 231 μ g/mL, 381 μ g/mL and 337 μ g/mL, respectively. Considering the weak potency of ginsenosides in inhibiting acetylcholinesterase activity, ginseng k-g3 may have reversed scopolamine-induced amnesia through a mechanism not related with the cholinergic nervous system. Although basal forebrain cholinergic neurons appear to be targeted primarily in early stages of AD, other neurotransmitter systems can also be affected [39,40]. Furthermore, scopolamine has some direct effects on noncholinergic neuronal pathways, and cholinergic neurons have functional interactions with a wide variety of neurotransmitter systems that could be affected indirectly by the drug [36]. In this regard the influence of ginseng k-g3 on other neurotransmitter systems (e.g., γ -aminobutyric acid) should be explored. Moreover, earlier studies have attributed that the memory-enhancing effects of Rg3 to neuroprotection against excitotoxicity [18]. The AD drug donepezil has also been ascribed neuroprotective effects against glutamate-induced neurotoxicity, and this mechanism coupled with enhancement of cholinergic functions has been suggested to explain donepezil-induced amelioration of cognitive deficits [40]. Furthermore, scopolamine-induced memory impairment in mice is also associated with altered brain oxidative stress status [41]. Thus, the effects of ginseng k-g3 on oxidative stress need to be examined.

In summary, we have reported here a viable and cost-effective method of Rg3 enrichment. The Rg3-enriched fraction, ginseng k-g3, significantly reversed scopolamine-induced memory impairment in mice in the passive avoidance and Morris water maze tests, signifying a specific influence of the compound on

reference or long-term memory. Moreover, we suggest that Rg3 enrichment through the ginseol k-g3 fraction enhanced the efficacy of Rg3 in scopolamine-induced memory impairment in mice, as demonstrated in the Morris water maze task. However, considering that ginseol k-g3 also contained other ginsenosides aside from Rg3, enhanced efficacy of ginseol k-g3 may have been brought by modulatory effects exerted by other ginsenosides (e.g., Rg5 and Rk1). It is noteworthy that both Rg5 and Rk1 have been shown to protect against scopolamine-induced memory deficits in mice [18,42]. As stated previously, the presence of other beneficial ginsenosides in ginseol k-g3 may be an important feature of the preparation when used as a formulation for AD. Furthermore, the mechanism underlying the reversal of scopolamine-induced amnesia by ginseol k-g3 is not yet known, but is not related to inhibition of AChE activity. Further optimization of Rg3-enriched preparations is suggested because it may aid the development of Rg3-enriched nutraceuticals with therapeutic potential for AD. Additionally, it would also be beneficial to evaluate the memory enhancing effects of ginseol k-g3 in normal animals.

Conflicts of interest

All authors have no conflicts of interest to declare.

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