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# Memory Enhancing and Neuroprotective Effects of Selected Ginsenosides

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The effects of ginsenosides $Rg_3(R)$, $Rg_3(S)$ and $Rg_5/Rk_1$ (a mixture of $Rg_5$ and $Rk_1$, 1:1, w/w), which are components isolated from processed *Panax ginseng* C.A. Meyer (*Araliaceae*), on memory dysfunction were examined in mice using a passive avoidance test. The ginsenosides $Rg_3(R)$, $Rg_3(S)$ or $Rg_5/Rk_1$, when orally administered for 4 days, significantly ameliorated the memory impairment induced by the single oral administration of ethanol. The memory impairment induced by the intraperitoneal injection of scopolamine was also significantly recovered by ginsenosides $Rg_3(S)$ and $Rg_5/Rk_1$. Among the three ginsenosides tested in this study, $Rg_5/Rk_1$ enhanced the memory function of mice most effectively in both the ethanol- and scopolamine-induced amnesia models. Moreover, the latency period of the $Rg_5/Rk_1$-treated mice was 1.2 times longer than that of the control (no amnesia) group in both models, implying that $Rg_5/Rk_1$ may also exert beneficial effects in the normal brain. We also evaluated the effects of these ginsenosides on the excitotoxic and oxidative stress-induced neuronal cell damage in primary cultured rat cortical cells. The excitotoxicity induced by glutamate or *N*-methyl-D-aspartate (NMDA) was dramatically inhibited by the three ginsenosides. $Rg_3(S)$ and $Rg_5/Rk_1$ exhibited a more potent inhibition of excitotoxicity than did $Rg_3(R)$. In contrast, these ginsenosides were all ineffective against the $H_2O_2$- or xanthine/xanthine oxidase-induced oxidative neuronal damage. Taken together, these results indicate that ginsenosides $Rg_3(S)$ and $Rg_5/Rk_1$ significantly reversed the memory dysfunction induced by ethanol or scopolamine, and their neuroprotective actions against excitotoxicity may be attributed to their memory enhancing effects.

**Key words:** Ginsenosides, Memory enhancement, Ethanol, Scopolamine, Excitotoxicity, Cortical neurons

## INTRODUCTION

Korean ginseng (*Panax ginseng* C. A. Meyer, *Araliaceae*) is one of the most widely used medicinal plants, particularly in traditional oriental medicine, and it has a wide range of pharmacological actions on the cardiovascular, endocrine, immune, and central nervous systems (CNS) (Attele *et al.*, 1999). Among its diverse effects in the CNS, ginseng extracts have been shown to improve learning and memory in normal, aged or brain-damaged animals (Petkov and Mosharrof, 1987; Zhong *et al.*, 2000; Kennedy and Scholey, 2003). Ginsenosides are the saponin constituents in ginseng, and they are considered to be the bioactive ingredients that exert many beneficial effects such as the enhancement of memory function.

Several ginsenosides have been previously reported to ameliorate impaired memory function. For example, ginsenosides $Rb_1$ and $Rg_1$ have been shown to accelerate memory acquisition of rats on a Y-maze task (Saito, 1990), and they also enhanced the cognitive function of mice in a Morris water maze (Mook-Jung *et al.*, 2001). Yamaguchi *et al.* (1995) have also reported that $Rg_1$ improved the scopolamine-induced impaired performance of rats in a

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radial-arm maze. $Rb_1$ and its metabolite $M_1$ were recently reported to improve memory disorders, axonal atrophy, and synaptic loss in a mouse model of Alzheimers disease (AD) that was induced by an i.c.v. injection of $A_{\beta(25\text{-}35)}$ (Tohda *et al.*, 2004). Based on these findings, the memory enhancing effects of ginseng root have often been attributed to the ginsenosides $Rb_1$ and $Rg_1$ (Mook-Jung *et al.*, 2001), although other ginsenosides such as Re may also exert similar effects on memory function (Yamaguchi *et al.*, 1996). $Rb_1$ and $Rg_1$ are known to facilitate cholinergic function (Zhang *et al.*, 1990; Benishin *et al.*, 1991; Benishin, 1992), to exhibit neuroprotective effects (Li and Zhang, 1997; Kim *et al.*, 1998; Liao *et al.*, 2002) and to promote neurite outgrowth in cultured neurons (Nishiyama *et al.*, 1994). All of these actions may contribute to their memory enhancing effects.

It is generally believed that the pharmacological activities of red ginseng are stronger than those of white ginseng (Takaku *et al.*, 1990), and that may be due to the alterations in their chemical constituents during the steaming process. New ginsenosides, including $Rg_3$, $Rg_5$ and $Rk_1$, were recently identified and isolated from steam processed ginseng extracts (Kwon *et al.*, 2001). $Rg_3$ has been reported to attenuate glutamate-induced neurotoxicity and to inhibit the overproduction of nitric oxide and malondialdehyde formation induced by glutamate (Kim *et al.*, 1998).

To characterize the pharmacological actions of the newly identified ginsenosides in the CNS, we examined the effects of $Rg_3(R)$ and $Rg_3(S)$, the diastereoisomeric forms of $Rg_3$, and $Rg_5/Rk_1$, a 1:1 (w/w) mixture of $Rg_5$ and $Rk_1$, on the memory dysfunction induced in mice by ethanol or scopolamine. The structures of these ginsenosides are shown in Fig. 1. Alcoholic dementia is reported to be similar to AD dementia with regards to the affected mediator systems and the brain regions that are damaged (Raghavendra and Kulkarni, 2001), and ethanol-intoxicated mice have been used as an animal model for AD-associated dementia (Lee *et al.*, 1995; Cho *et al.*, 2003). Scopolamine is a muscarinic antagonist that decreases central cholinergic activity and causes impairment of learning and memory (Kopelman and Corn, 1988; Okaichi *et al.*, 1989), and thus, it is also commonly used to induce memory dysfunction in animals (Pitsikas *et al.*, 2001; Wallenstein and Vago, 2001).

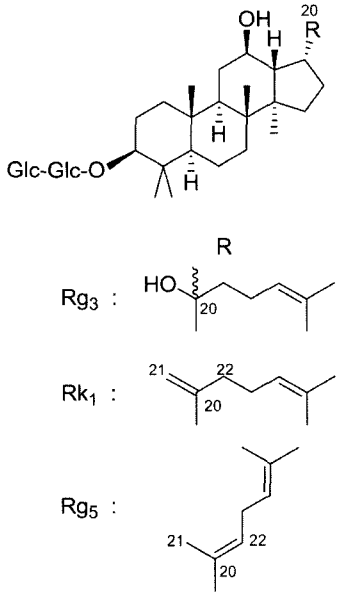


**Fig. 1.** The structures of ginsenosides $Rg_3(R)$, $Rg_3(S)$, $Rg_5$ and $Rk_1$.

L-glutamate is the principal excitatory amino acid neurotransmitter in the mammalian CNS. Excessive release of glutamate is known to be the major cause of neuronal damage in many acute and chronic neurodegenerative disorders including AD (Sauer and Fagg, 1992). Overstimulation of the glutamatergic system results in a massive influx of $Ca^{2+}$, mainly through the NMDA receptor, and this subsequently activates neurotoxic mechanisms such as the generation of reactive oxygen species (ROS) (Coyle and Puttfarken, 1993; Sengpiel *et al.*, 1998). Oxidative stress, including ROS, is also known to be associated with AD (Halliwell, 1992). In this study, we also examined the effects of the three ginsenosides on glutamate- or NMDA-induced excitotoxic damage and on the oxidative stress-induced neuronal damage by using primary cultured rat cortical cells.

## MATERIALS AND METHODS

### Instruments and chemicals

The Passive/Active avoidance system, PACS-30 (Columbus Co., OH, USA), was used for the passive avoidance test. Scopolamine hydrobromide, laminin, poly-L-lysine, L-glucose, cytosine arabinoside, L-glutamate, NMDA, $H_2O_2$, xanthine, xanthine oxidase, 3-(4,5-dimethylthiazoL-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and the lactate dehydrogenase (LDH) assay kit were purchased from Sigma Chemical Co. (MI, USA). The materials used for the cell culture including minimum essential medium (MEM) with Earles salts, L-glutamine, fetal calf serum, and horse serum were obtained from Gibco BRL (Gaithersburg, USA). All the solvents used in this study were of HPLC or analytical grade. The ginsenosides $Rg_3(R)$, $Rg_3(S)$ and $Rg_5/Rk_1$ (a mixture of $Rg_5$ and $Rk_1$, 1:1, w/w) were kindly donated by Ginseng Science Inc. (Seoul, Korea).

### Animals and dosing regimens

Male ICR mice (weighing 18-20 g) were purchased from Folas International Co. (Seoul, Korea). They were housed in an animal room with standard foods, ventila-

tion, a temperature of 24 ± 1 °C, a 55±5% relative humidity, and controlled lighting (from 6:00 to 18:00 daily). The ginsenosides were dissolved or suspended in water and administered orally once a day for 4 days at a dosage of 10 mg/kg body weight in a volume of 1 mL/100 g body weight. To induce memory impairment in the mice, the mice were administered either ethanol or scopolamine. Ethanol was given orally at a dosage of 3 g/kg at 30 min after the last administration of the ginsenosides. Scopolamine was intraperitoneally injected at a dosage of 3 mg/kg at 25 min after the last administration of the ginsenosides.

### Passive avoidance test

The memory acquisition tests were carried out by the previously described method (Lee *et al.*, 1995; Cho *et al.*, 2003). The apparatus consisted of two equal compartments ($15 \times 15 \times 22$ cm) separated by a wall with a guillotine door ($4 \times 3.5$ cm) in the lower middle part. One of the two compartments was illuminated and the other compartment was dark. The amnesia-induced mice were first placed in the dark room 1 h before the trials began. The test was conducted on two consecutive days at the same time of each day. On the first day, each mouse was placed in the illuminated compartment (10 watts). After 30 s, the guillotine door was raised, allowing the mouse access to the dark compartment. Once the mouse entered the dark compartment, the guillotine door was lowered and the mouse then received an electric shock (0.6 mA for 5 s). On the second day, the testing trial was carried out according to the same procedure of the learning trial. The mouse was again placed in the illuminated compartment, the guillotine door was opened and the step-through latency period was measured. An upper cut off time for the latency period was set, which allowed the mice exposed in the light compartment to stay there for a maximum of 300 s. The latency period in seconds and the number of mice that did not enter the dark compartment were recorded during the testing trials.

### Primary cultures of rat cortical cells

Pregnant Sprague-Dawley (SD) rats, whose length of pregnancy was known, were obtained from Daehan Biolink (Chungbuk, Korea), and they were maintained with Purina laboratory chow and water *ad libitum* until they were used in our experiments. Cortical cell cultures containing neuronal and non-neuronal cells were prepared from the cerebral cortices of SD rat embryos at 16-18 days of gestation, and these cells were maintained as previously described (Cho *et al.*, 2000). In brief, the embryos cortices were dissected free and then mechanically dissociated into single cells by triturations through fire-polished Pasteur pipettes. The cells were plated at a density of $4\text{-}5 \times 10^5$ cells per well on 24-well culture plates that were coated with poly-L-lysine and laminin. The cultures were incubated at 37°C within a humidified atmosphere of 95% air/5% $CO_2$ in a medium consisting of MEM supplemented with L-glucose (final concentration, 25 mM), L-glutamine (2 mM), fetal calf serum (5%), and horse serum (5%). Proliferation of the non-neuronal cells was arrested by the addition of 10 mM cytosine arabinoside at 7 days after plating (Cho *et al.*, 2001).

### Experimental treatment of cultured cells and the assessment of cell damage

To induce excitotoxic neuronal damage, the cortical cells that had been maintained for 10-12 days *in vitro* were washed with HEPES-buffered salt solution (HBSS) and then exposed to either 100 μM glutamate or NMDA for 15 min in $Mg^{2+}$-free HBSS, as was previously described (Cho *et al.*, 2001, Cho and Lee, 2004), in the absence or the presence of the test samples. To induce oxidative damage, the cultured cells were exposed to either 100 μM $H_2O_2$ for 5 min or to xanthine/xanthine oxidase (0.5 mM/10 mU/mL) for 10 min in the absence or presence of the test samples (Jung *et al.*, 2002; Dok-Go *et al.*, 2003). After this exposure, the cells were washed and maintained for 20-24 h at 37°C in MEM supplemented with 21 mM L-glucose. The extent of cell damage was then quantified by measuring the amount of LDH released into the culture media by cell damage (Cho *et al.*, 2001, 2002). Stock solutions of ginsenosides were prepared in 100% DMSO at 200x the highest concentration required for testing and then the solutions were serially diluted to the desired concentrations. For the control treatment, sister cultures were exposed to 0.5% DMSO, which was the DMSO concentration previously shown to exhibit no effect on cell viability (Cho *et al.*, 2000).

### Assays of DPPH radical scavenging activity and lipid peroxidation

The DPPH radical scavenging activity, the lipid peroxidation induced by $Fe^{2+}$ and by ascorbic acid in the rat brain homogenates were all measured according to the methods previously described (Cho and Lee, 2004).

### Statistical analysis

The data acquired from the step-through tests were expressed in terms of medians and interquartile ranges, and this was analyzed by Mann-Whitney U-tests for comparison of the control group and the treated group, and by the Wilcoxon signed rank test for comparison of learning and testing procedures. The criterion for statistical significance was $P<0.05$ on all the evaluations. The $IC_{50}$ values, the 50% inhibitory concentrations, were determined by nonlinear regression using Prism (GraphPad Software Inc., CA, USA).

## RESULTS

### Effects of the ginsenosides on the ethanol-induced memory impairment in mice

To induce memory dysfunction in mice, either ethanol or scopolamine was given to the mice before their training. A single oral administration of ethanol (3 g/kg) produced severe memory impairment, which is in agreement with our previous report (Cho *et al.*, 2003). The step through latency period of the mice treated with ethanol was reduced to 24.9% of the control mice (no ethanol-treatment) in the passive avoidance test (Fig. 2). The reduced latency period of the ethanol-treated mice was significantly increased by the oral administration of ginsenosides $Rg_3(R)$, $Rg_3(S)$ or $Rg_5/Rk_1$ for 4 days (Mann-Whitney U-test, $P<0.01$). Although all three of the ginsenosides were effective, $Rg_5/Rk_1$ exhibited the most effect for reversing the shorting of the latency period. The latency period of the mice treated with $Rg_5/Rk_1$ was even longer than that of the control mice (a 1.2-fold increase). Both $Rg_3(R)$ and $Rg_3(S)$ treated mice exhibited about a 2.7 times longer latency period in comparison with the latency period of the ethanol-treated mice.

### Effects of the ginsenosides on the scopolamine-induced memory impairment in mice

We then induced memory impairment by the single i.p. injection of scopolamine (3 mg/kg), and we examined the effects of ginsenosides on the memory impairment. Scopolamine produced a similar degree of memory impairment as the ethanol, and it reduced the latency period of the mice to 23.2% (Fig. 3). The latency period of the scopolamine-treated mice was significantly increased by the oral administration of ginsenosides $Rg_3(S)$ or $Rg_5/Rk_1$ (Mann-Whitney U-test, $P<0.01$). Again, $Rg_5/Rk_1$ exhibited the most effective reversal for the shorting of the latency period among the three ginsenosides that were tested. The latency period in the scopolamine-treated mice was increased by $Rg_5/Rk_1$ up to 1.2-fold of the latency period for the control mice. Although $Rg_3(S)$ exhibited similar effects in both the ethanol-induced and scopolamine-induced amnesia models, $Rg_3(R)$ did not significantly increase the latency period in the scopolamine-induced amnesia group.

### Effects of ginsenosides on excitotoxicity in primary cultured rat cortical cells

To examine the effects of ginsenosides on the excito-

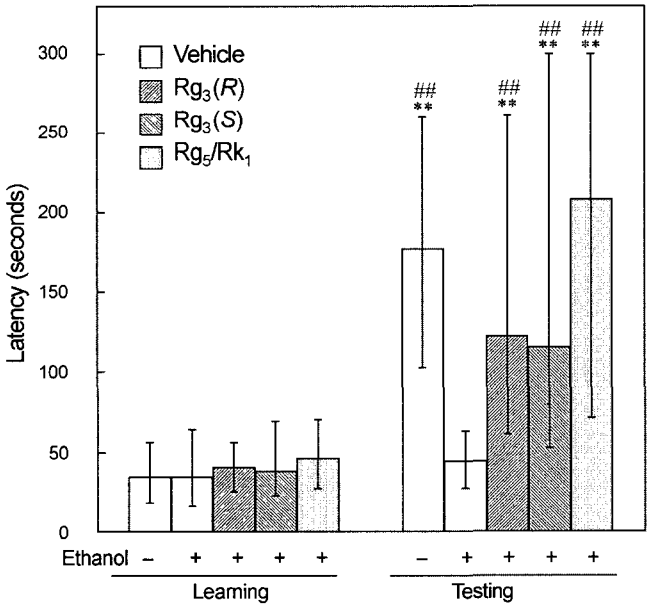


**Fig. 2.** Effects of ginsenosides $Rg_3(R)$, $Rg_3(S)$ and $Rg_5/Rk_1$ on the cognitive performance in ethanol-induced amnesia mice. The animals were orally administered with saline (vehicle), ginsenoside $Rg_3(R)$, $Rg_3(S)$ or $Rg_5/Rk_1$ (10 mg/kg) once a day for 4 days, and their memory function was examined by a passive avoidance test after the induction of amnesia with ethanol. Each column represents the median with the vertical bars indicating the interquartile ranges of n = 25 to 32 (*p<0.05, **p<0.01, compared with the ethanol-treated vehicle group; #p<0.05, ##p<0.01, compared with the respective learning latency).

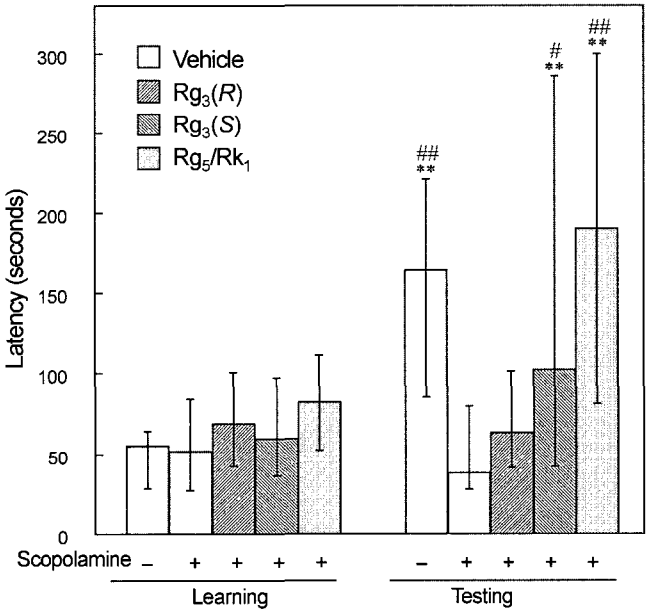


**Fig. 3.** Effects of ginsenosides $Rg_3(R)$, $Rg_3(S)$ and $Rg_5/Rk_1$ on the cognitive performance in scopolamine-induced amnesia mice. The animals were orally administered with saline (vehicle), ginsenoside $Rg_3(R)$, $Rg_3(S)$ or $Rg_5/Rk_1$ (10 mg/kg) once a day for 4 days, and their memory function was examined by a passive avoidance test after the induction of amnesia with scopolamine. Each column represents the median with the vertical bars indicating interquartile ranges of n = 20 to 26 (*p<0.05, **p<0.01, compared with the scopolamine-treated vehicle group; #p<0.05, ##p<0.01, compared with the respective learning latency).

toxic or oxidative neuronal cell damage, we employed primary cultured rat cortical cells that had been maintained for 10-12 days *in vitro*. Excitotoxicity was induced by the exposure of the cultured cells for 15 min to either 100 μM glutamate or NMDA. Consistent with our previous reports (Cho *et al.*, 2000, 2002) and other reports (Choi *et al.*, 1987), prominent neuronal damage was produced after 20-24 h of exposure to glutamate or NMDA. Based on the LDH measurements, approximately 70-80% of the cells were damaged compared to the vehicle-treated control cells. As illustrated in Fig. 4, the excitotoxicity induced by glutamate (A) or NMDA (B) was dramatically inhibited by $Rg_3(R)$, $Rg_3(S)$ or $Rg_5/Rk_1$ in a concentration-dependent manner. Fifty percent inhibition of the glutamate-induced toxicity was achieved at 75.3 μg/mL, 16.0 μg/mL, and 14.7 μg/mL concentrations of $Rg_3(R)$, $Rg_3(S)$ or $Rg_5/Rk_1$, respectively. Similarly, the $IC_{50}$ values for the NMDA-induced toxicity were 27.8 μg/mL, 9.3 μg/mL, and 6.9 μg/mL for $Rg_3(R)$, $Rg_3(S)$ or $Rg_5/Rk_1$, respectively. $Rg_5/Rk_1$ exhibited the most potent inhibition of the excitotoxic neuronal damage.

## Effects of ginsenosides on oxidative damage in primary cultured rat cortical cells

Exposure of the cultured cells to $H_2O_2$ or xanthine/xanthine oxidase produced severe oxidative neuronal cell

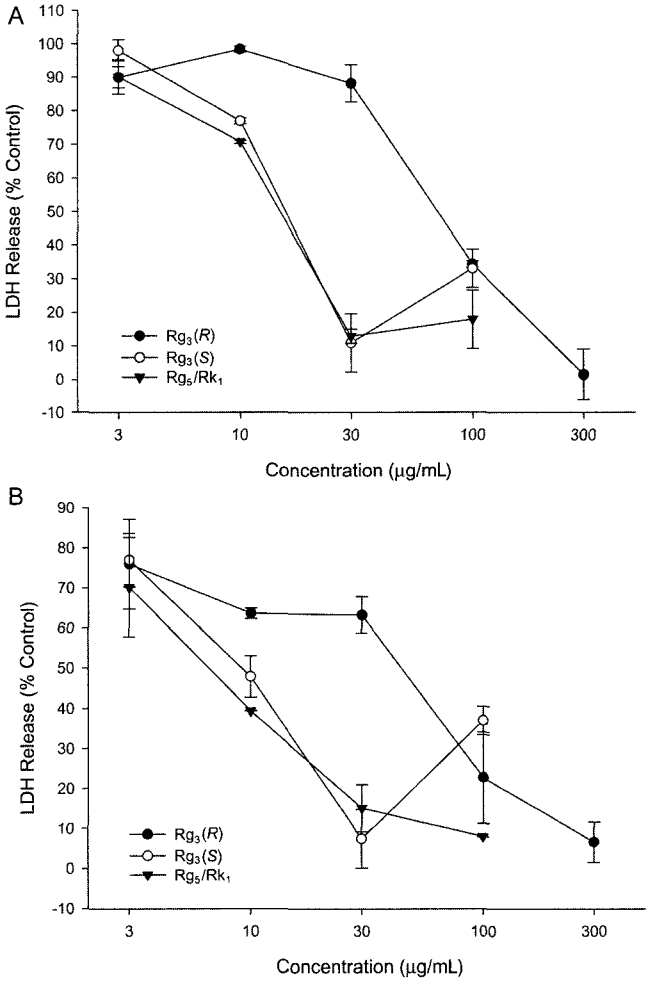


**Fig. 4.** Effects of ginsenosides $Rg_3(R)$, $Rg_3(S)$ and $Rg_5/Rk_1$ on the excitotoxicity induced by glutamate (A) or NMDA (B) in primary cultured cortical cells. Cultured cells (cultured 10-12 days *in vitro*) were exposed to 100 μM glutamate or NMDA for 15 min in the absence or presence of the indicated ginsenoside concentrations, and the cell damage was assessed after 20-24 h by measuring LDH activity in the culture media. Data were calculated as the percent of control LDH activity measured in the medium from cells exposed to the respective excitotoxic insults without ginsenosides. Each point represents the mean ± S.E.M. from 4 measurements.

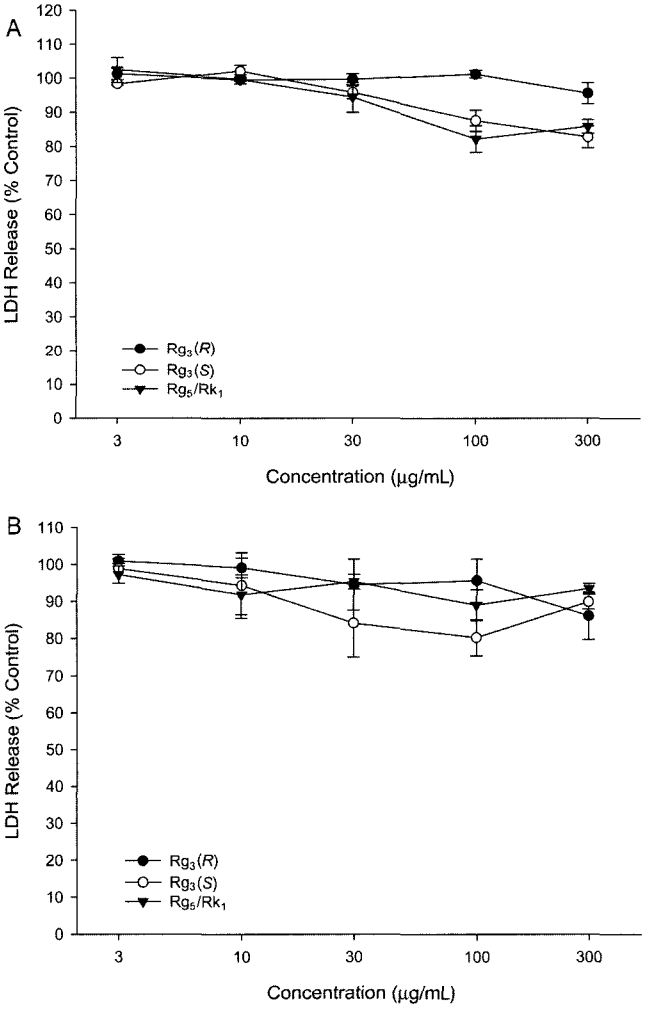


**Fig. 5.** Effects of ginsenosides $Rg_3(R)$, $Rg_3(S)$ and $Rg_5/Rk_1$ on the oxidative damage induced by $H_2O_2$ (A) or xanthine/xanthine oxidase (B) in primary cultured cortical cells. Cultured cells (10-12 days *in vitro*) were exposed to $H_2O_2$ (100 μM) for 5 min or xanthine (0.5 mM)/xanthine oxidase (10 mU/mL) for 10 min in the absence or presence of the indicated concentrations of ginsenosides. Cell damage was assessed by LDH assay after 20-24 h and calculated as described in the legend of Fig. 4.

damage. Based on LDH activity measurements, approximately 80-90% of the cells were damaged (Jung *et al.*, 2002; Dok-Go *et al.*, 2003; Cho and Lee, 2004). As shown in Fig. 5, the $H_2O_2$- (A) or xanthine/xanthine oxidase- (B) induced oxidative damage was not effectively inhibited by the ginsenosides that were tested in this study. Moreover, the three ginsenosides showed no significant effects on the DPPH radicals. Only $Rg_3(S)$ exhibited a modest inhibition ($22.9 \pm 1.4\%$) on the lipid peroxidation in rat brain homogenates induced by $Fe^{2+}$ and ascorbic acid at concentrations of 300 μg/mL.

## DISCUSSION

Extracts of Korean red ginseng have been reported to improve scopolamine-induced learning disability and spatial working memory in mice (Jin *et al.*, 1999). In the present study, we have demonstrated the memory enhancing effects of $Rg_3(R)$, $Rg_3(S)$ and $Rg_5/Rk_1$, the newly isolated ginsenosides from Korean red ginseng, in mice. In addition, we have also shown their neuroprotective actions against excitotoxicity in primary cultured cortical cells.

The memory deficits induced by ethanol were significantly recovered by the administrations of ginsenosides $Rg_3(R)$, $Rg_3(S)$ or $Rg_5/Rk_1$ (Fig. 2). Ginsenosides $Rg_3$(S) and $Rg_5/Rk_1$ also improved the scopolamine-induced memory impairment in mice (Fig. 3). Among the three ginsenosides we tested, $Rg_5/Rk_1$ was the most effective; its administration resulted in a complete reversal of both the ethanol-induced and scopolamine-induced memory deficits in mice. The latency period in the $Rg_5/Rk_1$-treated group was even longer (1.2 fold) than that of the control (no amnesia) group (Figs. 1 and 2), suggesting that $Rg_5/Rk_1$ may be able to enhance memory function in normal animals.

In the ethanol-induced amnesia model, $Rg_3(R)$ exerted similar effects as $Rg_3(S)$ (Fig. 2). However, in the scopolamine-induced amnesia model, $Rg_3(S)$ was more effective than $Rg_3(R)$ (Fig. 3). Although $Rg_3(R)$ appeared to improve cognitive function in the scopolamine-treated mice, its effect was not statistically significant (Fig. 3). The discrepancy for the effects of $Rg_3(R)$ in the two amnesia models may reside in the differences in the mechanisms involved for inducing memory impairment. Scopolamine induces amnesia mainly by antagonizing the muscarinic receptor, and so it inhibits cholinergic transmission (Kopelman and Corn, 1988; Okaichi *et al.*, 1989). Ethanol is known to exert a variety of effects in the brain that involve many different brain processes and neurotransmitters (Nevo and Hamon, 1995; Tracy *et al.*, 1997). Ethanol has been reported to not only inhibit muscarinic action (Brioni *et al.*, 1989), but it also alters dopamine (Boileau *et al*, 2003) or serotonin levels (Hayashi *et al.*, 2003) in the brain. Thus, it could be possible that $Rg_3$ may also affect other pathway(s) in addition to the cholinergic system, and that the site of action in the cholinergic system may be stereoselective.

We then examined the effects of the three ginsenosides on the excitotoxic neuronal cell damage in the cultured cortical cells. $Rg_3$, which is a mixture of (*R*) and (*S*) isoforms, has previously been reported to attenuate glutamate-induced neurotoxicity in cultured cortical cells (Kim *et al.*, 1998). We confirmed this prior observation in our study by showing that both $Rg_3(R)$ and $Rg_3(S)$ dramatically inhibited the excitotoxic neuronal damage induced by glutamate or NMDA (Fig. 4). All three ginsenosides exhibited a more potent inhibition of the NMDA-induced excitotoxicity than for the glutamate-induced toxicity, and these findings are consistent with the previous observations (Kim *et al.*, 2002). Using fura-2-based digital imaging techniques in cultured hippocampal neurons, Kim and coworkers found that the total saponins in ginseng inhibited the NMDA-induced $Ca^{2+}$ influx, but the glutamate-induced $Ca^{2+}$ influx was less effectively inhibited. Furthermore, they also demonstrated that $Rg_3$ is the active component mediating these neuroprotective actions (Kim *et al.*, 2002). In our study, however, $Rg_5/Rk_1$ exhibited even more potent inhibition of the excitotoxic neuronal damage than either form of $Rg_3$ (Fig. 4), which is in agreement with our *in vivo* data, (see Figs. 2 and 3).

We then examined the effects of the ginsenosides on the oxidative neuronal damage induced by $H_2O_2$ or xanthine/xanthine oxidase in the cultured cells. Kim *et al.* (1998) have showed that $Rg_3$ inhibited malondialdehyde formation in cultured cells exposed to glutamate, and this protected neurons from oxidative damage. However, the ginsenosides were unexpectedly found to be ineffective against the $H_2O_2$- or xanthine/xanthine oxidase-induced oxidative damage in the present study (Fig. 5). This may be due to the different chemical insults used to induce oxidative damage ($H_2O_2$ or xanthine/xanthine oxidase treatment in our study vs glutamate treatment in the report by Kim *et al.*, 1998). In addition, the duration of the ginsenosides treatment may have also had an influence on their effects. We treated the cultured cells with the ginsenosides only during the periods they were exposed to the respective insults, whereas Kim and coworkers treated the cells with $Rg_3$ before, during and after the glutamate treatment. Based on our additional experiments examining the antioxidant properties of these ginsenosides, $Rg_3(R)$, $Rg_3(S)$ and $Rg_5/Rk_1$ had no effects on DPPH radicals, although $Rg_3(S)$ exhibited only a modest inhibition of lipid peroxidation in rat brain homogenates. These findings may explain why the ginsenosides were incapable of inhibiting the oxidative damage in the cultured cells (Fig. 5).

Taken together, these results indicate that the ginsenosides $Rg_3(S)$ and $Rg_5/Rk_1$ significantly reversed the memory dysfunction that was induced by ethanol or scopolamine, and the ginsenosides protected the cultured cortical cells from excitotoxic damage. This is the first report demonstrating the memory enhancing effects and neuroprotective effects of $Rg_5/Rk_1$ and $Rg_3(S)$. Among the ginsenosides that we tested, $Rg_5/Rk_1$ was the most effective and potent. Based on these findings, $Rg_5/Rk_1$ and $Rg_3(S)$ may have useful therapeutic potentials for the clinical management of memory loss that is observed in aging or neurodegenerative disorders such as AD.

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