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Anxiolytic and Antidepressant Activities of Ginsenoside Rb1

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Abstract – The psychopharmacological profile of ginsenosides has not yet been confirmed systematically although various neuropharmacological activities associated with them have been investigated. In the present study, the psychological activities of Rb1 were investigated to evaluate whether it can be used in treatment or prevention of psychological disorders. Rb1 was intravenously injected at doses of 0.2, 2, 5 and 10 mg/kg. The effects of Rb1 on the Cl^- ion influx were investigated using IMR-32 human neuroblastoma cells. Moreover, locomotor activity, forced swimming activity, activity on rotating rod and activity in elevated plus-maze were tested in mice. Rb1 increased the Cl^- influx into the intracell region in a dose-dependent manner. Rb1 did not cause change in behavior in total open field when locomotor activity was tested, however it increased activities, especially, such as rearing frequency in center area. Administration of Rb1 at 0.2 mg/kg significantly reduced activities on rotating rod however administration at high dosages had no effect on them. Rb1 administration decreased animal immobile time in a water chamber in a dose dependent manner, and increased the strong mobile time of animals. In conclusion, the present results demonstrate that Rb1 contributes to the psychopharmacological effects of ginseng and may be used in treatment or prevention of psychological disorders such as anxiety or depression.

Key words □ ginsenoside Rb1, anxiolytic, antidepressant

INTRODUCTION

Phytochemicals may be biologically active and therefore capable of modulating certain physiological and pathological processes. *Panax ginseng* (ginseng) is one of the most medicinally important genera in the oriental medicine and has been used for thousands of years as a traditional medicine in many Asian countries (Hsu *et al.*, 1985). The active constituents of ginseng are ginsenosides, which have varying biological activities and high medicinal values (Zhou & Yan, 2003). Ginsenosides are normally fractioned into two groups, the panaxadiol group (e.g., Rb1 and Rc) and the panaxatriol group (e.g., Rg1 and Re), based on the types of aglycone they contain. Ginsenosides Rg1 and Rb1 play a major role in exerting effects on various aspects of the central nervous system (CNS), such as learning, memory, neuroprotection and modulation of neurotransmitters (Tsang *et al.*, 1985; Benishin, 1992; Xue *et al.*, 2006).

Rg1 exerts a number of pharmacological effects, including reductions in levels of the Alzheimer's amyloid beta peptide (Chen *et al.*, 2006), potential neurotrophic and neuroprotective effects (Rudakewich *et al.*, 2001; Radad *et al.*; 2004, Chen *et al.*, 2002), anti-aging and neurotropic effects (Liu, 1996), an ameliorating effect on performance impairment induced by scopolamine (Yamaguchi *et al.*, 1996), promotion of functional neovascularization into a polymer scaffold *in vivo* and the proliferation, chemoinvasion, and tubulogenesis of endothelial cells *in vitro* (Liang *et al.*, 2005; Sengupta *et al.*, 2004). Further, in addition to aforementioned properties, Rg1 possess an estrogen-like activity (Chan *et al.*, 2002). Ginsenoside Rb1 is considered the most abundant of the more than 20 ginsenosides (Attele *et al.*, 2002; Lim *et al.*, 2005; Washida and Kitanaka, 2003). Rb1 promotes neurotransmitter release (Xue *et al.*, 2006), attenuates stress-induced increases in neurosteroids (Lee

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et al., 2006), blocks homocysteine-induced endothelial dysfunction in porcine coronary arteries (Zhou *et al.*, 2005), and can improve scratching behaviors (Shin *et al.*, 2005). The Rb1 containing fraction of ginseng has also been demonstrated to elicite a balanced Th1 and Th2 immune response (Rivera *et al.*, 2005) and significant anticonvulsant effects in animal models of acutely induced seizures have been observed in Rb1 and Rb3 (Lian *et al.*, 2006).

In spite of their various neuropharmacological activities being investigated, the psychopharmacological profile of ginsenosides have not yet been systematically confirmed. We decided to study the psychopharmacological activities of Rb1 based on an *in vitro* study and a literature review. In this study, the psychological activities of Rb1 were investigated to evaluate if it could be used in treatment or prevention of psychological disorders.

MATERIALS AND METHODS

Experimental animals

Male ICR mice (20-25 g) used in this study were obtained from Hanlim Laboratory Animals Co. (Hwasung, Korea). All animals were maintained on a standard light-dark cycle, at ambient temperature (22±2°C) and humidity (55±5%) with free access to chow pellets and water. All animals were acclimated to their home cages for at least 6 days before the experiment began. The experimental groups, which consisted of 8-10 animals per each drug and dose, were chosen by means of a randomized schedule and all mice were used only once. All tests took place between 10:00 and 16:00 h.

Materials

Ginsenosides, Rb1, Rc, Rg1, and compound K, were supplied by BTgin Co. (Choongnam, Korea). Imipramine and diazepam were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA). Imipramine, diazepam, and ginsenoside Rb1 were dissolved in sterile distilled water immediately before injection. Rb1 was intravenously injected at doses of 0.2, 2, 5 or 10 mg/kg. 0.5 mg/kg Diazepam was intravenously injected and 5 mg/kg imipramine was orally administered to test mice. Animals in the control group were injected with the same volume of saline. Behavioral activities were measured 15 minutes after i.v. injection or 1 hour after oral administration.

Intracellular Cl^- ion measurement

Relative changes in the intracellular Cl^- concentration ($[Cl^-]_i$) in IMR-32 human neuroblastoma cells were monitored using the Cl^--sensitive indicator, *N*-(6-methoxyquinolyl) acetoetylester (MQAE), developed by Verkman *et al.* (1989). Experiments were performed as described by West and Molly (1996). Cells were washed twice and re-suspended at a concentration of 4×10^5 cells/ml in Hank's solution. Cells were incubated with 5 mM MQAE dye overnightat at room temperature to load the dye into cell. Fluorescence (excitation wavelength at 365nm, emission wavelength at 450 nm) was monitored in a well-stirred cuvette. Experiments were performed at room temperature to minimize fluorescent dye loss. Data are presented as relative fluorescence F_0/F, where F_0 is the fluorescence without Cl^- ions and F is the fluorescence as a function of time. The F_0/F values are directly proportional to $[Cl^-]_i$. All fluorescence values were corrected for background fluorescence which was separately determined using a HEPES-buffered KSCN solution containing 5 μM valinomycin to maximally quench the MQAE ion-selective signal (Shumaker *et al.*, 1999). In separate experiments the F_0 value was determined by bathing the cells with Cl^--free (KNO_3) solution containing 10 mM tributyltin and 10 mM nigericin.

Locomotor Activity

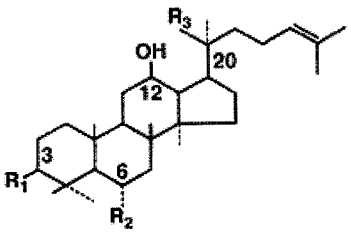
The observation apparatus consisted of nine plastic boxes (47×47 cm), and its field was bordered by 42 cm high sidewalls. The total distance moved and the total movement times were monitored for 10 min after administration (Kim *et al.*, 2003; Noldus *et al.*, 2001).

Rota rod test

The rota rod test was used to assess whether Rb1 caused myorelaxation or gross motor impairment in animals. Twenty-four hours before experiment, all mice were habituated to running on a rota rod at a speed of 60 rpm until they could remain there for 60s without falling. After the experiments, latency to fall was recorded (Lee *et al.*, 2006).

Elevated plus-maze test

The elevated plus-maze box and arms were made of plastic. The apparatus consisted of two open arms (30×6 cm in mice), alternating at right angles, with two arms enclosed by high walls of 20 cm. Each arm had a delimited central area of 6×6 cm. The whole apparatus was placed 50 cm above the floor. Animals were placed in the central square and allowed to explore the maze freely for 5 minutes, and the time spent in spent in open and closed areas were measured (Kim *et al.*,



Ginsenosides	R1	R2	R3
Ginsenoside-Rg1	-OH	-O-Glc	-O-Glc
Ginsenoside-Rb1	-O-Glc2-Glc	-H	-O-Glc6-Glc

Fig. 1. Structures of Rg1 and Rb1. Abbreviations for carbohydrates: Glc, glucopyranoside; superscripts indicate the carbon in the glucose ring that links the two carbohydrates.

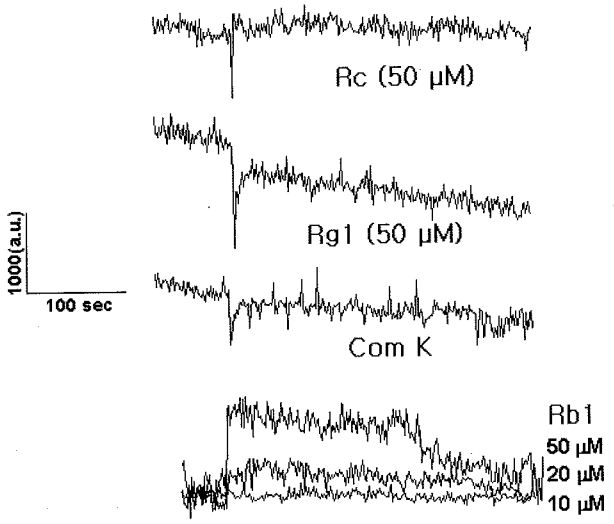


Fig. 2. Effects of ginsenosides on $[Cl^-]_i$ in neuroblastoma cells. Fluorescence was monitored in the excitation wavelength at 365nm and the emission wavelength at 450 nm using the Cl^--sensitive indicator, *N*-(6-methoxyquinolyl) acetoetylester. Contents of influx Cl^- ion was expressed as a peak (a.u.). Rc, ginsenoside Rc: Rg1, ginsenoside Rg1: Com K, compound K: Rb1, ginsenoside Rb1.

2003; Noldus *et al.*, 2001).

Forced swimming test

The mice were individually placed in a glass cylinder (20 cm high, 10 cm in diameter) containing 10 cm of water maintained at 23-25°C for 6 minutes. A mouse was regarded as immobile when it remained still in the water, making only small movements to keep its head above water. The total duration of immobility was measured after a 2 minute, habituation period (Park *et al.*, 2006).

Statistical analysis

Data are expressed as the mean±S.E.M.. ANOVA was used to compare the scores among the groups for one variable; followed by post hoc comparisons using the Newman-Keuls test.

RESULTS

Cl^- ion influx

Figure 2 shows the elelctrophysiological changes induced by ginsenosides treatment. Treatment with Rb1 increased the Cl^- influx into the intracell region in a dose-dependent manner. Whereas another ginsenosides didn't influence on Cl^- ion influx.

Locomotor Activity

As shown in figures 3 and 4, Rb1 affects locomotor activity in mice. Diazepam, a typical sedative drug, significantly decreased all the activities in the open field. However, although Rb1 did not significantly influence on locomotor activities such as distance moved, movement duration, rearing behavior or turn angle. But, activities of Rb1 treated animals in the center area increased in a dose-dependent manner, although this increase was not statistically significant. The rearing frequency of mice treated with 10 mg/kg was significantly higher than that of control animals. The tendency to increase activities in the center area represents enhanced exploratory, anxiolytic or anti-depressive behavior response.

Rota rod test

Activities on the rotating rod reflect the level of myorelaxation or sedation. Diazepam significantly decreased activities on the rotating rod. Administration of 0.2 mg/kg Rb1 significantly reduced the endurance time on the rotating rod and increased the falling frequency, however administration of high doses or Rb1 had little effect (fig. 5).

Elevated plus-maze test

The activities of animals on the elevated plus-maze reflect levels of anxiety. Percentage of time spent in open area was 30.82±6.133. Animals administered 0.2 mg/kg Rb1 stayed in the open arms longer than control animals, however administration of high doses had little effect (fig. 6).

Forced swimming test

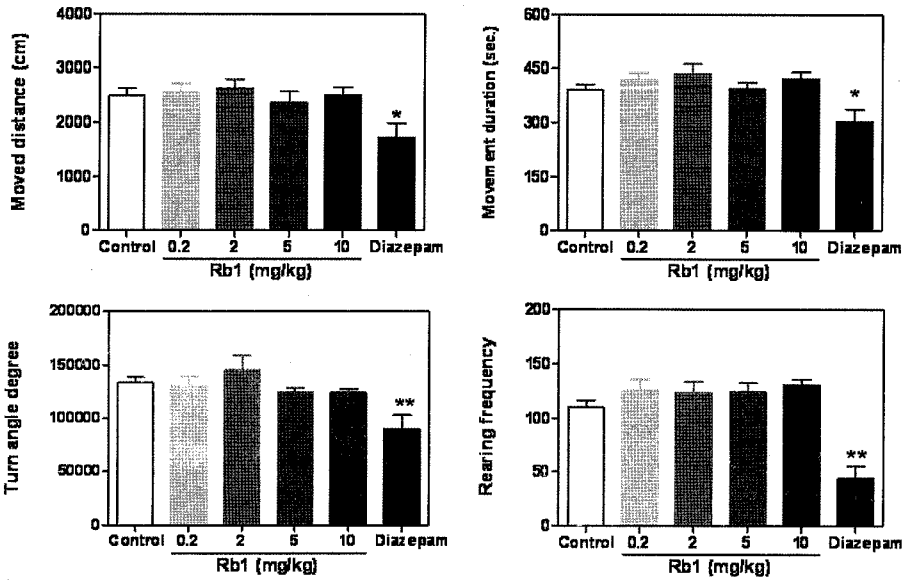


Fig. 3. Effects of Rb1 on locomotor activity in mice (n=8-10). Each bar represents the mean±S.E.M of the moved distances, movement durations, turn angle degrees or rearing frequencies for 10 minutes. Diazepam (0.5 mg/kg) was used as a positive control (*$p < 0.05$, **$p < 0.01$ versus control group).

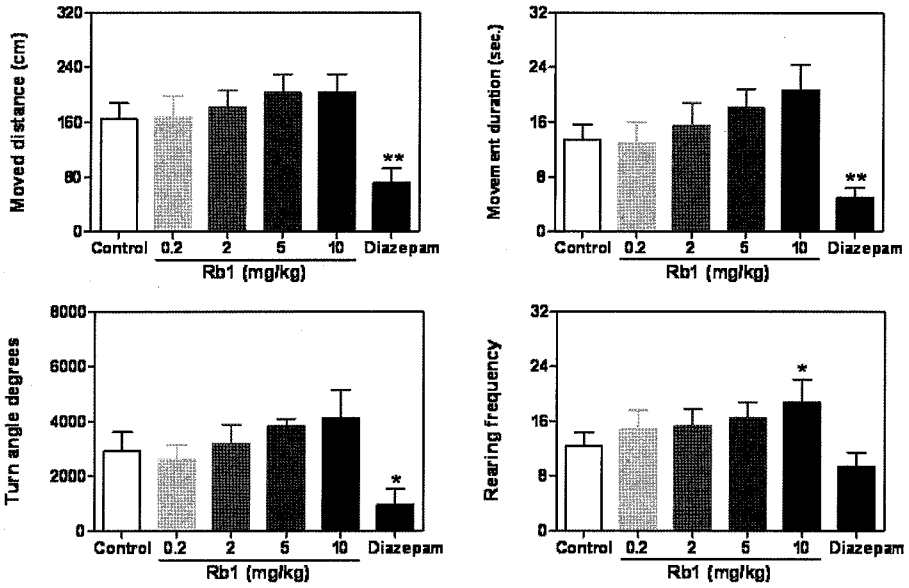


Fig. 4. Effects of Rb1 on locomotor activity in center area in mice (n=8-10). Each bar represents the mean±S.E.M of he moved distances, movement durations, turn angle degrees or rearing frequencies for 10 minutes. Diazepam (0.5 mg/kg) was used as a positive control (* $p < 0.05$, ** $p < 0.01$ versus control group).

The possible antidepressant effect of Rb1 was evaluated using the forced swimming test. As shown in figure 7, immobile duration in 5, 10 mg/kg Rb1 were 204.7±5.105, 191.6± 8.777 and strong mobile duration in 5, 10 mg/kg Rb1 were 18.54±3.745, 27.16±4.178. Rb1 administration decreased the immobile time in the water chamber in a dose dependent manner, as well as increased the strong mobile time. The effect of 10 mg/kg Rb1 on the immobility in the water chamber was similar to that of imipramine, an antidepressant drug (the positive control), however the effect of Rb1 on strong mobility was more potent than that of imipramine.

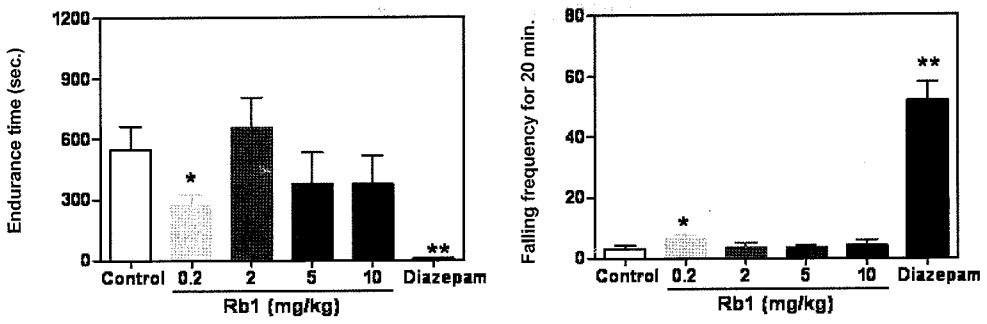


Fig. 5. Effects of Rb1 on activity on the rotarod in mice (n=8-10). Each bar represents the mean±S.E.M of the endurance times or falling frequencies for 20 minutes. Diazepam (0.5 mg/kg) was used as a positive control (*$p < 0.05$, ** $p < 0.01$ versus vehicle control group).

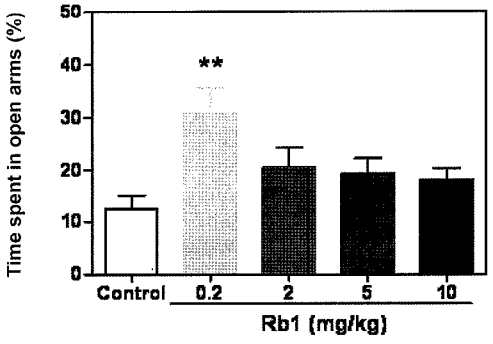


Fig. 6. Effects of Rb1 on activity on elevated plus maze in mice (n=8~10). Each bar represents the mean±S.E.M of the spent time percentages in open arms (*$p < 0.05$, **$p < 0.01$ versus control group).

DISCUSSION

We studied the psychopharmacological activity of Rb1 because in a previous *in vitro* test. Rb1 dominantly increased the Cl^- influx while other ginsenosides had no impact Cl^- ion influx. Rb1 has been found to increase a GABA-induced inward peak current (Choi *et al.*, 2003) and inhibit pentylenetetrazole (GABA antagonist) induced seizure (Lian *et al.*, 2006). Our results seems to support these findings. Rb1 did not cause a change of behavior in the total open field, but Rb1 treatment increased locomotor activities, such as rearing frequency in the center area. Since changes in locomotor activities may indicate the animal's motor function, emotional state or psychic conditions (such as wakefulness, sedation, anxiety, depression), (Meyer *et al.*, 2005; Fox *et al.*, 2001) these results suggest that Rb1 may act on psychic conditions such as wakefulness, sedation, anxiety, or depression. Administration of 0.2 mg/kg Rb1 significantly reduced activities on the rotating rod although administration of high doses had no effect, which indicates that low doses of Rb1 can induce myorelaxation or slight sedation without changes in locomotor activities. Administration of only 0.2 mg/kg Rb1 also exhibited anxiolytic activity in the plus-maze test, however high doses were not effective. The rota rod test and plus-maze test results demonstrate that Rb1 can induce anxiolytic activity with mild myorelaxation at a low dose. Previous studies indicate that Rb1 may be one of the anxiolytic

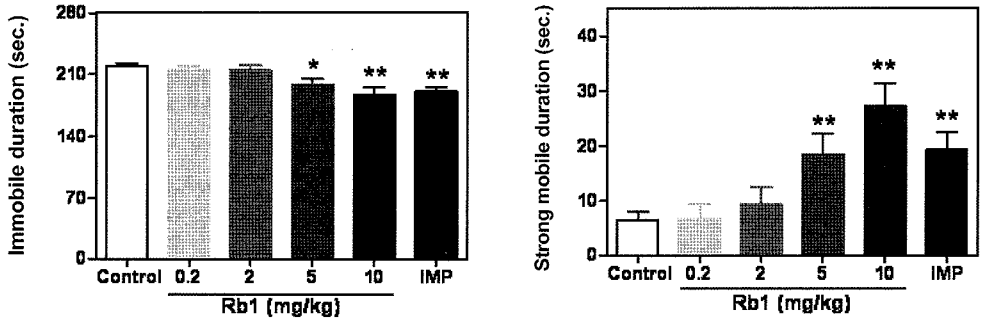


Fig. 7. Effects of Rb1 on forced swimming activity in mice (n=8-10). Each bar represents the mean±S.E.M of the immobile or strong mobile duration for 4 minutes (*$p < 0.05$, **$p < 0.01$ versus control group). IMP: imipramine (5 mg/kg, p.o.).

components found in ginseng (Carr *et al.*, 2006; Cha *et al.*, 2005). Although the action mechanism of the ginsenosides is still unclear, the anxiolytic effect of Rb1 may be related to the $GABA_A$ receptor channel (Choi *et al.*, 2003).

The forced swimming test (FST) is widely used for screening antidepressant drugs (Porsolt *et al.*, 1977). The immobile duration in the FST was shortened by administration of *P. ginseng* (Shin *et al.*, 2006). Administration of high doses of Rb1 (5 and 10 mg/kg) ameliorated depressive response and increased locomotor activities in the center area, however low doses had no effect. The antidepressant effect of Rb1 is an important finding in this study, however its mechanism needs to be studied further.

Ginseng has both stimulatory and inhibitory effects on the central nervous system and its constituents, the ginsenosides, possess various pharmacological activities (Attele *et al.*, 1999; Cheng *et al.*, 2005). Rg1 and Rb1 play a major role in exerting these effects on the central nervous system (Tsang *et al.*, 1985; Benishin, 1992). Rb1 also exhibited a specific effect based on dosage level, similar to the effects observed in other natural products (Louis *et al.*, 2006; Hui *et al.*, 2002). Comparative or different effects of ginseng might be caused by the variety of ginsenosides or a specific dose-related profile of ginsenoside. Therefore, dosage determination of ginsenoside when used for initial application or research procedures would be very important.

In conclusion, these results demonstrate that Rb1 does not influence locomotor activity, but exhibits anxiolytic effects at the low doses (0.2 mg/kg) and antidepressant effects at high doses (5 and 10 mg/kg). Rb1 contributes to psychopharmacological effects of ginseng and the action of Rb1 might be related to the $GABA_A$ receptor channel. These results also suggest that Rb1 might be useful in treatment or prevention of psychological disorders such as anxiety or depression.

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