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Anxiolytic-like Effects of Saponin and Polysaccharide Fractions Extracted from White and Red Ginsengs in the Elevated Plus-Maze Model

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Abstract Ginseng has been widely used for the management of anxiety and emotional instability, but there is little experimental evidence supporting these clinical applications. The anxiolytic-like effect of ginseng saponin and polysaccharide fractions of white (WG) and red ginsengs (RG) was investigated using the elevated plus-maze test. The saponin (SF) and polysaccharide (PF) fractions were orally administered to male ICR mice for 3 days and behavioral test for the anxiolytic activity were performed. SF significantly increased the time-spent open arms and number into the in the open arm entries. However, PF weakly increased the time-spent in the open arms, but did not increase number into the open arm entries. The WG showed more potent anxiolytic-like effect than that of RG. The anxiolytic-like activities were antagonized by flumazenil, but not by esmolol. These findings suggest the saponin fractions of WG and RG promote the anxiolytic-like activity by antagonizing GABA/benzodiazepine receptors in mice.

Key words : *Panax ginseng,* anxiety, saponin, polysaccharide, GABA receptor

INTRODUCTION

Anxiety affects 80% of the total population and has become a very important area of research in psychopharmcology during this decade[1]. The bezodiazepines in the 1960s have been widely used anxiolytics in general clinical practice for many years[1,2]. Although these medicines are the mainstay for anxiety disorders, they have many side-effects such as sedation, myorelaxation, ataxia, amnesia and pharmacological dependence. Recent researches have been conducted to identify safer, more specific, and perhaps lower cost therapies[2-4].

Ginseng (*Panax ginseng* C.A. Meyer, Araliaceae) is one of the most commonly and widely used herbal medicines in Korea, Japan and China. Ginseng has been used for the treatment of psychiatric diseases such as anxiety and depression[5]. Ginseng has diverse effects on the central nervous system, and promotes stimulation as well as inhibits cortical activity. Lee *et al.* reported that the ginseng extract stabilized sleeping and wakefulness in food-deprived rats[6]. Ginseng saponins prolong pentobarbital sleeping time and delay the onset of convulsions when administered at a high dose, effects which appear to be related to the GABA-benzodiazepine-chloride channel receptor complex[7]. Kimura *et al.* reported that ginseng saponins increased the affinity of specific binding of [^{3}H]baclofen and [^{3}H]flunitrazepam in crude synapse membranes from the rat frontal cortex[8]. Park *et al.* reported that Sun-ginseng processed in more than 120°C showed more potent anxiolytic-like effects than red ginseng[9]. Cha *et al.* reported that the ginsenosides Rg5 and Rk2 in Sun-ginseng exhibited the anxiolytic-like effects[10]. However, the anxiolytic-like effect of white ginseng was not studied in detail.

Therefore, this study was aimed to isolate the saponin and polysaccharide fractions from white and red ginsengs and characterize their anxiolytic-like effects.

MATERIALS AND METHODS

Material

Buspirone, flumazenil and esmolol were purchased from the Sigma Chem. Co., (U.S.A.).

The saponin and polysaccharide fractions of white (WG) and red ginsengs (RG) were prepared according to

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the previous method of Trinh *et al.*[11]. WG was prepared by the dryness of fresh root of *Panax ginseng* C.A. Meyer, (cultured for 4 years at Keumsan, Chungcheung-nam-do, Korea) for 24 h for 50°C. RG was prepared by steaming the fresh ginseng roots at 98-100°C for 4 h and dried for 5 h at 60°C. The WG and RG were extracted with 70% ethanol, evaporated and freeze-dried (Yield 52 and 55%, respectively).

WG and RG extract (10 g) were dissolved in 100 ml of distilled water, extracted with BuOH three times and the BuOH fractions were combined, evaporated, suspended in distilled water and then freeze-dried. It was used as a saponin fraction (SF). The residual water layer was precipitated by the addition of the same volume of cold ethanol. The precipitate was dissolved in distilled water and then dialyzed against water for 5 days. The dialysate was freeze-dried and then it was used as a polysaccharide fraction (PF).

Animals

Male ICR mice, weighing 25-30 g, were purchased from the Orient Co. (Seoul, Korea). The animals were housed 5 to 6 per cage, allowed access to water and food ad libitum, and maintained under a constant temperature (23 ± 1°C) and humidity (60 ± 10%) under a 12-h high/dark cycle (light on 07:30-19:30). Animal treatment and maintenance were carried out in accordance with the principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Kyung Hee University, Seoul, Korea.

Elevated plus-maze (EPM) test

EPM test for mice was performed according to the previous methods[4,12]. EPM consisted of two perpendicular open arms (30 × 7 cm) and two enclosed arms (30× 7 cm) with 20cm high walls, extending from the central platform (7× 7 cm). The open and closed arms were connected by a central square, 7× 7 cm, to give an apparatus of a plus sign appearance. The floor and walls of the maze were constructed from the dark opaque polyvinyl plastic. The maze was raised to a height of 50 cm above the floor level in a dimly lit room (20 lux) and a video camera was suspended above the maze to record the movements for analysis. Each mouse was placed at the center of the platform, its head facing an open arm. The animals were tested individually and only once for 5 min. The maze was cleaned after each trial so as to remove any residue or odors.

Each ginseng (50 and 100 mg/kg, p.o.) orally administered to mice once a day for 3 days. One hour after the final sample administration, the mice were placed in the EPM. The mice in the control group were given the vehicle alone, and animals were tested individually once only for 5 min. In a separate antagonism study, the mice were subjected to the coadministration of the ginseng administration (100 mg/kg) for 3 days and esmolol (10 mg/kg, i.p.) or flumazenil (3 mg/kg, i.p.) 30 min prior to testing and then the mice were placed in the EPM.

Statistics

The values are expression as mean±SEM. The data was analyzed by a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test for the multiple comparisons. Statistical significance was set at $p<0.05$.

RESULTS

Anxiolytic-like effects of SF and PF of red ginseng

Behavior observed in the elevated plus-maze confirmed the anxiolytic activity of buspirone as reported previously. Buspirone increased open arm entries and time spent on open arms (Fig. 1; Fig. 2). Therefore, we also investigated the anxiolytic effect of red ginseng saponin (RSF) and polysaccharide fractions (RPF). Of RSF and RPF, the RSF exhibited anxiolytic-like effect. The RSF more

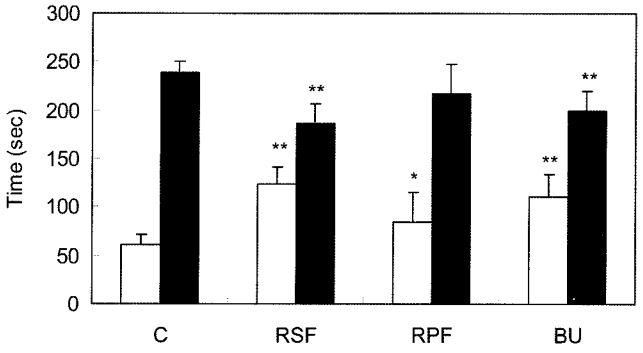

Fig. 1. Effect of saponin and polysaccharide fractions from red ginseng on the time spent in the open arms (white bar) and closed arms (black bar) of the elevated plus-maze over 5-min test in mice. RPF and RSF indicate polysaccharide and saponin fractions of red ginseng, respectively. C, treated with vehicle alone; RSF, orally administered 100 mg/kg of saponin fraction; RPF, orally administered 100 mg/kg of polysaccharide fraction; BU, intraperitoneally administered 1 mg/kg of buspirone. Values indicate mean ± SEM obtained from 10 mice. *Significantly different, compared with the control group (*$p<0.05$; **$p<0.01$).

potently increased the time spent on open arms and open arm entries than that of RPF. However, the RPF inhibited

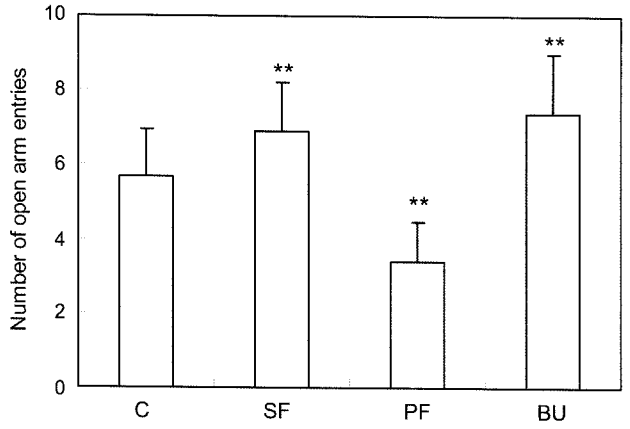

Fig. 2. Effect of saponin and polysaccharide fractions from red ginseng on the number of arm entries into the open arms of the elevated plus-maze over 5-min test in mice. RPF and RSF indicate polysaccharide and saponin fractions of red ginseng, respectively. C, treated with vehicle alone; RSF, orally administered 100 mg/kg of saponin fraction; RPF, orally administered 100 mg/kg of polysaccharide fraction; BU, intraperitoneally administered 1 mg/kg of buspirone. Values indicate mean ± SEM obtained from 10 mice. *Significantly different, compared with the control group (*p<0.05; **p<0.01).

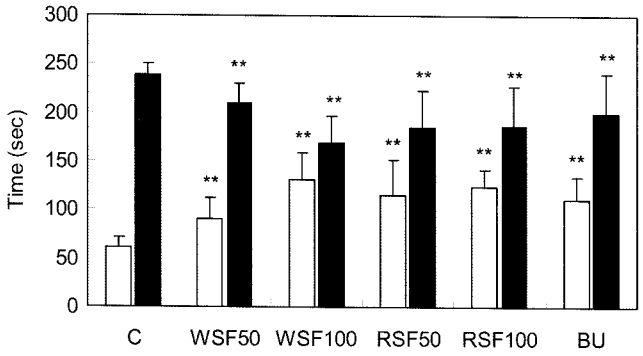

Fig. 3. Effect of saponin fraction from WG and RG on the time spent in the closed arms (white bar) and open arms (black bar) of the elevated plus-maze over 5-min test in mice. WSF and RSF indicate the saponin fractions of white and red ginseng, respectively: WSF50, orally administered 50 mg/kg of WSF; WSF100, orally administered 100 mg/kg of WSF; RSF50, orally administered 50 mg/kg of RSF; RSF100, orally administered 100 mg/kg of RSF; BU, intraperitoneally administered 1 mg/kg of buspirone. Values indicate mean ± SEM obtained from 10 mice. *Significantly different, compared with the control group (*p<0.05; **p<0.01).

number in the open arm entries.

Anxiolytic-like effect of SFs of white and red ginsengs

Of RSF and RPF, RSF exhibited anxiolytic-like effect. Therefore, we next compared the anxiolytic effect of SF of white ginseng to that of red ginseng (Fig. 3; Fig. 4). All the SFs of white and red ginseng increased the time spent on open arms. Of them, saponin fraction of white ginseng more potently increased the time spent on open arms and open arm entries. Of them, the SF of white ginseng at a dose of 100 mg/kg potently increased these behaviors.

Effect of esmolol and flumazenil in the anxiolytic-like activity of WG and RG saponin fractions

In order to investigate whether anxiolytic-like effect of these SFs is exerted via the serotonergic or GABAergic nervous system, these SFs treated mice were intraperitoneally administered with esmolol or flumazenil and their behaviors observed in the elevated plus-maze. As shown in Fig. 5, the anxiolytic-like effects of SFs of white and red ginsengs were antagonized by flumazenil, although esmolol and flumazenil all antagonized the number of open arm entries. However, esmolol did not antagonize the anxiolytic-like effect of RG and weakly block that of WG.

Discussion

Ginseng has been used traditionally for the treatment of

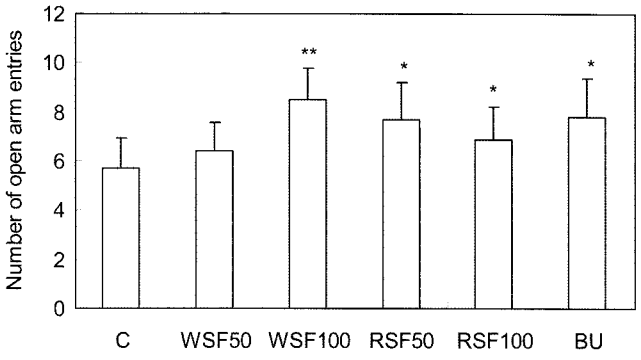

Fig. 4. Effect of saponin fractions from WG and RG on the number of arm entries into the open arms of the elevated plus-maze over 5-min test in mice. WSF and RSF indicate the saponin fractions of white and red ginseng, respectively: WSF50, orally administered 50 mg/kg of WSF; WSF100, orally administered 100 mg/kg of WSF; RSF50, orally administered 50 mg/kg of RSF; RSF100, orally administered 100 mg/kg of RSF; BU, intraperitoneally administered 1 mg/kg of buspirone. Values indicate mean ± SEM obtained from 10 mice. *Significantly different, compared with the control group (*p<0.05; **p<0.01).

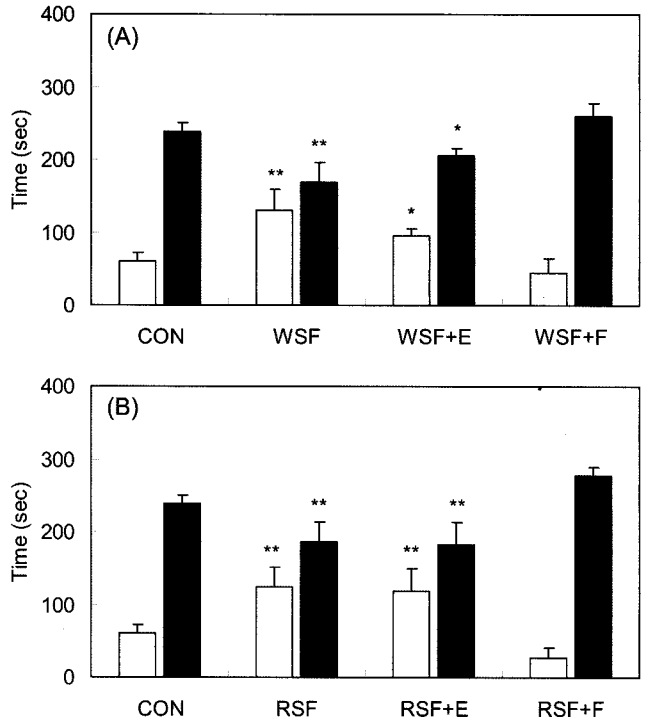

Fig. 5. Effect of esmolol and flumazenil on the anxiolytic-like activities of WG (A) and RG saponin fractions (B) in mice. Esmolol (10 mg/kg) and flumazenil (3 mg/kg) was administered 30 min before the elevated plus-maze test. WSF and RSF indicate the saponin fractions of white and red ginseng, respectively: WSF and RSF were orally administered at a dose 100 mg/kg. Esmolol (E) and flumazenil (F) were intraperitoneally administered at a dose of 10 mg/kg and 3 mg/kg, resepectively. Values indicate mean ± SEM obtained from 10 mice. *Significantly different, compared with the control group (*p<0.05; **p<0.01).

psychiatric disorders, such as anxiety and depression[5]. It has been reported that ginseng exhibits anxiolytic effects and the saponin fraction plays an important role in anxiolytic effects of the ginseng[13]. The SF of *Panax quinquifolium* also showed the potent anxiolytic-like effect in mice, compared with its PF[14]. Among the three types of pure ginsenoside (Rb1, Rg1 and Ro) isolated from red ginseng, only ginsenoside Rb1 significanlty increased both the frequency and duration of open arm entries[15]. The ginsenoside Rb1 is one of the active anixolytic-like components of the ginseng. However, Cha *et al.* reported that, when the anxiolytic-effects of Rb1, Rg1, Rg3-R, Rg3-S, and the Rg5/Rk mixture were investigated in the elevated plus-maze system, ginsenosides Rb1, Rg1 and Rg5/Rk mixture increased the number of open arm entries or the time spent on the open arm[10]. Of them, ginsenoside Rb1 exhibited the most potent anxiolytic-like effect. In the present study, SFs of white and red ginsengs significantly increase the time spent in the open arms and number of the open arm entries in mice using the EPM test, but PF weakly increased the time spent in the open arms, although it did not increase number of the open arm entries. The SFs showed more potent anxiolytic effect, compared with PF, which contains polysaccharide, adenosine, pyroglutamic acid, encichine, etc[16]. The SF of white ginseng more potently showed the anxiolytic-like effect than that of RG. In addition, when the ginsenoside contents of white and red ginsengs are compared, the content of ginsenoside Rb1 in white ginseng is higher than that in red ginseng and those of ginsenoside Rg5/Rk or ginsenoside Rg3 of red ginseng are higher than that in white ginseng[17,18]. However, PF may cause anxiety, although its activity is weak.

Based on these findings, the anxiolytic effect of white and red ginsengs may be mainly due to that of ginsenosides, particularly ginsenoside Rb1, even if the PF showed the weak anxiolytic-like effect. The anxiolytic-like effect of the SFs was antagonized by flumazenil, which is an antagonist for $GABA_A$ receptors[19]. This demonstrated that GABA/benzodiazepine receptors may be involved in the ginseng-induced anxiolytic effect.

In conclusion, we believe that the SF of white and red ginseng shows anxiolytic-like effect via GABA/benzodiazepine receptors and white and red ginsengs can ameliorate the psychiatric disorder such as anxiety and depression.

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