Anti-stress Effects of 20(S)-Protopanaxadiol and 20(S)-Protopanaxatriol in Immobilized Mice

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Panax ginseng C.A. MEYER (Araliaceae), which contains ginsenosides as its main components, has been shown to have various biological effects, including anti-inflammatory, anxiolytic, anti-stress, and anti-tumor effects. Orally administered ginsenoside Rb1 and Re are metabolized to 20(S)-protopanaxadiol (PPD) and compound K via ginsenoside Rd and 20(S)-protopanaxatriol (PPT) and ginsenoside Rh1 via ginsenoside Rg1 by gut microbiota, respectively. Therefore, we investigated the anti-stress effects of these metabolites, PPD and PPT, by measuring their anxiolytic and anti-inflammatory effects in immobilized mice. Treatment with PPD and PPT prior to immobilization stress increased the time spent in open arms and open arm entries in the elevated plus-maze (EPM) test. The anxiolytic effects of PPD (10 mg/kg) and PPT (10 mg/kg) were comparable to that of buspirone (1 mg/kg). This observed anxiolytic effect of PPD was significantly blocked by flumazenil or bicuculline, and the effect of PPT was blocked by WAY-100635. Treatment with PPD also potently suppressed immobilization stress-induced serum levels of corticosterone and interleukin (IL)-6 by the enzyme-linked immunosorbent assay. However, PPT treatment did not suppress them. Based on these findings, PPD and PPT may exhibit the anxiolytic effect via γ-aminobutyrate (GABA) receptor(s) and serotonergic receptor(s), respectively, and PPD may have an anti-inflammatory effect that is more potent than that of PPT.

Key words Panax ginseng; 20(S)-protopanaxadiol; 20(S)-protopanaxatriol; anxiety; inflammation; immobilization stress

Stress is a common and unavoidable phenomenon in human life.1,2 Exposure to stress induces glucocorticoid release via the activation of the hypothalamo–pituitary–adrenal (HPA) axis and it modulates immune cells and further modulates cytokine production.3–5 Among these cytokines, proinflammatory interleukin (IL)-6 and tumor necrosis factor (TNF)-α are up-regulated by stress and are associated with host defense systems.6 The failure to cope with stress exacerbates both psychological and immune disorders, including anxiety, depression, cardiovascular disturbances, neuronal degeneration of the central nervous system and chronic fatigue syndromes.7,8

Ginseng (the root of Panax ginseng C.A. MEYER, Araliaceae) has been widely used as a traditional Chinese medicine for the treatment of various maladies such as inflammation, anxiety, depression, fatigue, and stress. Its representative constituents are dammarane-type ginsenosides, which are classified into protopanaxadiols, such as ginsenosides Rb1, Rb2, Re, and Rd, and protopanaxatriols such as ginsenosides Re and Rg1.9,10 These ginsenosides have been previously reported to show various biological activities, including anti-inflammatory,10 anti-allergic,12 anxiolytic,13 anti-stress4 and anti-tumor activities.15 Orally administered ginsenosides are metabolized to compound K (20-Oβ-D-glucopyranosyl-20(S)-protopanaxadiol)20(S)-protopanaxadiol and ginsenoside Rh1/20(S)-protopanaxatriol by gut microbiota before absorption into the bloodstream.16–19 These metabolites have many pharmacological activities similar to ginseng, including anti-tumor, anti-diabetic, and anti-inflammatory effects.20–22 Therefore, to understand the pharmacological effect of ginseng, understanding the biological activities of two metabolites, 20(S)-protopanaxadiol and 20(S)-protopanaxatriol, of various ginsenosides is important. Nevertheless, the pharmacological effects of the ginsenoside metabolites have not been studied thoroughly.

In our preliminary study, we also found that ginseng saponin extract showed anti-stress effect in mice. Therefore, in the present study, we investigated further the anti-stress effects of ginseng saponin metabolites, 20(S)-protopanaxadiol (PPD) and 20(S)-protopanaxatriol (PPT), in immobilized mice.

MATERIALS AND METHODS

Materials Bicuculline, buspironie, dexamethasone, flumazenil and WAY-100635 were purchased from Sigma (St. Louis, MO, U.S.A.). 20(S)-Protopanaxadiol (purity ≥98% by HPLC) and 20(S)-protopanaxatriol (purity ≥98% by HPLC) were purchased from Ambo Institute (Daejeon, Korea). Orally administered 20(S)-protopanaxadiol and 20(S)-protopanaxatriol were suspended in 0.5% carboxymethylcellulose (CMC) solution and intraperitoneally injected buspironie, flumazenil, bicuculline, and WAY-100635 were dissolved in sterilized saline.

Animals Male ICR mice (age, 7 weeks-old; weight, 24–28 g) were purchased from Samtako Biokorea (Seoul, Korea) and acclimated for 1 week before use. All animals were maintained under a constant temperature (24±2°C) and humidity (60±10%) with an alternating 12h light–dark cycle. They were fed standard laboratory chow (Samyang Co., Seoul, Korea) with tap water ad libitum. Each group consisted of 7 mice for all experiments. The mice were randomly divided

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into 8 groups: normal control (N), non-immobilization and 0.5% CMC per os (p.o.); control (C), immobilization and 0.5% CMC (p.o.); 20(S)-protopanaxadiol (PPD), immobilization and 5 or 10 mg/kg of PPD (p.o.); 20(S)-protopanaxatriol (PPT), immobilization and 5 or 10 mg/kg of PPT (p.o.); and positive control, immobilization and 1 mg/kg of buspirone (Busp, intraperitoneally (i.p.)) or dexamethasone (Dexa, p.o.). Buspirone was administered 30 min prior to immobilization and the other test agents were administered to mice 1 h prior to immobilization. For the anxioselectic antagonism study, flumazenil (F, 3 mg/kg), bicuculline (B, 0.5 mg/kg) or WAY-100635 (W, 0.5 mg/kg) was intraperitoneally administered 30 min after the oral administration of PPD (10 mg/kg) or PPT (10 mg/kg), and then mice were subjected to immobilization stress for 2 h.

All experiments were performed in accordance with the National Institutes of Health and Kyung Hee University guides for Laboratory Animals Care and Usage. The protocol was approved by the Institutional Animal Care and Use Committee of the Kyung Hee Medical Center and Kyung Hee University.

**Immobilization Stress** Immobilized stress was performed with slight modification according to the previously reported method. It was accomplished by placing the mouse vertically in a 50 mL conical tube (3 cm in diameter and 10 cm in length) inserted gauze to limit mobility. A hole (0.3-cm-diameter) was made on the tip of the tube to allow the mouse to breathe and mice were immobilized in the tubes for 2 h.

**Elevated Plus Maze (EPM) Test** The EPM test was performed as previously described. The plus-maze apparatus consists of two open arms (30 × 7 cm) and two enclosed arm (30 × 7 cm) with 20 cm high walls, extending from a central platform (7 × 7 cm). The maze made of the black plexiglass was raised 50 cm above the floor in a dimly lit room (20 lux) and the other test agents were administered to mice 1 h prior to immobilization stress. For the anxioselectic antagonism study, serotoninergic receptors were blocked with flumazenil (3 mg/kg, i.p.), which is a 5-HT1A receptor antagonist, or WAY-100635 (0.5 mg/kg, i.p.), which is a 5-HT1A receptor antagonist, in mice exposed to immobilization stress (Fig. 2). Treatment with PPD and PPT without flumazenil, bicuculline or WAY-100635 significantly increased OT and OE. This anxiolytic effect of PPD was significantly suppressed by flumazenil or bicuculline (p < 0.01) and that of PPT was significantly suppressed by WAY-100635 (p < 0.01). Thus, the anxiolytic effect of PPD was exerted via GABA_A receptors and that of PPT was exerted via serotonergic receptors.

**Effect of PPD and PPT on Serum Corticosterone and IL-6 Levels in Immobilized Mice** Immobilization stress markedly increased serum levels of corticosterone and IL-6 compared to normal control group (p < 0.01, Fig. 3). Treatment with PPD inhibited the induction of serum levels of corticosterone and IL-6 in mice exposed to immobilization stress. The anti-inflammatory effect of PPD (10 mg/kg) was comparable to that of dexamethasone (1 mg/kg). Contrarily, treatment with PPT did not reverse the immobilization stress-induced serum levels of corticosterone and IL-6.

In order to understand the anxioselectic mechanism of PPD and PPT, we examined the effect of PPD (10 mg/kg) and PPT (10 mg/kg) with or without flumazenil (3 mg/kg, i.p.) or bicuculline (0.5 mg/kg i.p.), which are γ-aminobutyrate_A (GABA_A) receptor antagonists or WAY-100635 (0.5 mg/kg, i.p.), which is a 5-HT1A receptor antagonist, in mice exposed to immobilization stress (Fig. 1). Treatment with PPD and PPT in immobilized mice attenuated the decrease of OT and OE. PPD (10 mg/kg) and PPT (10 mg/kg) induced the significant anxioselectic-like effects. Their anxioselectic effects were comparable to that of buspirone (1 mg/kg), an anxioselectic agent, which significantly increased OT and OE (p < 0.01).

**Results**

**Effect of PPD and PPT on Immobilization-Induced Anxiety-Like Behaviors in the EPM Test** Mice exposed to immobilization stress showed significant decrease on time spent in open arms (OT) and open arm entries (OE) of EPM test (p < 0.01, Fig. 1). Treatment with PPD and PPT in immobilized mice attenuated the decrease of OT and OE. PPD (10 mg/kg) and PPT (10 mg/kg) induced the significant anxioselectic-like effects. Their anxioselectic effects were comparable to that of buspirone (1 mg/kg), an anxioselectic agent, which significantly increased OT and OE (p < 0.01).

**Effect of Flumazenil, Bicuculline and WAY-100635 on the Anxioselectic Activity of PPD and PPT in the EPM Test**

**Fig. 1** Effects of 20(S)-Protopanaxadiol and 20(S)-Protopanaxatriol on Immobilization-Induced Anxiety-Like Behavior in the Elevated Plus Maze Test

Mice were administered 20(S)-protopanaxadiol (PPD, 5, 10 mg/kg, p.o.), 20(S)-protopanaxatriol (PPT, 5, 10 mg/kg, p.o.), or buspirone (Busp, 1 mg/kg, i.p.). Data are expressed as the mean ± S.E.M. of 7 mice; **p < 0.01 vs. normal group; ***p < 0.01 vs. control group.
DISCUSSION

Exposure to stress stimulates the release of corticosterone from the adrenal cortex via the activation of the HPA axis and it up-regulates pro-inflammatory cytokines, such as IL-3, IL-6, and TNF-α to provoke immune-mediated inflammatory reactions. Glucocorticoids also alter the uptake and release of GABA, decrease benzodiazepine receptor binding in the hippocampus and amygdala, and attenuate 5-HT₁A receptor function, which induced anxiety-like behaviors in animal models.

Ginseng has been traditionally used for the treatment of psychiatric disorders, such as anxiety and depression: it attenuates stress-induced corticosterone and IL-6 by regulation of cortical cells of adrenal and pituitary adrenocorticotropic hormone (ACTH) secretion and induces anxiolytic-like effects in the elevated plus-maze (EPM) test in mice. Of its constituents, ginsenosides Rb1, Rh2, Rg5/Rk mixture, and Rg1 also showed an anxiolytic effect (i.e., mice treated with these ginsenosides increased the time spent in the open arm (OT) or open arm entries (OE) in the EPM test). However, orally administered protopanaxadiol and protopanaxatriol ginsenosides are metabolized to their aglycones, 20(S)-protopanaxadiol (PPD) and 20(S)-protopanaxatriol (PPT), via compound...
K and ginsenoside Rh1, respectively, by gut microbiota. 16–19 Nevertheless, the anxiolytic effects of their aglycones have not been studied.

In the present study, we investigated the anxiolytic effects of PPD and PPT in mice exposed to immobilization stress to understand the pharmacological effects of ginsenosides. PPD and PPT potently inhibited immobilization stress-induced anxiety-like behaviors. Furthermore, the anxiolytic effect of PPD was antagonized by flumazenil and bicuculline, antagonists of GABA_A receptors, and the anxiolytic effect of PPT was antagonized by WAY-100635, an antagonist of serotonergic receptors. These results suggest that the anxiolytic effect of ginseng may be exerted via GABA_A and serotonergic receptors. Furthermore, immobilization stress disturbed immune system function by decreasing corticosterone and IL-6 expression; this finding was consistent with the previously reported. 6,32 Treatment with PPD potently suppressed the stress-induced inflammation by decreasing serum corticosterone and IL-6 levels, but not PPT. These results were supported by the previous reports that PPD ginsenosides exhibit anti-inflammatory effects in mice, but not PPT ginsenosides. 12,13,22 Thus, PPD ginsenosides alone, particularly PPD, exhibit potent anti-inflammatory effects in immobilization stress-stimulated inflammation.

On the basis of these findings, we conclude that PPD and PPT may exhibit the anxiolytic effect via GABA_A receptor(s) and serotonergic receptor(s), respectively, and PPD may also have anti-inflammatory effect more potently than PPT.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES


