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The antistress effect of ginseng total saponin and ginsenoside Rg3 and Rb1 evaluated by brain polyamine level under immobilization stress

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Abstract

The present study aims to demonstrate the ability of ginseng total saponin (GTS), ginsenosides Rg3 and Rb1 to reduce brain polyamine levels in immobilization-stressed gerbil mice. A previous study reported that ginsenosides had an anti-stress property. So, we tested the anti-stress effect of ginseng by investigating the brain level of polyamine, a well-known stress stimuli marker. We determined the brain polyamine levels under 30-min immobilization stress in pretreating GTS (100 mg kg^{-1}, oral), ginsenosides Rg3 and Rb1 (10 mg kg^{-1}, oral, respectively). Then, we compared polyamine levels between the non-stressed mouse and the stressed mouse which had taken saline orally to check the placebo effect. Putrescine (PUT) levels were significantly increased ($P < 0.01$) in the stressed condition, but it was reduced in pretreatment of GTS, ginsenosides Rg3 ($P < 0.01$, respectively) and Rb1 ($P < 0.001$) under 30-min immobilization stressed-mouse. However, other polyamine levels did not change regardless of stressed condition or GTS-, ginsenosides Rg3- and Rb1-treated stressed condition. These results mean that only PUT could be a marker for stress and GTS, ginsenosides Rg3 and Rb1 administration lead to an anti-stress effect. Thus, our studies indicate that GTS, ginsenosides Rg3 and Rb1 may play a neuroprotective role in the immobilization-stressed brain.

Keywords: Immobilization stress; Brain; Polyamine; Ginseng total saponin (GTS); Ginsenosides Rg3 and Rb1

1. Introduction

Putrescine (PUT), spermidine (SPD), and spermine (SP) are endogenous polyamines which are essential for cellular growth, proliferation, regeneration, and differentiation [1]. The metabolism and catabolism of polyamines is highly regulated by the concerted action of six enzymes [2,3], such as ornithine decarboxylase (ODC), *S*-adenosyl-L-methionine decarboxylase (AdoMetDC), spermidine synthases, spermine synthases, spermidine/spermine N^1-acetyltransferase (SSAT), and polyamine oxidase (PAO). Various forms of stresses result in the alteration of polyamine metabolism [4,5]. The ODC has particularly been considered as a biochemical hallmark of brain damage. The ODC or SSAT activity is particularly increased in the stressed condition, and it leads to the accumulation of putrescine levels [1,6]. Therefore, inhibiting the activity of ODC or SSAT could result in an anti-stress effect against stressed brain damage.

Panax ginseng C.A. Mayer (Araliaceae), one of the most popular herbal medicines, has been widely used for the therapy of stress disorders [7]. There are eight major ginsenosides in ginseng. Rb1 is the most abundant ginsenoside in ginseng, and reportedly, it has a neuroprotective effect against ischemia [8], glutamate neurotoxicity [9,10], seizures [11], motor impairment, and cell loss in the striatum [12]. Rg3 is well known as a potent ginsenoside which has a neuroprotective effect in fermented red-ginseng [13] and an anti-stress effect [14].

Therefore, the present study was investigated whether or not the administration of ginseng total saponin (GTS) and ginsenosides Rg3 and Rb1 can attenuate polyamine levels in brain damage after an experience with immobilization stress.

2. Materials and methods

2.1. Animals and immobilization stress

Male Mongolian gerbils (60–80 g) were housed in a temperature-controlled environment under 12-h dark:12-h light

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cycle under condition where food and water were freely available. All experiments conformed to the animal care guidelines of the Korean Academy of Medical Sciences, and all efforts were made to minimize their suffering. Stress procedures were approved and monitored by the ethical committee of Kyung Hee University. For stress experiments, gerbils were immobilized for 30 min in tightly fitted, ventilated plastic bags. At the end of the stress period, the animals were immediately decapitated, and the brain was rapidly removed and frozen for polyamines analysis. Animals that were set free in their home cage in the absence of any stressors served as controls (the normal control group) [15]. The non-stressed, non-stressed + GTS, saline + stressed, GTS + stressed, Rg3 + stressed, and Rb1 + stressed animals were housed in separate rooms during immobilization protocol. To revise the placebo effect, we administrated saline instead of GTS, ginsenosides Rg3 and Rb1 to stressed mouse (the stressed control group), and we compared brain polyamine levels between this group and the GTS- and ginsenosides Rg3- and Rb1-treated groups.

2.2. Treatment

Gerbils were divided into six groups: saline, GTS, Rg3, and Rb1 were administered in the stressed mouse, GTS-treated non-stressed mouse, and non-stressed mouse group was used as a control. GTS (100 mg kg^{-1}, oral), ginsenosides Rg3 and Rb1 (10 mg kg^{-1}, oral, respectively) were administrated at 30 min before the application of immobilization stress. We set up acute stress model to give 30-min immobilization stress to gerbil. The GTS, ginsenosides Rg3 and Rb1 were dissolved in saline for oral administration.

2.3. Polyamines extraction and GC-NPD analysis

We analyzed brains polyamine levels to determine the effect of pretreatment with saline, GTS, ginsenosides Rg3 and Rb1 ($n = 5$, respectively). We modified Lee et al. method [16] to determine the polyamine concentration in mouse brain. We placed the gerbils brain tissue (100 mg) were taken into a glass test tube and added 20 μL of 1,6-diaminohexane solution (100 μg mL^{-1}). The tissues were homogenized with 10 volumes of 0.2 M perchloric acid on ice and then centrifuged (3000 rpm, 5 min). The supernatant of brain homogenate were diluted to 5 mL with distilled water. Samples were then loaded on the OASIS HLB cartridges (60 mg, 3 mL; Waters), which were equilibrated with methanol (1 mL) and methanol/H_2O (5/95, v/v; 1 mL) successively before using. The sample was passed through the cartridge using a vacuum and the eluent was discarded. The cartridge was then washed with MeOH/H_2O (50/50, v/v; 1 mL). The polyamine fraction was eluted with 0.1N methanolic HCl (5 mL). The eluent was evaporated to dryness in vacuo. The residue was dissolved with a mixture (100 μL) of ethyl acetate (EA) and heptafluorobutyric anhydride (HFBA) (1:1, v/v) for the derivatization. This solution was heated at 80 °C for 1 h. After cooling, the solvent was evaporated at room temperature under a stream of air. The residue was reconstituted with ethyl acetate (30 μL) for the direct injection to the gas chromatograph with nitrogen–phosphorus detection (GC-NPD) analysis. For GC-NPD, a model 5890 gas chromatograph equipped with a model 7673 automated sampler (both from Agilent Co., Palo Alto, CA, USA) was used. An Ultra-2 (SE-54 bonded phase) fused-silica capillary column (25 m, length × 0.2 mm I.D., 0.33 μm film thickness; Agilent Co.) was used. The gas flow rate (helium) was 0.8 mL/min (split ratio 1:10). The detector and injector temperature were 280 °C and 260 °C, respectively. The oven temperature program was a gradient system: the initial temperature was 140 °C, increased to 270 °C at a rate of 5 °C/min. Then, it was increased to 315 °C at a rate of 30 °C/min and held for 3 min.

2.4. The ratio of spermidine/N^1-acetylspermidine and spermine/N^1-acetylspermine

SSAT is the enzyme which converts spermidine to N^1-acetylspermidine and spermine to N^1-acetylspermine. We examined the SSAT activity after immobilization stress by calculating the ratio between the precursor and the product (metabolite); spermidine/N^1-acetylspermidine (SPD/N^1-acSPD) and spermine/N^1-acetylspermine (SP/N^1-acSP).

2.5. Extraction of corticosterone in the plasma and GC–MS analysis

The corticosterone was extracted based upon the method of Vallee et al. [17] with some modification ($n = 5$). The mouse plasma (500 μL) was taken into a glass test tube and the 10 μL of a stock solution of 17β-estradiol-d_4 (internal standard, 0.1 μg mL^{-1}) were added. The plasma was diluted with 5 mL of distilled water. Samples were then extracted with OASIS HLB cartridges (60 mg, 3 mL; Waters), which were equipped in a vacuum manufolder and equilibrated with MeOH (1 mL) before sample loading. The cartridge was washed with methanol/H_2O (5/95, v/v; 1 mL) successively. Then, the sample was passed through the cartridge using a vacuum. The corticosterone fraction was eluted with methanol (5 mL) and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was dissolved in 30 μL of a silylating-reagent mixture MSTFA/NH_4I/DTE (1000:4:5, v/w/w) and heated at 60 °C for 15 min [18]. We analyzed corticosterone derivatives on a GC–MS system (5973 mass selective detector combined with a 6890 Plus gas chromatograph, Agilent Co.). The gas chromatograph was equipped with a Ultra-1 column (25 m × 0.2 mm I.D., 0.33 μm film thickness; Agilent Co.). The initial GC temperature was 180 °C. Followed by a temperature program to 260 °C at 10 °C/min, and finally to 315 °C at 20 °C/min, where it was held for 2.25 min. The gas flow rate (helium) was 0.8 mL/min (split ratio 1:5).

2.6. Statistical analysis

The data was expressed as mean ± standard deviation (S.D.). Comparisons between the groups were performed by Kruskal–Wallis variance analysis and a P value < 0.05 was considered as statistically significant.