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Research article

Chronic dietary ginseng extract administration ameliorates antioxidant and cholinergic systems in the brains of aged mice

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ABSTRACT

Background: Black ginseng has a more potent biological activity than non-steamed ginseng. We investigated the effects of long-term intake of dietary black ginseng extract (BG) on antioxidant activity in aged mice. We also compared the effects of BG on cognitive deficits with those of white ginseng extract (WG) and red ginseng extract (RG).

Methods: Ten-month-old mice were fed an AIN-93G-based diet containing 10 g/kg (low dose, L) or 30 g/kg (high dose, H) WG powder, RG powder, or BG powder for 24 wk. We measured serum lipids, the activities of antioxidant enzymes, and malondialdehyde levels. Additionally, the protein expression levels of choline acetyltransferase and vesicular acetylcholine transporter, which are presynaptic cholinergic markers in the cortex and hippocampus of the brain, were measured by western blotting.

Results: Triglyceride levels were reduced in all the extract-treated mice, except those in the LBG group. High-density lipoprotein cholesterol levels in the HBG group were higher than those in the control group. Total cholesterol levels were reduced in the LBG group. Additionally, glucose levels in the HBG group were significantly reduced by 41.2%. There were lower levels of malondialdehyde in the LBG group than in the control group. Furthermore, glutathione reductase activity increased in the HWG group and the HRG group. The protein expression levels of choline acetyltransferase and vesicular acetylcholine transporter significantly increased in all the ginseng-treated groups.

Conclusion: The results suggest that supplementation with the tested ginseng extracts may suppress the cognitive decline associated with aging, via regulation of the cholinergic and antioxidant defense systems.

1. Introduction

Aging is a natural process associated with gradual decline in biological function and increased incidence of degenerative diseases and mortality [1]. According to the free radical theory of aging, imbalance between the production of reactive oxygen species and antioxidant activity causes age-related functional deterioration [2,3]. A major concern in research on aging and aging-related disorders has been the impact of oxidative stress and the damage it induces [4,5]. In addition, age-related neurodegenerative diseases cause severe damage to the cholinergic system, resulting in cognitive and memory impairments [6]. Cortical cholinergic innervation has been widely studied because of its role in memory and learning [7]. A significant decrease in cholinergic markers such as choline acetyltransferase (ChAT) and acetylcholinesterase has been observed in Alzheimer's disease [8]. Therefore, it is very important to find materials that can inhibit or delay age-associated memory loss, and to investigate defense mechanisms that enhance healthy aging [9].

Panax ginseng Meyer, a traditional herbal medicine, is a tonic used to improve physiological functions and is a key ingredient in several herbal products [10,11]. *P. ginseng* is classified as white, red, or black based on its color, which is caused by repetitive steaming. It has been reported that ginsenoside content in *P. ginseng* is affected by the steaming process [12]. Black ginseng has been reported to have more potent pharmacological activities than white ginseng and red ginseng do. These activities include radical scavenging, anticancer, cholinesterase inhibition, and anti-

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inflammatory activities, and are due to the new ginsenosides that are formed during the steaming process [13–16]. Recently, many studies have indicated that treatment with ginseng or individual ginsenosides has a protective effect on oxidative damage and improves learning and memory impairment caused by aging [17–19]. Although various pharmacological effects of black ginseng have been studied extensively, studies on its effects on antioxidant activity and cholinergic markers in the brains of naturally aging mice have not been conducted. Therefore, this study was performed to compare serum lipid levels, antioxidant activity, and cholinergic markers in the brains of aged mice fed an experimental diet based on an AIN-93G formula containing 1% or 3% of white ginseng extract (WG), red ginseng extract (RG), or black ginseng extract (BG) for 24 wk.

2. Materials and methods

2.1. Sample preparation

White ginseng, red ginseng, and black ginseng were obtained from Daeduck Bio Corp. (Daejeon, Korea). To prepare the ginseng extracts, each type of ginseng was ground and then subjected to extraction in 80% ethanol for 1 h in a sonicator. This extraction step was repeated three times. The solution thus obtained was filtered, evaporated, and freeze-dried. The compositions of the ginsenosides Rg1, Re, Rf, Rb1, Rc, Rb2, Rd, and Rg3 in each ginseng extract were determined via HPLC as previously described [20]. Rg3 was not detected in WG, and Rg1 and Re were not detected in BG.

2.2. Animals and diet

Twelve-week-old male Imprinting Control Region mice were purchased from Dahan BioLink (Eumseong, Korea). The animals were housed (5 mice per cage) under controlled temperature ($22 \pm 2^{\circ}C$), humidity (50–55%), and a 12/12 h light/dark cycle (light on at 08:00 AM). They were also allowed free access to food and water for 6 mo. Ten-month-old mice were divided into seven groups (5 mice per group) and fed an experimental diet based on the AIN-93G formula, containing 10 g/kg (low dose, L) or 30 g/kg (high dose, H) of WG powder, RG powder, or BG powder for 24 wk (Table 1). Food intake and body weight were measured once weekly. The dosages of the extracts used in this study were based on those used in previous studies on the efficacy of chronic intake of dietary ginseng extract on obesity in rats. In these studies, no effect was observed with the use of RG at 2,000 mg/kg/d [21,22]. All the experimental procedures used were approved by the Institutional Animal Care and Use Committee of Chungnam National University (CNU-00245; Daejeon, Korea).

Table 1
Experimental diet compositions (g/kg)

Ingredient	Control	Ginseng extract	
		1%	3%
Casein	200	200	200
Corn starch	457	447	427
Sucrose	200	200	200
Soybean oil	43	43	43
Cellulose	50	50	50
Choline bitartrate	2	2	2
L-Cysteine	3	3	3
t-Butylhydroquinone	0.014	0.014	0.014
Mineral mix[1)]	35	35	35
Vitamin mix[2)]	10	10	10
Ginseng extract powder[3)]	–	10	30
Total	1000	1000	1000

[1)] AIN-93G mineral mixture
[2)] AIN-93G vitamin mixture
[3)] Ginseng extract powder: white, red, or black ginseng extract powder

2.3. Preparation of serum and brain tissue samples

Blood samples were collected by cardiac puncture and centrifuged to obtain serum. Whole brains were removed and the hippocampus and cortex were dissected from each left hemisphere. The right hemispheres were homogenized in a 10-fold buffer (12.5mM sodium phosphate buffer (pH 7.0) and 400mM NaCl) and centrifuged to obtain the supernatant for enzyme assay.

2.4. Biochemical measurements

Serum levels of glucose, triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined using commercial kits (Young-Dong Pharmaceutical, Seoul, Korea). Malondialdehyde (MDA) levels were measured using the thiobarbituric acid-reactive substances method described by Mihara and Uchiyama [23]. Superoxide dismutase and glutathione reductase (GR) levels in brain homogenates were determined using commercial kits (Nianjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Western blot analysis

Proteins in the hippocampus and cortex were extracted using a lysis buffer (PRO-PREPTM; iNtRON Biotechnology, Seongnam, Korea). The extracted proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane. The membrane was blocked with 5% skim milk for 1 h and immunoblotted with primary antibodies against ChAT, vesicular acetylcholine transporter (VAChT), and β-actin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Immunoblotting was performed by incubating the membrane with horseradish peroxidase-conjugated secondary antibodies. Blots were developed using an enhanced chemiluminescence detection kit (WEST-ONE, iNtRON Biotechnology). ImageJ software (version 1.44p, National Institutes of Health, Bethesda, MD, USA) was used for densitometric analysis of the bands.

2.6. Statistical analysis

All data were analyzed using the SPSS statistical software package (version 20; IBM Corporation, Armonk, NY, USA). Differences between groups were analyzed using analysis of variance and Dunnett's multiple comparison test. A p value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Clinical observations

No death, significant abnormal behavior, or distinct clinical symptoms were observed in any of the groups during the experiment. The feed intake, body weight, and organ weights measured in the groups are presented in Table 2. The body weights of mice supplemented with dietary ginseng extract, as well as those of the control mice, were similarly maintained from the beginning to the end of the experiment. This indicates that chronic intake of dietary ginseng extract does not change appetite or cause malnutrition.

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