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Improved lipid profile in ovariectomized rats by red ginseng extract

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The effects of red ginseng extract on lipid metabolism were examined in ovariectomized rats. Twenty-four female Sprague-Dawley rats (210 ± 20 g) were studied for 10 weeks. The rats were divided into four groups: (I) "sham" non-ovariectomized rats treated with olive oil, (II) control ovariectomized rats treated with olive oil, (III) ovariectomized rats treated with 0.5 mg/kg 17β-estradiol in olive oil, and (IV) ovariectomized rats treated with 5 mg/kg red ginseng extract in olive oil. Red ginseng extract induced significant reductions in total cholesterol, low density lipoprotein cholesterol/total cholesterol, high density lipoprotein cholesterol/total cholesterol, and low density lipoprotein cholesterol/high density lipoprotein cholesterol, implying the effectiveness of ginseng in targeting postmenopausal symptoms.

1. Introduction

Studies have shown that menopause is associated with an increased risk of coronary heart disease as a result of changes in lipid profile, clotting and fibrinolytic factors, and vessel function (Colacurci et al. 2005). To improve blood metabolism, estrogen replacement therapy has been used for more than 30 years, and combined estrogen–progesterone therapy has been widely used for at least 20 years (Glazier and Bowman 2001). However, adverse effects such as vaginal bleeding, endometrial cancer, breast cancer, and interference with a 'natural' process have led to concerns regarding synthetic estrogen (Ravnikar 1992; Coope and Marsh 1992). Phytoestrogens are assumed to produce minimal side effects, and studies have shown that phytoestrogens are effective in the prevention and amelioration of metabolism of sex hormones and blood lipids in postmenopausal women (Yan et al. 2008).

A number of components have been isolated and characterized from ginseng including ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids (Liu et al. 2000). Several studies have shown that ginseng contains estrogenic activity, providing a scientific rationale for its use on postmenopausal symptoms (Cho et al. 2004). Ginsenoside Rg1, one of the most abundant compounds in ginseng, has been shown to activate estrogen receptors (ER) at the picomolar range (Lau et al. 2009). Ginsenoside Rg1 and Rb1, the two main components of ginsenoside, have been found to be effective in preventing osteoporosis in ovariectomized (OVX) rats (Gong et al. 2006). In oriental medicine, ginseng is extracted with boiling water and used for medicinal purposes. Processing steps such as steaming, drying, and/or pressurizing to make a dietary supplement can affect the pharmacological activity by changing the constituent ginsenosides (Kim et al. 2007). Commercially available ginseng is classified as either white or red ginseng. White ginseng is made by peeling fresh ginseng roots and drying them without steaming (Lee et al. 2009). To preserve ginseng for an extended period of time, red ginseng is made by steaming and drying the fresh ginseng, leading to the chemical transformation of some components (Park 1996).

In this study, we hypothesized that red ginseng extract contained estrogenic components and elicited hypolipidemic effects as with exogenously administered estrogen in OVX rats. We examined the impact of red ginseng extract on body weight, uterine weight, and serum plasma lipids.

2. Investigations and results

2.1. ER-mediated transcription activation in MCF-7 cells

Several studies have indicated that ginseng contains estrogenic activity (Cho et al. 2004; Lee et al. 2003). Since steaming in the preparation of red ginseng may affect estrogenic characteristics, we examined whether red ginseng extract activates transcription of an estrogen response element (ERE)-containing reporter plasmid in a human breast adenocarcinoma cell line, MCF-7.

Red ginseng extract activated transcription of the estrogen-responsive luciferase reporter gene in MCF-7 breast cancer cells at 200 μg/mL (Fig. 1A). To confirm that the activities of red ginseng were ER-mediated, we co-incubated the cells with the pure anti-estrogen ICI 182,780. Transcriptional activation of the reporter plasmid by red ginseng was blocked by ICI 182,780 (Fig. 1B), indicating that the estrogenic effect of red ginseng is ER dependent.

2.2. Effects on body and uterine weights

Estrogen increased high-density lipoproteins (HDL) and triglycerides (TG), and decreased low-density lipoproteins (LDL) and fat deposition in OVX animal models (Saengsirisuwan et al. 2009). To examine whether red ginseng extract affects lipids and lipoproteins in the same manner as 17β-estradiol (E2) does, we used OVX rats as a model system. The body weight decreased

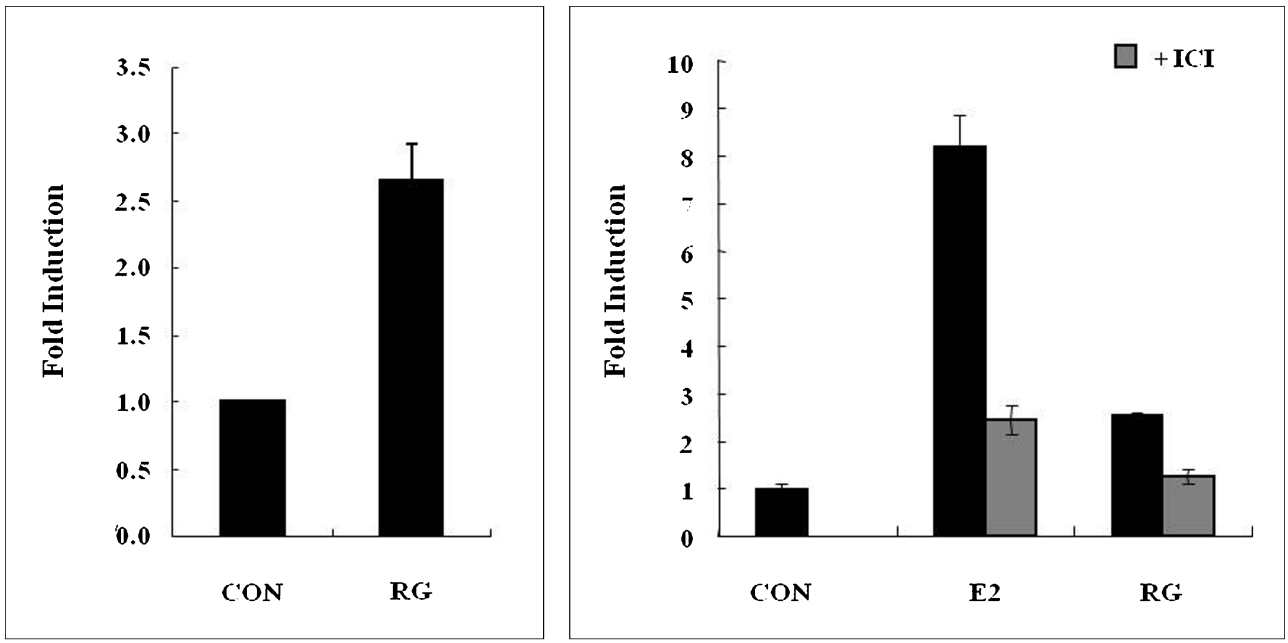

Fig. 1: Activation of estrogen-responsive reporter gene in MCF-7 cells. Cells were transiently transfected with ERE2-tk81-luc and treated with 1 μM ICI 182,780 or 10 nM E2 or 200 μg/ml red ginseng extract, and assayed for luciferase activity after 48 h treatments. Data are representative of at least three independent experiments performed in triplicate

compared with the control OVX group in both the red ginseng and E2 groups (Fig. 2A).

To evaluate the impact of ovariectomy, E2 (0.5 mg/kg), and red ginseng (5 mg/kg) on uterine growth, we measured uterine weight following 5 weeks of a feeding period. The uterine weights were 3.12 ± 0.99 g/kg body weight in the sham non-ovariectomized group, 0.61 ± 0.21 g/kg body weight in the control OVX group induced by ovarian hormone deficiency, 2.30 ± 0.26 g/kg body weight in the E2 group, and 1.17 ± 0.70 g/kg body weight in the red ginseng group. Atrophy of uteri in the OVX group demonstrated the decrease in estrogen secretion. Administration of red ginseng extract increased uterine weight, although not as potently as E2 (Fig. 2B). These results imply that red ginseng extract contains a weak estrogenic effect *in vivo* as well as *in vitro*.

2.3. *Effects on the serum lipid profile*

We have studied the effects of ginseng on the serum lipid profile (Table). A decrease in estrogen due to ovariectomy elevated

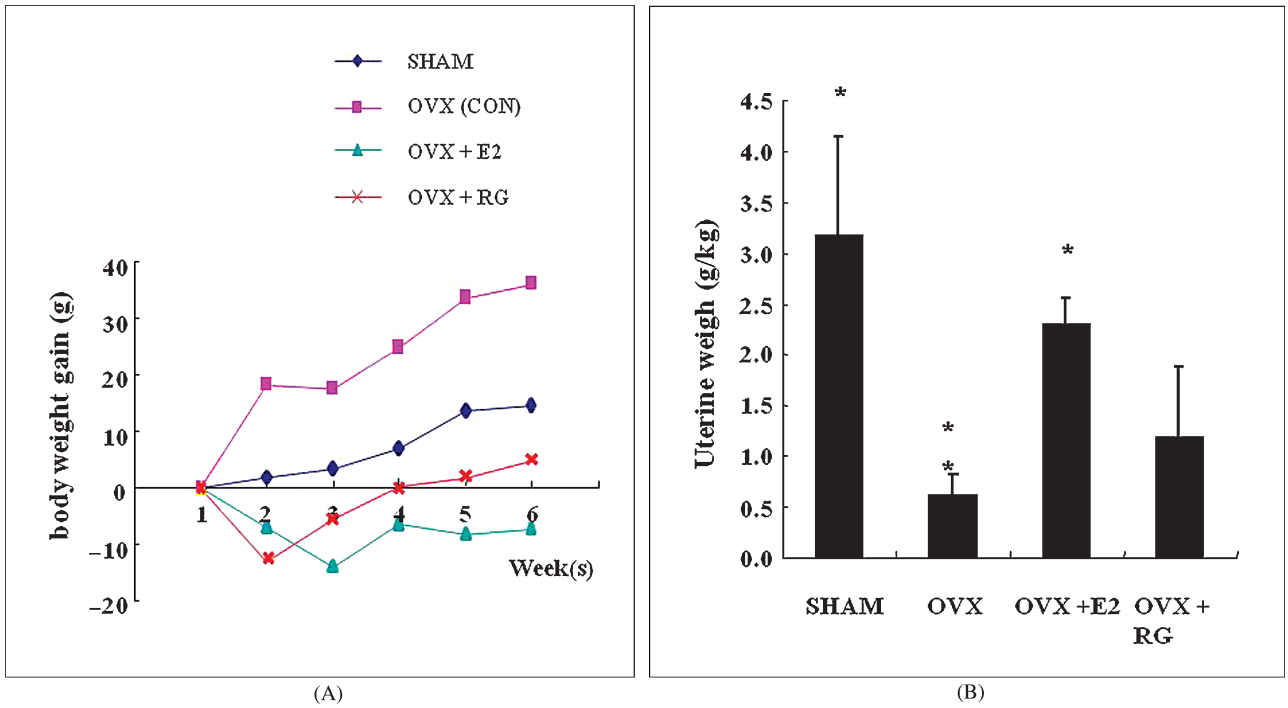

Fig. 2: Effects of red ginseng extract on weight of the body and the uterus. (A) SD rats were ovariectomized. After 4 weeks, weights of operated rats were measured every week. Rats were orally injected with compounds for 5 weeks as indicated in the figure. Treatment groups are: sham (-♦-), control (-■-), E2 0.5 mg/kg (-Δ-), and red ginseng extract 5 mg/kg (-□-). (B) Uterus were isolated 5 weeks after oral injection and measured. The asterisk * and ** shows a statistically significant difference from control and sham group at $p < 0.05$, respectively

Table: Effects of ovariectomy, estrogen, and red ginseng on lipid profile

	SHAM	OVX(CON)	OVX + E2	OVX + RG
TG(mg/dl)	36.12 ± 5.27*	51.42 ± 10.40	119.68 ± 19.0*	36.20 ± 4.34*
TC(mg/dl)	93.43 ± 8.84*	137.30 ± 19.95	43.78 ± 16.87*	109.58 ± 17.20
HDL (mg/dl)	43.75 ± 19.93	54.75 ± 5.36	28.50 ± 7.92*	30.50 ± 4.03*
LDL (mg/dl)	10.94 ± 7.46*	32.83 ± 10.07	6.27 ± 2.98*	14.12 ± 2.48*
HDL/TC	0.40 ± 0.19	0.40 ± 0.02	0.52 ± 0.04*	0.28 ± 0.01*
LDL/TC	0.15 ± 0.07	0.22 ± 0.01	0.14 ± 0.03	0.13 ± 0.01*
LDL/HDL	0.23 ± 0.09*	0.54 ± 0.03	0.22 ± 0.01*	0.41 ± 0.03*

The asterisk * shows a statistically significant difference from control group at $p < 0.05$.

serum total cholesterol (TC) levels, and E2 supplementation significantly lowered cholesterol levels. Red ginseng extract induced a reduction in TC compared to the control OVX. E2 and red ginseng extract induced a reduction in LDL/TC. The levels of HDL/TC in E2 showed an increase of 30%, but red ginseng extract showed a decrease of 30% compared to the control. The levels of LDL/HDL in E2 and red ginseng extracts showed a decrease of 59.3% and 24.1%, respectively, compared to the control. Interestingly, red ginseng decreased TG levels, whereas E2 groups significantly ($P < 0.05$) increased TG levels.

3. Discussion

Ginseng has been used to alleviate menopausal symptoms (Newton et al. 2005; Haimov-Kochman et al. 2008). Several studies have demonstrated that ginseng exerts estrogenic activity, although no clinical data have confirmed its efficacy. However, it should be noted that most clinical studies have focused on effectiveness on vasomotor symptoms, especially hot flashes associated with menopause (Yoshihiro et al. 2003). Further study is necessary to validate herbal alternatives for various other menopausal symptoms.

Aside from uncomfortable symptoms such as hot flashes, night sweats, and sleep disturbances, postmenopausal women may gain weight and develop abdominal obesity. Additionally, the lipid profile is shifted towards atherosclerosis characteristics; an increase in LDL and TG, and a decrease in HDL (Rachoń et al. 2008).

We studied the effects of ginseng extract on lipid profiles to provide a scientific background for using ginseng for vascular symptoms related to the menopausal decrease in estrogen. Our results indicate that ginseng extract may exert vascular protective effects as well as attenuation of body weight in postmenopausal women. Further studies identifying the active components responsible for the estrogenic effects will improve our understanding of the clinical applications of ginseng.

4. Experimental

4.1. Plant material and preparation of crude ginsenosides

Red ginseng extract was obtained from BioPia Co., Ltd (Gyeonggi-Do, Korea). Briefly, the dried root powders were extracted with 80% methanol; the solvent was evaporated *in vacuo*, and then the residue was treated with diethyl ether to remove fats. The resulting extract was fractionated successively with *n*-butanol and water, the *n*-butanol phase was concentrated and lyophilized into crude ginsenosides with a yield of 7.0% of red ginseng.

4.2. Plasmids

ERE2-tk81-luc constructed by inserting the fragment of the herpes simplex thymidine kinase promoter and two copies of the vitellogenin ERE into pA3luc. Expression vector for ERα was from Dr. Pierre Chambon.

4.3. Cell cultures

ER-positive human breast adenocarcinoma, MCF-7 cells were grown at 37 °C in a humidified atmosphere of 95% air/5% CO_2 and fed every 2–3 days. Before hormone induction, the cells were washed with phosphate-buffered saline and cultured in DMEM/10% charcoal-dextran stripped FBS (CD-FBS) for 2 days. All treatments were done with DMEM/10% CD-FBS.

4.4. Transient transfection and luciferase assays

Cells were seeded in 24-well plates at a density of 7×10^4 cells/well. After 24 h, plasmids were transiently transfected into the cell by calcium phosphate-DNA coprecipitation method. Luciferase activity was determined 24 or 48 h after drug treatments with an AutoLumaat LB953 luminometer using the luciferase assay system (Promega, Madison, WI) and expressed as relative light units. All transfection experiments were repeated three or more times with similar results.

4.5. Animals

Eight weeks-old female Sprague-Dawley (SD) rats were purchased from Koatech, (Pyoung-taek, Gyeonggi-Do, Korea). At the end of the 10 weeks experimental period, animals were 18 weeks. The animals were kept at a temperature of 23 ± 2 °C with a 12 h light and dark cycle and had free access to food and water. The study was carried out on four groups of animals each consisting of six rats. Three groups of rats were ovariectomized and one group of rats was used as a sham group. For 5 weeks every day, rats were orally injected with E2 (0.5 mg/kg, in olive oil), red ginseng (5 mg/kg, in olive oil) or olive oil as a control. Group 1 non ovariectomized rats were sham-operated. The uteri were dissected out to measure their weight and used to show the estrogenic hypertrophy.

4.6. Measurement of serum lipids

After the bloods were put at room temperature for 1 h, serum was separated by centrifugation and was stored at –20 °C until assayed. The levels of serum lipid profile were measured by Wet chemistry method from Samsung Medical Center (Seoul, Korea).

4.7. Statistical analysis

Values shown represent mean ± SD. Statistical analysis was performed by Student's t test with a P value of less than 0.05 being considered statistically significant.

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