Effects of Traditional Chinese Medicines on Serum Lipid Profiles and Homocysteine in the Ovariectomized Rats

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Abstract: To investigate the effects of the traditional Chinese medicines, Kuei-lu-erh-hsien-chiao and Chia-wei-hsiao-yao-san, on the cardiovascular systems of mimic menopausal rats, five groups were formed: group 1 (the control group) was given a sham operation and received distilled water, while groups 2, 3, 4 and 5 were ovariectomized and received distilled water, Kuei-lu-erh-hsien-chiao, Chia-wei-hsiao-yao-san and 17-β-estradiol, respectively, for 4 months. Our results demonstrated that the mean differences of the estrogen levels in groups 3 or 5 were significantly higher than those of group 2. These data suggest that there might be some estrogen-like substances in Kuei-lu-erh-hsien-chiao. However, the function of these estrogen-like substances was unknown. The mean differences of the triglyceride (TG) levels, high-density lipoprotein cholesterol (HDL-C) levels, low-density lipoprotein cholesterol (LDL-C) levels, and the ratios of TC/HDL-C and LDL-C/HDL-C in groups 1, 3, 4 and 5 were not significantly different from those in group 2. The mean differences of the total cholesterol (TC) levels in group 5 were significantly higher than those in group 2 (p < 0.05), but no obvious difference of the TC levels was found between groups 2 and 4. Nevertheless, the mean differences of the homocysteine (Hcy) levels in groups 4 and 5 were statistically lower than those of group 2. Therefore, administration of Chia-wei-hsiao-yao-san declines the Hcy levels in OVX rats and does not affect the TC levels in these animals. In conclusion, our results indicate that Chia-wei-hsiao-yao-san shows a more profound effect than 17-β-estradiol in the prevention of atherosclerosis in these OVX rats.

Keywords: 17-β-Estradiol; Kuei-lu-erh-hsien-chiao; Chia-wei-hsiao-yao-san; Ovariectomized; Lipid Profiles; Homocysteine.
Introduction

It has been established that high serum cholesterol levels are a major risk factor for atherosclerosis (Stamler et al., 1986). Estrogen has a beneficial effect in the prevention of cardiovascular diseases in premenopausal women (Barrett-Connor and Bush, 1991). Postmenopausal women lose this protection because of dramatic decreases in their serum estrogen levels as a result of natural atrophy of the ovaries. Premenopausal women who received a bilateral oophorectomy also demonstrate a high incidence of the coronary heart diseases (Rosenberg et al., 1981). Although the high-density lipoprotein cholesterol (HDL-C) concentrations were not significantly different among the premenopausal, postmenopausal and surgically menopausal women, an unchanged plasma HDL-C level may also be one of the risk factors for coronary heart diseases in women with a lack of estrogen owing to the increased plasma concentrations of low-density lipoprotein cholesterol (LDL-C) after natural or surgical menopause (Wakatsuki and Sagara, 1995). It has been suggested that the lack of estrogen in postmenopausal and surgically menopausal women might enhance the lipoprotein lipase activity and reduce the LDL receptor activity leading to the accumulation of plasma LDL-C (Wakatsuki and Sagara, 1995).

Homocysteine (Hcy), a sulfur-containing amino acid involved in the re-methylation and transulfuration metabolic pathways (D'Angelo and Sehlub, 1997), is an independent risk factor for atherosclerosis in hyperlipidemic patients (Glueck et al., 1995). Christodulakos et al. (2001) demonstrated that Hcy levels increased significantly in postmenopausal women at the age of 60 to 64 years.

Some investigations showed that women receiving hormone replacement therapy reduced the risk of cardiovascular diseases by increasing the HDL-C levels and reducing the LDL-C and Hcy levels. Therefore, estrogen replacement therapy in postmenopausal women restores the protective effects against cardiovascular diseases (Giri et al., 1998; Stampfer et al., 1991; Barrett-Connor et al., 1999). However, an increase in side effects, such as increase of risk for breast cancer, resumption of menses and weight gain, is consistently accompanied with this treatment (Norman et al., 2000; Henderson et al., 1993). Because traditional Chinese medicines have fewer side effects and are more suitable for long-term use compared to chemically synthesized medicines, the effect of Chinese medicines has been recently re-evaluated by clinicians (Pao and Chang, 1998). Kuei-lu-erh-hsien-chiao (Liu, 1998) is well known to be used to treat hormonal disturbance, and Chia-wei-hsiao-yao-san used to treat postmenopausal symptoms, such as severe flushes, insomnia muscle aches and pains, formication, fatigue, palpitations and mood swings, also reveals effectiveness in treating premenstrual tension syndrome, and is a novel formula for hypofunction of the reproductive system (Wang, 1994).

Although some investigators (Handdock et al., 2000) reported that serum cholesterol concentrations decreased after treatment with estrogen in postmenopausal women, Ferreri and Natio (1977) showed that serum cholesterol concentrations increased in estradiol-treated rats. The results vary from study to study. Many factors may play significant roles in determining the alteration of serum lipids and one of them is duration of treatment. In order
to evaluate the long-term effectiveness of Kuei-lu-erh-hsien-chiao and Chia-wei-hsiao-yao-san on the cardiovascular system in postmenopausal status, we adopted an ovariectomized (OVX) rat model which artificially produces the depleted state of estrogen for the postmenopausal studies (Kalu, 1991), and analyzed the changes of lipid profiles and Hcy levels after 4 months of treatment with the traditional Chinese medicines and estrogen in the OVX rats.

**Materials and Methods**

**Chinese Medicines and Chemicals**

Gel and liquid extracts of Kuei-lu-erh-hsien-chiao and Chia-wei-hsiao-yao-san were supplied by Chuang Song Zong Pharmaceutical Company, Kaohsiung, Taiwan. They have been reported to be effective for gynecological diseases (Liu, 1998; Wang, 1994). Their herbal constituents and contents are shown in Table 1. 17-β-estradiol was purchased from Sigma Chemicals (St. Louis, MO). All other reagents were purchased from chemical companies with high quality.

**Ovariectomy and Administration of Chinese Medicines**

Five- to six-week-old female Long-Evans rats with body weight from 100–180 g were purchased from the National Laboratory Animal Breeding and Research Center (Taiwan). All the animals went under regulated 12-hour/12-hour light-dark illumination cycles at constant temperature (24 ± 0.5°C) and humidity (45%–50%). The animals had free access to standard chow. When rats were three months old (Albright *et al.*, 1941), they were divided at random into five groups (Table 2); the first group was given a sham operation, and the others were ovariectomized under nembutal (Pentobarbital sodium; 50 mg/kg body weight: Abbott Laboratories, IL) anesthesia. A small midline dorsal skin incision (1–2 cm) was made just caudal to the 13th rib. Bilateral ovariectomy was performed according to the procedures described by Waynforth and Flecknell (1992). Animals were kept warm during the surgical procedures and recovery. The next day, the first and second groups started to feed distilled water as drinking water; the third group received Kuei-lu-erh-hsien-chiao (2.5 g/kg/day); the fourth group received Chia-wei-hsiao-yao-san (2.5 g/kg/day), and the fifth group received 17-β-estradiol (10 µg/kg) by the gastric gavage during the experimental period. The 17-β-estradiol and Chinese medicines were given daily for 4 months and the total volume of solution gavaged for each dose was controlled between 1 to 1.5 ml.

**Biochemical Analysis**

Blood samples were taken from the retro-orbital plexus before the operation and 4 months later at termination of the experiment for all rats. After serum was separated by centrifugation at 2000 rpm for 10 minutes at 4°C, the levels of estrogen, urea nitrogen (UN), creatinine...
(CRTN), aspartate transaminase (AST), alanine transaminase (ALT), total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were determined in the serum.

TC and TG were determined by a Beckman Coulter biochemical analyzer (SYNCHRON CX-5CE, Fullerbon, CA, USA). The TC and TG levels were analyzed by the CHOD-POD method and the Lipase-GOD-POD method (Beckman reagent kit, Fullerbon, CA, USA), respectively. The HDL-C and LDL-C fractions were determined by an electrophoresis analyzer (Helena rep, Texas, USA). Then, the HDL-C and LDL-C levels were obtained by the HDL-C and LDL-C fractions times the TC levels.

The estrogen and hcy levels were measured by the chemiluminescence immunoassay with a DPC Immulite 2000 analyzer.

**Statistics**

Data were expressed as the mean ± standard error. The mean differences of the analyzed values were calculated from the values that subtracted the baseline (pre-operation) from the
analyzed values after 4 months of post-operative treatment. The mean difference of biochemical parameters of group 2 were compared to those in groups 1, 3, 4 and 5, respectively, by using the Wilcoxon (Rank-Sums) method. When the p value was less than 0.05, the difference was considered statistically significant.

**Results**

*Liver and Renal Function Tests*

As liver and renal function tests, the values of BUN and CRTN, and the activities of AST and ALT were determined during the experiment for all groups; there were no significant differences (p > 0.05, data not shown). It excludes the differences in other tests due to the effects of liver or renal damages.

*Estrogen Values*

To certify the efficiency of the administration of 17-\(\beta\)-estradiol, we determined the serum estrogen levels during the experimental period. From the results, we found that although the estrogen levels after the operation were higher than those before the operation in all the rats, the mean differences of the estrogen levels in group 5 were higher than those of the other groups. The mean differences of the estrogen levels in groups 3 and 5 were significantly higher than those of group 2 (Fig. 1A). The mean differences of the estrogen levels in group 2 were lower than those in group 1, although there were no significant differences.

*Lipid Profiles*

TC levels (Fig. 1B): The mean differences of the TC levels in groups 3, 4 and 5 were higher compared to those in group 2; however, only group 5 showed significant differences.

TG levels (Fig. 1C): The mean difference of the TG levels of group 5 was higher compared to the levels in other groups. The mean difference of the TG levels of group 5 was about four times of those in group 2, but no significant differences were found.

HDL-C levels (Fig. 1D): Although the mean difference of the HDL-C levels in group 2 was higher than those in group 1, there were no significant differences. The mean differences of the HDL-C levels were higher in groups 3 and 5, and lower in group 4 compared to group 2. Nevertheless, there were not significant differences.

LDL-C levels, and the ratios of TC/HDL-C and LDL-C/HDL-C (Figs. 1E, F and G): Although the mean differences of the LDL-C levels, TC/HDL-C and LDL-C/HDL-C ratios in group 2 were higher than those in group 1, there were not significant differences between them. The mean differences of these biochemical parameters were higher in group 4, and lower in groups 3 and 5 compared to group 2. However, no significant differences were found among them.
Figure 1. The mean differences of (A) estrogen levels (pg/ml), (B) TC levels (mg/dL), (C) TG levels (mg/dL), (D) HDL-C levels (mg/dL), (E) LDL-C levels (mg/dL), (F) TC/HDL-C ratios, (G) LDL-C/HDL-C ratios, and (H) Hcy levels from the values that subtracted the baseline (pre-operation) from the analyzed values after 4 months of treatment after the operation in groups 1, 2, 3, 4 and 5. * The mean differences differed from those in group 2 were significant (p < 0.05). The standard error of the mean differences.
Hcy levels (Fig. 1H): The mean differences of the Hcy levels in group 2 were higher than those of group 1, but the mean difference of the Hcy levels decreased in groups 3, 4 and 5. Furthermore, there were significant differences between group 2 to groups 4 and 5, respectively.

Discussion

In this study, we found that the estrogen levels in the OVX rats administrated with Kuei-lu-erh-hsien-chiao or 17-β-estradiol were significantly higher than those with distilled water. There might be some estrogen-like substances in Kuei-lu-erh-hsien-chiao, but the function of these estrogen-like substances was unknown. After the ovariectomy, the estrogen levels still increased. It could be the outcome of estrogen production by other organs besides the ovaries.

Some investigators have shown that estrogen could dramatically lower the TC levels in OVX rats (Handdock et al., 2000), but there were some contrary reports regarding this issue (Ferreri and Natio, 1977). A number of possible variables may play a significant role in the effects of estrogens on the serum TC levels, e.g. age, sex, diet, species and strains of tested animals, type and dose of estrogen, duration of treatment, and presence of synergistic hormones (Ferreri and Natio, 1978). In one study by Gökmen and Y apar (1999), they showed that serum TC levels slowly increased with time in the estradiol-treated rats. In this study, the mean differences of the TC levels in the OVX rats with estrogen treatment were significantly higher than those in the control, which was not consistent to the previous report (Handdock et al., 2000). It may be due to the difference of the duration of treatment. Our duration of treatment was longer than that of the previous study (Gökmen and Y apar, 1999). Besides, the estradiol dose may be another influential factor of the TC levels in the OVX rats. The pharmacological doses (1–2 mg/kg) (Frolik et al., 1996; Fewster et al., 1967) of estrogen, which were higher than those in our study (10 μg/kg, about 2–3 μg/day), can induce profound hypolipidemia. Furthermore, Ferreri et al. (1978) reported that lower doses of the estradiol levels (0.25, 2.5 and 25 μg/day) effectively raised the TC levels after 5 weeks of treatment. However, a higher dose of estrogen (2 mg/day) significantly reduced the TC levels by the 8th week.

Campos et al. (1990) found that estrogen therapy decreased the population of large LDL-C and increased the population of small LDL-C, which was associated with a significant increase in plasma TG levels. Although the mean differences of the TG levels were not significantly altered among all groups in our study, the mean difference of the TG levels in group 5 were higher than those in group 2. Our results were consistent with the previous report (Campos et al., 1990). It may be owing to the difference of estrogen doses.

The mechanism of the hypocholesterolemia effect of estrogen in rats is different from that observed in humans. In humans, estrogen up-regulates LDL receptors in the liver, resulted in an increased removal of TC from the circulation (Brown and Goldstein, 1980). The administration of estrogen inhibits the postheparin plasma hepatic TG lipase activity and elevates the plasma HDL-C levels in postmenopausal women (Colvin et al., 1991). Both
LDL-C and HDL-C levels decrease after estrogen treatment in the rats because rat HDL-C contains higher amounts of apoprotein E than does human HDL-C (Windler et al., 1980). The rat LDL receptor has a higher affinity for apoprotein E. Therefore, HDL-C particles are cleared from the blood at a higher rate after estrogen treatment in rats than in human. The mean differences of the HDL-C and LDL-C levels in groups 3, 4 and 5 did not significantly differ from those in group 2; the results showed contrast to previous studies (Frolik et al., 1996; Windler et al., 1980; Ma et al., 1986). There were no significant changes of the mean differences of HDL-C and LDL-C levels in our results; the dosage of both 17-β-estradiol and traditional Chinese medicines may have influenced the outcome.

As an important risk factor for coronary atherosclerosis, elevated plasma total Hcy concentration has recently received greater attention than any other conventional risk factors. Ozkan et al. (2002) reported that postmenopausal women had higher Hcy levels than premenopausal women. Women who received hormone replacement therapy had significantly lower Hcy levels than women in the control group (Madsen et al., 2002). In the present study, the mean differences of the Hcy levels in groups 4 and 5 were significantly lower than those in group 2, which suggest that 17-β-estradiol and Chia-wei-hsiao-yao-san have beneficial effects in the prevention of atherosclerosis in OVX rats. Nevertheless, the mean differences of the TC levels of group 5 were significantly higher than those of group 2. In conclusion, administration of Chia-wei-hsiao-yao-san for 4 months is better than 17-β-estradiol in preventing the atherosclerosis in OVX rats.

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References


