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Effect of ultra-low-dose estradiol and dydrogesterone on arterial stiffness in postmenopausal women

S. Matsui*, T. Yasui†, A. Tani*, T. Kato*, H. Uemura‡, A. Kuwahara*, T. Matsuzaki*, K. Arisawa‡ and M. Irahara*

Departments of *Obstetrics and Gynecology, †Reproductive Technology, and ‡Preventive Medicine, The University of Tokushima Graduate School, Tokushima, Japan

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ABSTRACT

Background Ultra-low-dose estradiol is known to improve menopausal symptoms and increase bone mineral density. However, the effect of ultra-low-dose estradiol on vascular function has not been clarified.

Objectives We examined the effects of ultra-low-dose estradiol on brachial-ankle pulse wave velocity (baPWV) and circulating markers of cardiovascular risk.

Patients and methods Twenty-eight postmenopausal women were enrolled in this study. Fourteen women received oral estradiol (0.5 mg) and dydrogesterone (5 mg) every day for 12 months (ultra-low-dose group) as hormone replacement therapy (HRT) and 14 women as a control group did not receive HRT. The baPWV, lipid profiles, homeostasis model assessment of insulin resistance (HOMA-IR) and vascular inflammatory markers were measured.

Results The baPWV level significantly decreased in the ultra-low-dose group ($p = 0.037$), while the baPWV level did not significantly change in the control group. HOMA-IR tended to decrease in the ultra-low-dose group ($p = 0.076$). Systolic blood pressure and diastolic blood pressure did not change significantly in either group.

Conclusion An HRT regimen using oral ultra-low-dose estradiol and dydrogesterone has an effect on arterial stiffness and insulin resistance.

INTRODUCTION

The effects of hormone replacement therapy (HRT) on vascular function have been demonstrated by using flow-mediated dilation (FMD) as a marker of endothelial function and carotid intima-media thickness (IMT) and pulse wave velocity (PWV) as indicators of arterial stiffness[1], which are useful for predicting the onset of cardiovascular disease[2]. It has been reported that the FMD of the brachial artery was increased by conventional doses of conjugated equine estrogen (CEE), oral estradiol and transdermal estradiol[3–5]. The progression of carotid IMT was prevented by conventional doses of CEE and transdermal estradiol in postmenopausal women without pre-existing cardiovascular disease[6,7]. The effects of conventional doses of CEE and transdermal estradiol on PWV have been controversial, although at least no adverse effects have been reported[8–10].

The dosage of estrogen has been considered as one of the key factors for controlling unfavorable actions. The lowest effective dosage of estrogen for the treatment of menopausal symptoms is recommended by current guidelines[11] in order to minimize adverse effects and risks. Previous studies have shown the effects of low-dose estrogen on FMD and IMT[3,12]. However, the effect of low-dose estrogen on PWV has not been clarified.

Recently, ultra-low-dose estradiol (0.5 mg) has been used as an HRT regimen. It has been reported that HRT using ultra-low-dose estradiol administered orally is effective for improving vasomotor symptoms and increasing bone mineral density (BMD)[13–17]. It has also been shown that the triglyceride level was not affected by ultra-low-dose estradiol[18] and that the incidence of adverse effects in patients receiving ultra-low-dose estradiol was lower than that in patients receiving low-dose estradiol (1 mg)[14,16].

Correspondence: Dr S. Matsui, The University of Tokushima Graduate School, Department of Obstetrics and Gynecology, Tokushima, Japan; E-mail: matsui.sumika@tokushima-u.ac.jp

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To date, the effect of HRT using ultra-low-dose estradiol on vascular function has not been clarified. We examined the effects of ultra-low-dose estradiol on brachial-ankle PWV (baPWV) and circulating markers of cardiovascular risk.

PATIENTS AND METHODS

Patients

The subjects of this study were recruited from patients visiting the outpatient clinic of the Department of Obstetrics and Gynecology, Tokushima University Hospital with complaints of menopausal symptoms. Twenty-eight postmenopausal women were enrolled in this study between July 2009 and January 2012. Postmenopausal status was confirmed by follicle stimulating hormone (FSH) concentration $\geq$40 mIU/ml and estradiol concentration $\leq$20 pg/ml in women with no natural menstruation for at least 1 year or women who had undergone bilateral oophorectomy. Fourteen women received oral estradiol (0.5 mg) and dydrogesterone (5 mg) for endometrial protection every day for 12 months (ultra-low-dose group). Fourteen women acting as a control group did not want to receive HRT. Before recruitment in the study, patients underwent gynecological and biochemical examinations that included bimanual examination and transvaginal ultrasonography. Reviews of medical histories and results of physical examinations and blood chemistry tests showed that all the women were in good health. Exclusion criteria for this study were a history of any cardiovascular disease, breast cancer, venous thromboembolic disease, uncontrolled hypertension, diabetes mellitus, renal dysfunction and liver disease. Women who had received hormone therapy in the past were not included in this study.

Venous blood samples were drawn into tubes between 08.00 and 10.00 after a 12-h fast before and at 12 months after commencement of the study. Blood samples obtained were frozen at −70°C until used for analysis. Informed consent for participation in this study was obtained from each woman. The Ethics Committee of Tokushima University Hospital approved the study.

Measurement of pulse wave velocity

The baPWV was measured using volume-plethymographic apparatus (BP-203RPEIII, Colin Co. Ltd., Japan), which allows pulse wave recording and automatic calculation of baPWV as previously described and validated[19]. The subjects were examined after 10 min of bedrest with cuffs wrapped around both brachia and ankles and the electrodes of the electrocardiogram placed on both wrists. The pulse volume waveforms at the brachium and ankle were recorded using a semiconductor pressure sensor. Pearson's correlation coefficients of inter- and intra-observer reproducibility were $r = 0.98$ and $r = 0.87$, respectively[19].

Measurement of serum concentrations of estradiol and FSH

The serum estradiol concentration was measured by using a kit (Siemens Healthcare, Los Angeles, CA, USA). Intra- and inter-assay coefficients of variation (CVs) were 4.5–8.0% and 3.2–12.1%, respectively, and the sensitivity of the assay was 2.5 pg/ml. The serum FSH concentration was measured by an IRMA using a commercially available kit (TFB Co., Tokyo, Japan). The intra- and inter-assay CVs ranged from 3 to 4% and from 3 to 4%, respectively. The range of measurement was 0.5–200 mIU/ml and the sensitivity of the assay was 0.5 mIU/ml.

Measurement of concentrations of glucose, insulin and lipid profiles

The plasma glucose level was measured by using the glucose oxidase method on an Automated Glucose Analyzer GA04 (A&T, Kanagawa, Japan). The intra- and inter-assay CVs ranged from 0.8 to 1.3% and from 0.6 to 1.3%, respectively. The serum insulin level was measured by using an enzyme immunoassay on AIA2000 (Tosoh Co., Tokyo, Japan). The intra- and inter-assay CVs ranged from 1.1 to 3.2% and from 1.9 to 3.3%, respectively, and the sensitivity of the assay was 1.0 μU/ml. Insulin resistance was evaluated with the homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated for all subjects by the following formula: [fasting serum insulin (μU/ml) × fasting plasma glucose (mg/dl)]/405.

Serum total cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride levels were assessed by using an enzymatic calorimetric method. Low density lipoprotein (LDL) cholesterol was estimated by the Friedewald equation (total cholesterol − triglyceride/5 − HDL cholesterol).

Measurement of vascular inflammatory markers

Assays for soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1) and E-selectin were carried out using an ELISA kit (R&D Systems, Minneapolis, MN, USA). Intra- and inter-assay CVs were 2.2–2.8% and 6.5–9.5% for sVCAM-1, 6.5–7.9% and 7.6–11.6% for sICAM-1, and 3.3–4.9% and 3.1–4.8% for E-selectin, respectively.

Statistical analysis

Data are presented as means ± standard deviations. Differences between the two groups in baseline characteristics were evaluated by an unpaired *t*-test. Baseline and follow-up levels of baPWV, hormones, glucose, insulin, lipid profiles and vascular inflammatory markers were compared across the

Table 1 Serum levels of hormones and blood pressure at baseline and after 12 months in the control group and ultra-low-dose group. Data are given as mean ± standard deviation

	Control group ($n = 14$)			*Ultra-low-dose group* ($n = 14$)		
	Baseline	*After 12 months*	*p Value*	*Baseline*	*After 12 months*	*p Value*
Age (years)	50.6 ± 4.9			50.1 ± 6.0		
Body mass index (kg/m^2)	21.8 ± 2.9	21.7 ± 2.9	0.783	20.8 ± 3.0	20.9 ± 2.9	0.617
Estradiol (pg/ml)	2.7 ± 1.0	4.2 ± 1.2	0.063	4.5 ± 2.5	15.4 ± 13.1	0.016
Follicle stimulating hormone (IU/l)	83.4 ± 39.7	79.1 ± 26.0	0.719	87.0 ± 30.8	66.2 ± 34.6	0.014
Systolic blood pressure (mmHg)	114.1 ± 20.8	115.6 ± 19.5	0.579	124.3 ± 16.0	128.8 ± 29.1	0.679
Diastolic blood pressure (mmHg)	69.8 ± 12.1	68.8 ± 10.0	0.461	71.1 ± 11.6	68.2 ± 14.0	0.210
Heart rate (beats/min)	66.5 ± 8.5	67.6 ± 9.6	0.551	73.7 ± 9.4	68.9 ± 7.5	0.203

same group by a paired *t*-test. We set the basal values as 100% and calculated the percentage changes. All statistical analyses were carried out using SPSS statistics version 20.0 (IBM, Armonk, New York, USA). *p*-Values less than 0.05 were considered to be statistically significant.

RESULTS

General characteristics

All participants originally enrolled in the study completed the 12-month study without severe adverse effects. Baseline characteristics are presented in Tables 1 and 2. Baseline demographic characteristics including age, body mass index (BMI), hormonal data and blood pressure did not show significant differences between the two groups. There was no significant difference between the two groups in baseline baPWV (ultra-low-dose group: 1498 ± 256 cm/s, control group: 1326 ± 201 cm/s). In addition, there were no significant differences in lipid profiles, glucose, insulin, HOMA-IR and vascular inflammatory markers between the two groups. In postmenopausal women, the mean durations since menopause were 3.2 years and 7.1 years in the ultra-low-dose group and control group, respectively, and there was no significant difference. Smoking rate showed no significant difference between the two groups (ultra-low-dose group: 7.1%, control group: 7.1%).

Levels of estradiol and FSH

As can be seen in Table 1, the estradiol level was significantly increased and the FSH level was significantly decreased at 12 months in the ultra-low-dose group ($p = 0.016$ and $p = 0.014$, respectively). The serum estradiol level at 12 months in the ultra-low-dose group was 15.4 ± 13.1 pg/ml.

Changes in baPWV

As can be seen in Figure 1, the baPWV level was significantly decreased at 12 months in the ultra-low-dose group ($p = 0.037$), while the baPWV level did not significantly change at 12 months in the control group. There was a significant

Table 2 Serum levels of lipid parameters, HOMA-IR and vascular inflammatory markers at baseline and after 12 months in the control group and ultra-low-dose group. Data are given as mean ± standard deviation

	Control group ($n = 14$)			*Ultra-low-dose group* ($n = 14$)		
	Baseline	*After 12 months*	*p Value*	*Baseline*	*After 12 months*	*p Value*
Total cholesterol (mg/dl)	217.3 ± 23.8	228.6 ± 20.9	0.049	204.6 ± 23.3	204.3 ± 24.9	0.970
Triglycerides (mg/dl)	89.0 ± 52.2	83.1 ± 37.6	0.628	87.8 ± 27.3	83.1 ± 32.6	0.713
HDL cholesterol (mg/dl)	80.0 ± 23.4	81.1 ± 23.3	0.595	77.6 ± 15.1	84.5 ± 11.8	0.226
LDL cholesterol (mg/dl)	119.5 ± 30.3	130.9 ± 24.2	0.069	111.5 ± 23.3	103.2 ± 23.9	0.186
Fasting blood sugar (mg/dl)	93.5 ± 7.0	97.1 ± 8.7	0.124	94.4 ± 13.5	83.8 ± 19.5	0.122
Insulin (μU/ml)	5.6 ± 3.2	5.0 ± 3.1	0.364	8.0 ± 8.7	3.7 ± 1.7	0.076
HOMA-IR	1.4 ± 0.9	1.2 ± 0.9	0.325	1.8 ± 2.0	0.8 ± 0.5	0.076
sVCAM-1 (ng/ml)	584.1 ± 101.1	592.0 ± 106.7	0.665	627.2 ± 150.9	577.8 ± 153.0	0.047
sICAM-1 (ng/ml)	170.0 ± 55.7	179.0 ± 55.7	0.236	209.9 ± 68.5	206.0 ± 64.1	0.707
E-selectin (ng/ml)	27.1 ± 10.5	27.0 ± 10.2	0.979	35.4 ± 10.0	33.1 ± 10.9	0.209

HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; sVCAM-1, soluble vascular cell adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule-1

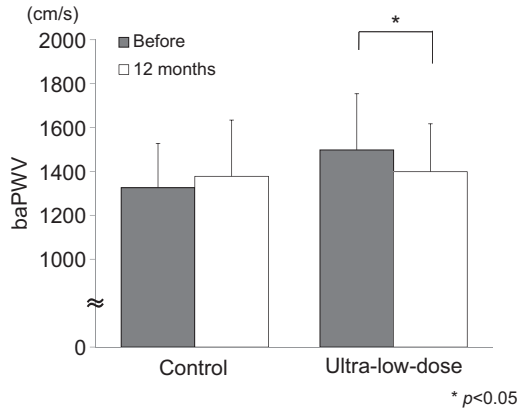

Figure 1 Changes in brachial-ankle pulse wave velocity (baPWV) in the control group and in the ultra-low-dose group (mean ± standard deviation)

difference in percentage changes of baPWV between the two groups ($p = 0.009$) and the difference remained significant after adjustment by age and years since menopause ($p = 0.005$). Systolic and diastolic blood pressures did not change significantly in either group (Table 1).

Lipid profiles, glucose, insulin, HOMA-IR and vascular inflammatory markers

As can be seen in Table 2, serum insulin levels and HOMA-IR tended to decrease in the ultra-low-dose group ($p = 0.076$ and $p = 0.076$). Serum sVCAM-1 levels were significantly decreased in the ultra-low-dose group ($p = 0.047$). Serum levels of total cholesterol were significantly increased in the control group ($p = 0.049$). There were significant differences in percentage changes in glucose and LDL cholesterol during the 12-month period between the two groups (data not shown).

DISCUSSION

We found that HRT using ultra-low-dose estradiol and dydrogesterone significantly decreased baPWV without significant changes in blood pressure, suggesting that ultra-low-dose estradiol improves arterial stiffness independently of blood pressure. In addition, sVACM-1 was significantly decreased and the triglyceride level was not significantly increased in the ultra-low-dose group. Sumino and colleagues reported that treatment with a conventional dose of transdermal estradiol, but not oral CEE therapy, significantly decreased baPWV after 12-month treatment, suggesting that baPWV is not improved by CEE administration because of increases in vascular inflammatory markers and triglycerides[8]. Therefore, the effect of oral ultra-low-dose estradiol on baPWV may be found since the first-pass effect in the liver by ultra-low-dose estradiol is weak and there is no interference with its anti-atherosclerotic effect.

The effects of conventional doses of oral estradiol on PWV have been controversial. It has been reported that oral cyclic HRT with estradiol valerate (2 mg/day) and cyproterone acetate significantly improved PWV after three cycles of treatment[20]. However, Teede and colleagues reported that estradiol (2 mg) combined with norethisterone (1 mg) orally during a 2-year period in healthy postmenopausal women did not improve aorto-femoral and femoro-dorsalis PWV in a randomized placebo-controlled trial[21]. In that study, the mean age of the subjects was 62 years and the mean BMI was 26 kg/m^2. The difference in the results may be associated with the differences in background characteristics, such as age and BMI, of the subjects and differences in measurement of PWV.

Effects of low-dose estrogens, such as CEE (0.3 mg) and estradiol (1 mg), on FMD and IMT have been reported[3,6,12]. However, to date, the effect of ultra-low-dose estradiol on endothelial function and arterial stiffness has not been clarified. In the present study, we showed that the mean estradiol level in the ultra-low-dose group at 12 months was 15.4 pg/ml and that this low estradiol level could affect arterial stiffness. Barbieri proposed the estrogen threshold hypothesis, suggesting that sensitivity to estradiol varied in each tissue[22]. Vascular function as well as bone metabolism and vasomotor symptoms might be affected by a low circulating estradiol level.

We found that ultra-low-dose estradiol had a favorable effect on insulin sensitivity. A previous study showed that a low dose of estradiol (1 mg) administered orally decreased HOMA-IR, although a standard dose of estradiol (2 mg) caused a slight deterioration in insulin sensitivity in healthy postmenopausal women. The results of that study suggested that a low dose of estradiol minimized the increase in exposure of the liver to high concentrations of estrogen and diminished adverse effects on hepatic metabolism[23]. The effect of oral estradiol on insulin resistance may be due to dose dependency. However, another study showed that both low-dose estradiol and standard-dose estradiol administered orally significantly reduced levels of fasting glucose and insulin. Insulin secretion in the pancreas during an intravenous glucose tolerance test and insulin elimination were increased in both treatments, while insulin sensitivity was unaffected[24]. Further studies on the effects of ultra-low-dose estradiol administration on insulin resistance by using the euglycemic-hyperinsulinemic clamp procedure and glucose tolerance are needed.

Terauchi and colleagues reported that levels of total cholesterol, HDL cholesterol, triglycerides and LDL cholesterol were not significantly changed after an 8-week treatment with ultra-low-dose estradiol[18]. Our results are consistent with the results of that study. Ultra-low-dose estradiol may have a neutral effect on lipid profiles without decreases in total cholesterol and LDL cholesterol or increases in HDL cholesterol and triglycerides. sICAM-1 and E-selectin, which are involved in the process of atherosclerosis, were also not also significantly changed, while only sVCAM-1 levels were significantly decreased. Störk and colleagues reported that oral estradiol (1 mg) reduced the levels of VCAM-1, ICAM-1 and E-selectin[25]. The circulating estradiol level in patients treated with ultra-low-dose estradiol may have no effect on lipid profiles

and a weak effect on vascular inflammatory markers. Based on the present results, a low estradiol level can affect vascular function and insulin sensitivity and a higher estradiol level is needed for changes in lipid profiles and vascular inflammatory markers.

The present study has several limitations. First, the sample size was small. Second, we used dydrogesterone as a progestogen for protection against endometrial hyperplasia. It has been reported that natural progesterone increased production of endothelial nitric oxide, while medroxyprogesterone acetate did not increase the production[26]. On the other hand, Fu and colleagues reported that dydrogesterone does not interfere with the anti-inflammatory effects of estradiol[27]. In a study on lipid metabolism and carbohydrate metabolism, which influence vascular function, Seeger and colleagues showed that addition of dydrogesterone intensified the beneficial estrogenic effects[28]. Therefore, dydrogesterone as well as estrogen may have a beneficial effect on vascular function and further study on the effect of estradiol alone on baPWV may be needed. Third, further investigation is needed to determine the difference in effects of oral administration and transdermal administration on PWV.

In conclusion, an HRT regimen using oral ultra-low-dose estradiol and dydrogesterone has an effect on vascular function and insulin resistance.

Conflict of interest The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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