Original Article

Effect of Transdermal Hormone Replacement Therapy on Carotid Artery Wall Thickness and Levels of Vascular Inflammatory Markers in Postmenopausal Women

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Carotid intima-media thickness (IMT) and vascular inflammatory markers have been shown to be involved in atherosclerosis. This study was designed to investigate the effect of transdermal hormone replacement therapy (HRT) on carotid IMT and vascular inflammatory markers in postmenopausal women and to explore the interrelationship between the change in carotid IMT and the changes in vascular inflammatory markers. Thirty-five postmenopausal women (mean age 57.0±7.7 years) received transdermal HRT (continuous 17β-estradiol patch [36 μg/day] plus cyclic oral medroxyprogesterone acetate [2.5 mg/day, for 12 days/month]) for 12 months, and 32 controls (mean age 58.0±7.5 years) did not. Carotid IMT, assessed by ultrasound, and circulating vascular inflammatory markers, *i.e.*, C-reactive protein (CRP), intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, E-selectin, monocyte chemoattractant protein (MCP)-1, and matrix metalloproteinase (MMP)-9 were measured before and after 12 months of treatment. In the HRT group, carotid IMT decreased significantly ($p<0.01$), from 0.71±0.13 mm to 0.65±0.12 mm, and the ICAM-1, VCAM-1, E-selectin, and MCP-1 levels decreased significantly ($p<0.01$ for all), but the CRP and MMP-9 levels remained unchanged. Carotid IMT and vascular inflammatory markers were unchanged in the control group. In the HRT group, the change in carotid IMT was significantly correlated with the change in serum E-selectin ($r=0.38$, $p<0.05$), but not with the changes in other vascular inflammatory markers. These results suggest that transdermal HRT reduced carotid artery wall thickness, and that the reduction may have been induced by an antiatherosclerotic effect combined with the direct effect of estrogen and decreased levels of estrogen-induced E-selectin. (*Hypertens Res* 2005; 28: 579–584)

***Key Words*: carotid artery, estrogen, inflammation, ultrasound, women**

Introduction

Carotid intima-media thickness (IMT) measurements are being widely used as a measure of atherosclerosis in studies on determinants of the presence and progression of atherosclerosis (*1*). Carotid IMT is a marker of generalized atherosclerosis, and increased carotid IMT is a strong predictor of coronary heart disease and stroke (*2–4*).

Vascular inflammation, in addition to lipids, has also been considered an important factor in the pathogenesis of atherosclerosis, and elevated circulating C-reactive protein (CRP)

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and markers of inflammation are independent risk factors for cardiovascular disease (*5*). Cell adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin, appear to be particularly important for the recruitment of monocytes from the blood into the intima of arteries, where they subsequently become lipid-filled foam cells and increase atherosclerotic plaque size, and the serum levels of these molecules are elevated in coronary and carotid artery disease (*6*). Macrophage recruitment to arterial lesions is predominantly mediated by monocyte chemoattractant protein (MCP)-1 (*7*), and the increased MCP-1 levels in human atherosclerotic arteries (*7*, *8*) and experimental atherosclerotic lesions (*7*) imply that this molecule plays an important role in the formation of vascular lesions. Macrophages in human atherosclerotic plaques produce matrix metalloproteinase (MMP), which degrades collagen and elastin, the major components of the extracellular matrix in the fibrin cap, and predispose it to rupture and thrombus formation (*9*). Increased serum levels of MMP, and especially of MMP-9, have been reported in patients with unstable angina or acute myocardial infarction (*10*) and acute cerebral ischemia (*11*).

The Women's Health Initiative (WHI) reported that oral hormone replacement therapy (HRT) slightly but significantly increases the risk of coronary heart disease and stroke in postmenopausal women without established coronary disease (*12*, *13*). However, the effect of transdermal HRT on this risk is unknown. Transdermal HRT reduces serum levels of soluble cell adhesion molecules and MCP-1 in postmenopausal women (*14*, *15*). In addition, carotid IMT is smaller in postmenopausal women who received non-oral HRT (percutaneous gel or patch) than in non-users (*16*). Since the circulating levels of cell adhesion molecules and MCP-1 are associated with the degree of carotid IMT in various populations (*6*, *8*, *17*, *18*), we hypothesized that transdermal HRT in postmenopausal women would decrease carotid IMT in combination with the changes in vascular inflammatory markers.

Thus, this study was designed to investigate the effect of transdermal HRT on carotid IMT and vascular inflammatory markers in postmenopausal women and to explore the interrelationship between the change in carotid IMT and the changes in vascular inflammatory markers.

Methods

Subjects

Seventy-two postmenopausal Japanese women participated in this study. Upon enrollment, all subjects had completed at least 1 year since their final menstrual period. Menopausal status was confirmed by a serum estradiol concentration <20 pg/ml and a serum follicle-stimulating hormone (FSH) concentration >40 mIU/ml. None of the subjects smoked or had a history of thyroid disease, liver disease, hypertension, diabetes mellitus, cardiovascular disease, hormone-dependent malignancy, or breast cancer; none were currently taking any medication known to influence lipoprotein metabolism; and none had received HRT, other steroid hormones, or any medication that might affect sex steroid metabolism before the present study. Written informed consent was obtained from each participant before admission to the study, and the Ethics Committee of the Cardiovascular Hospital of Central Japan approved the protocol.

Study Protocol

Subjects were assigned to one of two groups, a group of 35 postmenopausal women who received HRT (57.0±7.7 years; range 48 to 66 years; HRT group) and a group of 32 postmenopausal women who did not wish to receive HRT (58.0±7.5 years; range 49 to 65 years; control group). The subjects in the HRT group were treated with a continuous 17β-estradiol patch (absorption rate, 36 μg/day) combined with cyclic oral medroxyprogesterone acetate (2.5 mg/day, for 12 days/month) for 12 months. One subject in the HRT group and four subjects in the control group withdrew during the study period. Each subject in the HRT and control groups attended the HRT clinic at the Cardiovascular Hospital of Central Japan once a month for physical checkups that included blood pressure and heart rate measurements, and blood specimens were collected before HRT and 12 months after the start of HRT. The blood specimen collections and anthropometric measurements were performed between 9 AM and 10 AM after a 14-h overnight fast. Blood pressure in the right arm was measured with a mercury sphygmomanometer by the same investigator. Plasma and serum were separated by centrifugation for 15 min at $3{,}000\times g$ at 4°C and assayed immediately.

Assessment of Carotid Wall Thickness

The bilateral carotid arteries were evaluated with an ultrasonograph (Power Vision 6000; Toshiba, Tokyo, Japan) using a 7.5-MHz linear type-B-mode probe (*17–19*). After having the subject rest for at least 10 min in the supine position with the neck in slight hyperextension, we evaluated the optimal visualization of the bilateral common carotid arteries, carotid bulb, and extracranial internal and external carotid arteries. From anterior, lateral, and posterior approaches, the end-diastolic IMT of the far wall was measured in the bilateral common carotid artery 1 cm proximal to the bulb and averaged to obtain the mean IMT. The detection limit of the echo system at 7.5-MHz was 0.1 mm. All scans were performed by a physician who was unaware of the clinical characteristics of the subjects. The variability of the ultrasonographic measurements was assessed by performing 5 measurements over 1 month in 12 volunteers. The intraobserver coefficient of variation for IMT measurement was 5.5±0.8%.

Table 1. Changes in BMI, Blood Pressure, and Serum Hormone and Lipid Levels

	HRT (n=35)		Control (n=32)	
	Baseline	12 months	Baseline	12 months
BMI (kg/m^2)	22.0±3.1	22.1±3.0	22.6±2.1	22.6±2.4
SBP (mmHg)	122.1±12.6	119.6±13.9	123.0±9.9	123.5±12.2
DBP (mmHg)	76.7±8.4	75.6±7.8	78.3±7.1	78.8±6.5
Heart rate (bpm)	64.0±6.7	65.0±5.1	63.5±6.8	62.8±7.1
FSH (mIU/ml)	60.2±30.1	23.1±14.3*,†	58.0±16.9	58.7±17.0
Estradiol (pg/ml)	13.2±2.9	103.4±62.1*,†	14.0±3.7	13.7±3.3
Total cholesterol (mg/dl)	216.5±34.6	214.2±31.9	214.5±31.0	210.9±32.2
Triglycerides (mg/dl)	84.7±32.8	86.4±50.7	99.4±39.2	98.2±42.1
HDL cholesterol (mg/dl)	60.8±9.2	64.2±15.9	63.7±10.7	61.8±9.6
LDL cholesterol (mg/dl)	138.8±32.9	132.6±24.8	130.9±27.6	129.5±35.2

BMI, body mass index; HRT, hormone replacement therapy; SBP, systolic blood pressure; DBP, diastolic blood pressure; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein. The data shown are the means±SD. *p<0.001 *vs.* baseline. †p<0.05 *vs.* control group.

Table 2. Changes in Vascular Inflammatory Markers

	HRT (n=35)		Control (n=32)	
	Baseline	12 months	Baseline	12 months
hs-CRP (ng/dl)	40.8±32.2	35.9±40.2	46.0±40.5	48.0±30.6
ICAM-1 (ng/ml)	246.8±75.2	232.8±67.7*	251.0±74.8	252.9±68.1
VCAM-1 (ng/ml)	655.6±129.9	624.7±118.5*	625.6±114.1	624.6±106.0
E-selectin (ng/ml)	41.0±18.4	33.1±15.8†	37.8±10.0	42.1±24.2
MCP-1 (pg/ml)	192.6±42.7	167.5±35.3†,‡	206.6±65.6	211.2±99.3
MMP-9 (ng/m)	20.1±17.4	15.2±7.1	20.7±25.9	18.7±14.7

HRT, hormone replacement therapy; hs-CRP, high-sensitivity C-reactive protein; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase. The data shown are the means±SD. *p<0.01, †p<0.001 *vs.* baseline. ‡p<0.05 *vs.* control group.

Laboratory Assays

Serum total cholesterol and triglyceride concentrations were determined by enzymatic methods (Medca Japan, Konosu, Japan) with a Hitachi 7170S automatic analyzer (Hitachi, Tokyo, Japan). Serum high-density lipoprotein (HDL) cholesterol concentrations were determined electrophoretically with an HDL Cholesterol Supply Kit (Helena Laboratory, Beaumont, USA). Low-density lipoprotein (LDL) cholesterol concentrations were calculated according to the Friedewald formula. Serum FSH and estradiol concentrations were measured by radioimmunoassay with commercially available kits (Boehringer Mannheim, Mannheim, Germany). Plasma high-sensitivity CRP (hs-CRP) concentrations were determined with a Boering BNII nephelometer (Dade Boering, Marburg, Germany). Serum ICAM-1, VCAM-1, E-selectin, MCP-1, and MMP-9 concentrations were quantified by an enzyme-linked immunosorbent assay technique (R&D Systems, Minneapolis, USA). The intra- and inter-assay coefficients of variation were <6% for lipids, <7% for FSH, <5% for estradiol, <9% for hs-CRP, <8% for ICAM-1, <7% for VCAM-1, <8% for E-selectin, <9% for MCP-1, and <8% for MMP-9.

Statistical Analysis

Data are expressed as the means±SD. Student's t-test was used to analyze the differences between the two groups. The paired t-test was used to analyze the differences between the values recorded at baseline and again at 12 months. The associations between the change in carotid IMT and changes in vascular inflammatory marker were assessed by Pearson's correlation coefficient. All probability values were 2-tailed. A value of p<0.05 was considered statistically significant.

Results

There were no significant differences between the groups with respect to age or the baseline values (Tables 1 and 2). HRT increased the estradiol concentration and decreased the FSH concentration. Blood pressure, heart rate, and lipid profiles in the two groups remained unchanged throughout the study (Table 1).

Carotid IMT at baseline was similar in both groups. Carotid IMT decreased significantly, from 0.71±0.13 mm to 0.65±0.12 mm, in the HRT group (p<0.01), but did not

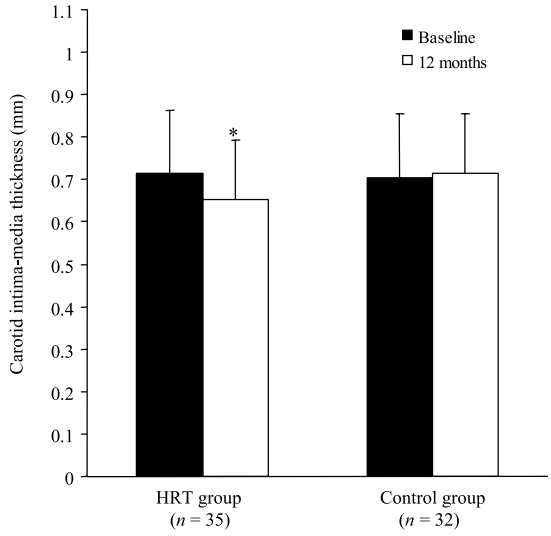

Fig. 1. *Changes in mean carotid intima-media thickness (IMT) in the hormone replacement therapy (HRT) group and the control group. HRT significantly decreased mean carotid IMT in the HRT group (*$p < 0.01$*), but did not affect it in the control group. Closed and open columns represent the values at baseline and 12 months, respectively. The data shown are the means±SD.* *$p < 0.01$ vs. *baseline.*

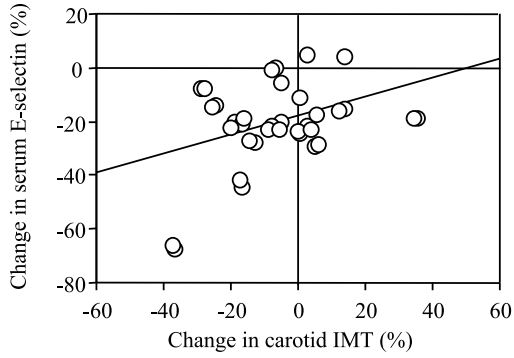

Fig. 2. *Relationship between mean changes in carotid intima-media thickness (IMT) and changes in serum E-selectin in the hormone replacement therapy group (*$y = 0.35x - 17.82$, $r = 0.38$, $p < 0.05$*).*

change in the control group (Fig. 1).

The ICAM-1, VCAM-1, E-selectin, and MCP-1 levels decreased significantly in the HRT group ($p<0.01$, $p<0.01$, $p<0.001$, and $p<0.001$, respectively), but did not change in the control group (Table 2). The hs-CRP and MMP-9 levels in the two groups remained unchanged throughout the study.

We evaluated the relationship between the change in carotid IMT and changes in vascular inflammatory markers in the HRT group. There was a significant correlation between the change in carotid IMT and the change in serum E-selectin in the HRT group ($r=0.38$, $p<0.05$; Fig. 2), but there were no significant correlations between the change in carotid IMT and the changes in other vascular inflammatory markers in the HRT group.

Discussion

The present study showed that transdermal HRT reduced the cell adhesion molecules and MCP-1 levels and the carotid IMT in postmenopausal women, but had no effect on their lipid, hs-CRP, or MMP-9 levels. The decrease in serum E-selectin was positively correlated with the reduction in carotid IMT after transdermal HRT.

Carotid IMT after non-oral HRT has been investigated in several studies. In a cross-sectional study, Tremollieres *et al.* (*16*) measured carotid artery IMT by B-mode ultrasonography and found that this parameter was significantly smaller in healthy postmenopausal women over 55 years of age who had received 7 years of 1.5 mg/day of 17β-estradiol in the form of a percutaneous gel and 50 μg/day of transdermal 17β-estradiol twice a week in combination with natural progesterone or 19-norprogesterone than in never-treated women. On the other hand, the present study was a longitudinal study and showed that 12 months of continuous 36 μg/day of transdermal 17β-estradiol combined with cyclic 2.5 mg/day of oral medroxyprogesterone acetate decreased the carotid IMT in healthy postmenopausal women with a mean age of 57 years. Although there were differences in the study design and the dosage, type, and duration of estrogen and progestin administration between this previous study and the present one, their results support our present findings. Thus, transdermal HRT may have a beneficial effect on the vascular wall.

The mechanisms by which transdermal HRT affects the vascular wall in postmenopausal women have not yet been identified. However, we can speculate some possibilities. First, the reduction in carotid artery wall thickness as a result of transdermal HRT may be partly due to the direct suppressive effect of estrogen on the growth of the arterial wall, since functional estrogen receptors are present on vascular smooth muscle cells and fibroblasts, and estrogen has been shown to inhibit fibroblast growth and collagen synthesis in experimental preparations (*20–22*). In this regard, Fischer and Swain (*21*) reported that estradiol treatment for 8 weeks reduced collagen synthesis and content in ovariectomized rabbit aortas. Second, the reduction in carotid artery wall thickness may also be attributable to an indirect effect of estrogen on the artery wall mediated by cell adhesion molecules and MCP-1. In the Atherosclerosis Risk In Communities (ARIC) study (*6*), higher serum levels of E-selectin and ICAM-1 were found in patients with coronary heart disease and carotid artery atherosclerosis than in healthy control subjects, and the E-selectin level was positively correlated with carotid artery thickness. Kohara *et al.* (*17*) and Tabara *et al.* (*18*) also reported significant positive correlations between plasma ICAM-1, VCAM-1, and MCP-1 levels and carotid

IMT in subjects over 50 years of age who were free from cardiovascular disease. Stork *et al*. (*8*) demonstrated a significant positive correlation between serum MCP-1 levels and mean maximum IMT in the carotid and femoral arteries of healthy postmenopausal women, but the correlation disappeared after oral HRT. In the present study, the mean change in carotid IMT was significantly positively correlated with the change in serum E-selectin level in the HRT group, but not with changes in other markers. Although the reason for this finding is unknown, the reduction in carotid IMT may contribute to the direct effect of estrogen as well as the decrease in estrogen-induced serum E-selectin.

Several clinical studies have reported that transdermal HRT had no effect on CRP (*15*, *23*) and MMP-9 (*15*, *24*) in postmenopausal women, findings which are consistent with the results of this study. CRP may contribute to atherogenesis by facilitating uptake of LDL by macrophages, thereby accelerating foam-cell formation (*25*). Thus, transdermal HRT may be less likely to promote atherogenesis as well as plaque destabilization and rupture than oral HRT.

Cell adhesion molecules are induced by inflammatory cytokines and facilitate leukocyte attachment to and migration across endothelial cells during vascular inflammation, and the presence of ICAM-1, VCAM-1, and E-selectin in atherosclerotic human arteries has been demonstrated immunohistochemically (*26*). In the ARIC study (*6*), higher serum levels of ICAM-1 and E-selectin were found in patients with coronary artery disease and carotid artery atherosclerosis than in healthy control subjects. Transdermal HRT decreases serum levels of soluble fragments of ICAM-1, VCAM-1, or E-selectin in postmenopausal women (*14*, *15*). These findings are compatible with the results of the present study showing that transdermal HRT reduced serum ICAM-1, VCAM-1, and E-selectin concentrations and suggest that transdermal HRT has a favorable effect on cell adhesion molecules.

An estradiol-induced reduction in MCP-1 has been observed in human studies (*8*, *27*, *28*). Estradiol has been found to dose-dependently down-regulate MCP-1 mRNA expression in human coronary artery endothelial cells (*28*). Our previous study showed that transdermal HRT decreases the serum MCP-1 levels of postmenopausal women (*15*). This finding is compatible with the results obtained in the present study. Estrogen causes an increase in nitric oxide (NO) production in vessel walls (*29*), and the NO, in turn, inhibits MCP-1 expression in vascular endothelial cells (*30*). Thus, it is possible that estrogen indirectly inhibits the expression of MCP-1 by increasing NO production, and transdermal HRT may exert an antiatherosclerotic effect by reducing MCP-1 levels.

Transdermal HRT has been reported to have little or no effect on lipid metabolism (*31–33*), consistent with the findings in our study showing that serum lipids did not change significantly after transdermal HRT. Because transdermal HRT stimulates the liver less, it may have less of an effect on lipid metabolism than oral HRT.

This study has several limitations that should be mentioned. The study was not designed as a double-blind randomized placebo controlled trial, and the number of subjects was relatively small. Also, the study may have had a bias in that the subjects who consented to receive HRT were generally more health-conscious than those who did not, and thus the former may have received an additional health benefit beyond the efficacy of HRT.

In conclusion, transdermal HRT was here shown to reduce carotid artery wall thickness, and this reduction may have been induced by an antiatherosclerotic effect combined with the direct effect of estrogen and decreased levels of estrogen-induced E-selectin.

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