CLINICAL STUDY

Effects of oral and transdermal estrogen therapies on circulating cytokines and chemokines in postmenopausal women with hysterectomy

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Abstract

Objective: The aim of the present study was to determine the different effects of oral estrogen therapy (ET) and transdermal ET on changes in circulating levels of cytokines and chemokines in relationship to changes in markers of inflammation in postmenopausal women with hysterectomy.

Methods: Fifty-five postmenopausal women with hysterectomy were randomly assigned in open, parallel-group fashion to an oral ET group and a transdermal ET group. Serum levels of cytokines and chemokines were simultaneously measured using a multiplexed human cytokine assay. Serum concentrations of high-sensitive C-reactive protein, soluble vascular cell adhesion molecule-1, soluble intercellular adhesion molecule-1, and E-selectin were measured as vascular inflammation markers.

Results: Both oral ET and transdermal ET significantly decreased serum interleukin (IL)-7 concentrations at 12 months ($P=0.020$ and $P=0.015$ respectively). Transdermal ET decreased serum concentrations of IL-8, monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1 β ($P=0.05$, $P=0.019$, and $P=0.029$), but oral ET increased IL-8 level ($P=0.025$). There were significant differences in percentage changes in IL-8 and MIP-1 β between the oral and transdermal ET groups. Oral ET significantly decreased E-selectin level after 12 months.

Conclusion: Transdermal ET reduces circulating levels of IL-8, MCP-1, and MIP-1 β , while both oral ET and transdermal ET reduce circulating level of IL-7.

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Introduction

The effects of hormone therapy (HT) on C-reactive protein (CRP) and cell adhesion molecules associated with development of atherosclerosis have been shown to be different depending on the route of estrogen administration (1–5). Cytokines and chemokines, such as interleukin (IL)-6, IL-1 β , tumor necrosis factor- α , IL-10, and monocyte chemoattractant protein (MCP)-1, related to the development of atherosclerosis have been reported to be changed by HT (1–8).

Recently, other cytokines and chemokines as factors involved in the development of atherosclerosis have been investigated. IL-7 has been reported to be involved in the first step of adhesion of leukocytes to endothelial cells (9, 10), and its receptor has been reported to be present in human microvascular endothelial cells (11). Circulating IL-8 level has been shown to be high in patients with

atherosclerosis (12). Macrophage inflammatory protein (MIP)-1 β has been reported to be expressed by T cells in advanced atherosclerotic lesions (13). The effects of HT on these cytokines and chemokines and the differences in these cytokines and chemokines between women receiving oral HT and women receiving transdermal HT have not been demonstrated.

In addition, results of studies regarding the action of progestogen in an HT regimen on vascular inflammation have been inconsistent (14, 15). The effects of estrogen therapy (ET) and estrogen–progestogen therapy on cytokines and chemokines may be different.

The aim of the present study was to determine the different effects of oral ET and transdermal ET on changes in circulating levels of cytokines and chemokines in relationship to changes in markers of inflammation in postmenopausal women who have undergone hysterectomy.

Subjects and methods

Subjects

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. Fifty-five postmenopausal women who had undergone hysterectomy were enrolled in this study between January 2006 and December 2007. All subjects were premenopausal women at the time of hysterectomy. Postmenopausal status was confirmed by FSH concentration ≥ 40 mIU/ml and estradiol (E_2) concentration ≤ 20 pg/ml.

Before recruitment in the study, patients underwent gynecological and biochemical examinations that included bimanual examination and transvaginal ultrasonography. Reviews of medical histories and the results of physical examinations and blood chemistry tests showed that all of the women were in good health. Exclusion criteria in the study were a history of any cardiovascular disease, hormone-dependent malignancy or breast cancer, venous thromboembolic disease, diabetes mellitus, renal dysfunction, liver disease, hypertension, and use of lipid-lowering drugs. Women who had received HT in the past were not included in the study. None of the subjects had taken any medication known to influence the immune system for at least 1 year. Subjects suspected of having infectious diseases, inflammatory disorders, malignancy or autoimmune diseases, of being undernourished, or of abusing alcohol or drugs were excluded according to the SENIEUR protocol (16). The SENIEUR protocol provides the structural basis for analyses of aging in a well-defined healthy population and strict criteria for human immunogerontologic studies.

Fifty-five postmenopausal women who had undergone hysterectomy were randomly assigned to one of two groups by registration number of the hospital for the duration of the 12-month study: a continuous oral-conjugated equine estrogen (CEE) therapy group (0.625 mg/day; $n=27$) and a continuous transdermal E_2 therapy group (50 μ g of E_2 released in 24 h by a transdermal patch; $n=28$). The patients who visited our hospital on odd-numbered days were treated with oral CEE, and those who visited our hospital on even-numbered days were treated with transdermal E_2 . We assessed compliance by pill count and sheet count. In addition, we assessed the status of hot flashes. Venous blood samples were drawn into tubes between 0800 and 1000 h after 12-h fasting before and at 12 months after commencement. 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.

Serum E_2 concentration was measured by a two-site immunoassay using a commercially available kit (TOSOH Co., Tokyo, Japan). The intra- and inter-assay coefficients of variation (CV) were 4–9 and 6–9% respectively. Serum FSH concentration was measured by an IRMA using a commercially available kit (TFB Co., Tokyo, Japan). The intra- and inter-assay CV were 3–4 and 3–4% respectively.

The concentration of high-sensitive C-reactive protein (hsCRP) was measured by the Behring Latex-Enhanced CRP assay using a Behring Nephelometer Analyzer System (Dade Behring, Tokyo, Japan). Assays for soluble vascular cell adhesion molecule (sVCAM)-1, soluble intercellular adhesion molecule (sICAM)-1, and E selectin were carried out using ELISA kits (R&D Systems, Minneapolis, MN, USA). The intra- and inter-assay CV were 0.9–1.7 and 2.3–3.0% for CRP, 2.2–2.8 and 6.5–9.5% for sVCAM, 6.5–7.9 and 7.6–11.6% for sICAM, and 3.3–4.9 and 3.1–4.8% for E-selectin respectively. All assays were carried out in duplicate.

Serum concentrations of seven cytokines and chemokines (IL-5, IL-6, IL-7, IL-8, IL-10, MIP-1 β , and MCP-1) were measured using a Bio-Plex human cytokine assay kit (Bio-Rad Laboratories) as previously reported (17). Assays of cytokines and chemokines were carried out in duplicate using a Bio-Plex human cytokine assay kit. The intra- and inter-assay CVs were 2.0–10.0 and 3.5–16.1% respectively. Intra-assay CV was calculated from eight samples within a single plate. Inter-assay CV was calculated from five samples each from three plates. The sensitivity levels were 1.1 pg/ml for IL-6 and MIP-1 β , 0.5 pg/ml for IL-7 and IL-8, 0.8 pg/ml for IL-5, 0.9 pg/ml for IL-10, and 6.7 pg/ml for MCP-1.

Statistical analysis

Based on the results of a previous study (3), sample size was estimated to detect at least 20% change in levels of cytokines and chemokines after administration with 80% power at the 0.05 level of significance. We defined the values below the detection limit as half of the detection limit in further analyses. Differences between oral and transdermal ET groups in subject characteristics and baseline serum concentrations of hormones were analyzed by an unpaired *t*-test, and values are presented as means $\pm$ S.D.s. Baseline serum levels of inflammatory markers, cytokines, and chemokines, which were not normally distributed, are presented as medians with 25th and 75th percentile ranges, and significance of those values was evaluated by the non-parametric Wilcoxon rank-sum test. Baseline and follow-up levels of inflammatory markers and cytokines were compared across the same therapy by the non-parametric Wilcoxon signed-rank test. The percentage changes from baseline levels in the oral and transdermal ET groups were compared by the non-parametric Wilcoxon rank-sum test. The relationship among continuous variables was determined by using

Spearman's rank-order analysis. *P* values <0.05 were considered to be statistically significant. The SAS software package (version 8.2, SAS Institute Inc., Cary, NC, USA) was used for both data management and analysis.

Results

General characteristics

Out of the 55 women, 53 who originally enrolled completed the 12-month study. Two women who received transdermal E₂ dropped out of the study at 2 months because of skin irritation. Baseline characteristics according to assignment are presented in Table 1. There were no significant differences between the two groups in age, body mass index (BMI), and serum concentrations of FSH and E₂.

Changes in serum concentrations of cytokines and chemokines

Baseline levels of cytokines and chemokines were not significantly different between the two groups. As can be seen in Table 2 and Fig. 1, serum IL-7 concentrations in the oral and transdermal ET groups were decreased significantly at 12 months ($P=0.020$ and $P=0.015$ respectively). There was no significant difference in percentage changes in IL-7 between the oral and transdermal ET groups. Serum IL-8 level at 12 months was significantly ($P=0.025$) increased in the oral ET group, but was decreased ($P=0.05$) in the transdermal ET group (Table 2 and Fig. 1). Serum MIP-1 β level at 12 months was significantly ($P=0.029$) decreased in the transdermal ET group, but tended to be increased ($P=0.069$) in the oral ET group. There were significant differences ($P=0.008$ and $P=0.017$ respectively) in percentage changes in IL-8 and MIP-1 β between the oral and transdermal groups. Serum MCP-1 level at 12 months was significantly ($P=0.019$) decreased in the transdermal ET group, but there was no significant change in the oral ET group. IL-6 level tended to increase ($P=0.052$) in the oral ET group, but remained unchanged throughout the study in the transdermal ET group.

Table 1 Baseline characteristics of postmenopausal women receiving oral and transdermal estrogen therapies.

	Oral	Transdermal	<i>P</i> value
Number	27	28	
Age (years)	50.8 (5.0)	50.0 (6.5)	0.606
Years since hysterectomy	5.4 (3.5)	4.3 (3.7)	0.224
BMI (kg/m ²)	22.0 (2.7)	22.9 (3.4)	0.243
FSH (mIU/ml)	103.3 (48.1)	90.2 (32.9)	0.244
Estradiol (pg/ml)	13.4 (4.0)	14.8 (5.0)	0.317

Values are mean $\pm$ (s.d.s). BMI, body mass index. FSH, follicle-stimulating hormone

Table 2 Serum cytokine concentrations at baseline and after 12 months and percentage changes from baseline levels in postmenopausal women receiving oral and transdermal estrogen therapies.

	Oral			Transdermal					
	Baseline	12 months	Percentage changes (%)	P value (baseline versus 12 months)	Baseline	12 months	Percentage changes (%)	P value (baseline versus 12 months)	P value (oral versus transdermal)
L-5 (pg/ml)	0.41 (0.40–0.93)	0.40 (0.40–0.55)	0	0.232	0.42 (0.40–0.66)	0.40 (0.40–0.72)	0	0.247	0.30
L-6 (pg/ml)	2.80 (1.40–13)	8.00 (3.01–15.8)	+69.0	0.052	1.51 (0.55–6.86)	2.29 (0.55–4.99)	–8.6	0.218	0.28
L-7 (pg/ml)	5.36 (3.13–11.8)	4.90 (2.21–7.00)	–19.4	0.020	4.90 (3.25–6.50)	3.77 (1.84–4.80)	–22.3	0.015	0.08
L-8 (pg/ml)	32.3 (17.0–44.7)	84.1 (39.3–211.1)	+111.0	0.025	44.2 (16.7–140.9)	25.0 (8.08–111.0)	–23.1	0.050	0.008
L-10 (pg/ml)	0.49 (0.45–1.20)	0.45 (0.45–1.05)	0	0.595	0.45 (0.45–1.02)	0.45 (0.45–0.69)	0	0.167	0.11
MCP-1 (pg/ml)	63.5 (55.0–93.0)	59.0 (48.2–81.3)	+1.7	0.259	61.0 (34.6–100.9)	52.1 (40.0–79.0)	–12.7	0.019	0.89
MIP-1β (pg/ml)	186.0 (158.9–299.0)	258.2 (213.4–408.8)	+50.6	0.069	196.6 (119.0–291.8)	172.0 (126.0–202.0)	–7.2	0.029	0.017

Values are medians (25–75 percentiles). Percentage changes are medians. IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MIP-1 β , macrophage inflammatory protein-1 β .

Table 4 IL-8 levels at baseline and after 12 months in postmenopausal women with and without hot flashes receiving oral and transdermal estrogen therapies.

	Oral		P value (baseline versus 12 months)	Transdermal		P value (baseline versus 12 months)
	Baseline	12 months		Baseline	12 months	
Women with hot flashes	100.2 (33.1–121.0)	69.1 (38.0–107.7)	0.546	130.8 (26.8–158.3)	18.0 (9.6–113.3)	0.015
Women without hot flashes	16.5 (13.0–22.4)	120.8 (76.7–302.0)	0.011	17.3 (7.7–56.7)	33.0 (6.8–103.9)	0.235

Values are medians (25–75 percentiles).

and at 12 months after treatment in women with hot flashes, and serum IL-8 level in women without hot flashes was significantly increased at 12 months after treatment.

Discussion

Both treatments with oral estrogen and transdermal estrogen decreased serum IL-7 levels after 12 months of therapy. IL-7 is a pleiotropic non-redundant cytokine for the development and homeostatic maintenance of T and B lymphocytes, and its receptor has been reported to be present in human microvascular endothelial cells (11). It has been demonstrated that IL-7 induced the binding of unstimulated T cells to vascular cell adhesion molecules (9), and that IL-7 stimulated the expression of adhesion molecules on the cell surface in a murine endothelial cell line (10). Recently, we have showed that circulating IL-7 level was reduced by administration of a selective estrogen receptor modulator (18). Therefore, estrogen may inhibit the induction of adhesion molecules due to suppression of the action of IL-7 via the estrogen receptor. Among cell adhesion molecules, we showed that oral ET significantly reduced E-selectin level, but that E-selectin level in the transdermal ET group did not change. Our results regarding the change in E-selectin caused by oral ET are consistent with the results of previous studies (19, 20). The inhibitory effect of oral estrogen on the secretion of E-selectin through IL-7 might be stronger than that of transdermal estrogen.

In the present study, we confirmed that serum IL-8 concentration in women with hot flashes was higher than that in women without hot flashes as shown in our previous study (21). In addition, we showed that oral ET increased IL-8 level, but that transdermal ET decreased IL-8 level. In our previous study, we found that elevated level of cytokine-induced neutrophil chemoattractant, a member of the IL-8 family, was reduced by E₂ and that elevated skin temperature in ovariectomized rats was reduced by treatment with injection of a luteinizing hormone-releasing hormone agonist (22). This result observed in rats was also observed in women with hot flashes in the transdermal

group in the present study. ET may reduce IL-8 level in order to modulate the homeostasis of body temperature in postmenopausal women. However, IL-8 level did not change significantly in women with hot flashes in the oral ET group. Estrogen administered orally is absorbed in the gut and moves to the liver. IL-8 has been reported to be produced in liver and bile ductular epithelial cells (23, 24). Therefore, production of IL-8 may have been increased due to action on the liver and bile duct through a first-pass hepatic effect in the oral ET group. On the other hand, it has been reported that IL-8 was induced by low shear stress in endothelial cells (25), and that the amount of IL-8 produced in visceral adipose tissue was greater than that in s.c. adipose tissue (26). The increase in IL-8 in the oral ET group may reflect the production from vascular endothelial cells and adipose tissue.

Results of previous studies showed that IL-6 level was not changed significantly (4, 27) and was increased (6, 20) by oral ET. In the present study, oral ET tended to increase IL-6 level, but IL-6 in the transdermal ET group did not change. It has been reported that IL-6 is synthesized by Kupffer cells in the liver (28). Therefore, increase in IL-6 by oral ET might be due to first-pass hepatic metabolism. On the other hand, it has been reported that production of IL-6 in blood mononuclear cells and IL-6 level in serum were decreased by transdermal ET (29, 30). IL-6 is involved in not only inflammation but also regulation of endocrine and metabolic functions, and it is produced and released from the adrenal gland by stimulation with corticotropin (31) as well as monocytes, T lymphocytes, endothelial cells, and adipocytes. Therefore, a possible reason for the finding of no change in IL-6 level in the transdermal ET group is that the production of IL-6 was induced in various tissues in response to estrogen.

It has been shown that E₂ inhibited the production of MCP-1 protein in human coronary artery smooth muscle cells (32), and that E₂ treatment reduced mRNA expression of *Mcp-1* in injured arteries of ovariectomized rats (33). In the present study, only transdermal ET significantly decreased MCP-1 level. It has been reported that the expression of MCP-1 was enhanced in endothelial cells (34) and that MCP-1 level was significantly correlated with maximum

intima-media thickness, which was associated with severity of atherosclerosis in coronary arteries (35). Transdermal ET may have a favorable effect on endothelial function due to reduction in MCP-1 level. Decrease in MCP-1 by estrogen might have been masked by first-pass hepatic metabolism in the oral ET group.

Serum MIP-1 β level at 12 months was significantly decreased in the transdermal group, but tended to be increased in the oral ET group. It has been reported that MIP-1 β was expressed in advanced atherosclerotic lesions. Therefore, transdermal ET may have a favorable effect on the development of atherosclerosis.

We demonstrated that CRP levels were not significantly different between the oral and transdermal ET groups, while oral ET but not transdermal ET has been shown to raise the level of CRP in postmenopausal women (1, 4, 19, 20). The differences in the changes in cytokines and inflammatory markers between the oral and transdermal ET groups may be due to differences in background characteristics of the subjects. The subjects in the present study were relatively young and their BMIs were relatively low compared to those in other studies. The small number of subjects and differences in the metabolism in the liver might also be relevant.

In the present study, we recruited postmenopausal women who had undergone hysterectomy in order to determine the effects of only ET. Progestogen is needed in order to prevent endometrial hyperplasia in women with an intact uterus. Estrogen–progestogen therapy may have different effects on cytokines and chemokines in postmenopausal women. Therefore, further study for assessment of cytokines and chemokines in women receiving estrogen–progestogen therapy is needed.

There are several limitations to this study. First, we compared the levels of cytokines and chemokines and the levels of inflammatory markers between women receiving oral CEE and women receiving transdermal E₂ at conventional doses, but different changes in circulating cytokines and chemokines may be observed with different doses of estrogen. Secondly, CEE contains at least ten natural estrogens, including equine, estrone, and E₂, while transdermal E₂ contains only E₂ (36). Further studies are needed.

Taking into consideration our results regarding cytokines and chemokines in women receiving oral ET and women receiving transdermal ET, estrogen administered transdermally may have favorable effects on vascular inflammation in relationship to changes in cytokines and chemokines in postmenopausal women.

In conclusion, transdermal ET reduces circulating levels of IL-8, MCP-1, and MIP-1 β , while both oral ET and transdermal ET reduce the circulating level of IL-7. A direct comparison of the effects of oral administration and transdermal administration is, however, not possible, given the differences in the two preparations as discussed above.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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