ORIGINAL ARTICLE

Beneficial influence of postmenopausal estrogen therapy on serum adhesion molecules is independent of the route and dose of administration

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Submitted: 2010-09-05 *Accepted: 2011-04-25* *Published online: 2011-06-29*

Key words: **adhesion molecules; combined estrogen-progestin therapy; menopause**

Neuroendocrinol Lett 2011; **32**(3):340–344 **PMID:** 21670727 NEL320311A03

Abstract

OBJECTIVE: In current literature, the immune-inflammatory theory of atherosclerosis is widely discussed. The role of how adhesion molecules contribute to the development of atheromatic plaques is especially underlined.

MATERIAL AND METHODS: 120 females in menopausal age were included in the study between 2004 and 2009. All the women were of menopausal age (51±3 years), from southern Poland, with FSH levels above 30 mIU/ml, and complaining of menopausal symptoms that disturbed normal daily activity. The study was conducted over a 6 month period. Three groups of 40 randomized patients were selected. The control group consisted of 40 volunteers, who were also from southern Poland, in good health, without menopausal symptoms, or menstrual periods in the last 6 months. Control subjects were match according to age and weight, with FSH levels above 25 mIU/ml and normal TSH and prolactin values. All patients, in the treatment and control groups were seronegative for Chlamydia pneumonia throughout the duration of the study.

RESULTS: After 6 months, hormonal therapy was found to significantly reduce levels of sICAM-1 and sVCAM-1 in all treated groups compared to the control group and the results were statistically significant. Alternatively, in the latter group, we observed increased levels of the investigated adhesion molecules (group I: 37.5 μg/24h transdermal estradiol + dydrogesteron; group II 50 μg/24h transdermal estradiol + medroxyprogesteron; group III 1mg of oral estradiol + noretisteron sICAM-1 and control group; using paired Wilcoxon test).

CONCLUSION: All of the investigated estrogen therapy schemas have a favorable impact on the blood levels of sICAM-1 and sVCAM-1 in postmenopausal women without cardiovascular risk factors, reducing their concentration.

Abbreviations:

BMI	- body mass index
CAM	- cell adhesion molecules
E2	- estradiol
FSH	- follicle stimulating hormone
HDL	- high density lipid
hsCRP	- high sensitive C-reactive protein
Ig	- immunoglobulin
IL	- interleukin
LDL	- low density lipid
PRL	- prolactin
sICAM-1	- intercellular adhesion molecule-1
sVCAM-1	- vascular cell adhesion molecule-1
TNF	- tumor necrosis factor
TSH	- thyroid stimulating hormone
WBC	- leucocytes
WHR	- waist and hipline ratio

INTRODUCTION

Certain inflammatory and immune markers in peri- and postmenopausal women play an important prognostic role in modern medicine. An especially interesting issue is the possible impact of postmenopausal declines in sex hormone production and medication therapy on a vessel's immune-inflammatory condition (Rossouw *et al.* 2002). Increased levels of inflammatory-immune markers are signs of chronic immune-inflammatory processes found in: atherosclerosis, arterial hypertension, obesity, diabetes mellitus, and others.

In current literature, the inflammatory theory of atherosclerosis is widely discussed. Markers associated with atherosclerotic vascular changes include: high sensitive C-reactive protein (hsCRP), interleukin (IL) 1β, IL 6, IL 8, tumor necrosis factor α (TNFα), leucocytes, along with cells adhesion molecules (VCAM-1 – vascular cell adhesion molecule-1, ICAM-1 – intercellular adhesion molecule-1), fibrinogen, amyloid A protein and others. Data demonstrates that circulating inflammatory products are chronic bacterial and atherosclerosis markers of inflammation, but furthermore they damage epithelium and directly facilitate the development of atherosclerosis (Danesh *et al.*1997; De Backer *et al.* 2002; Dechend *et al.* 1999).

The role of adhesion molecules in the development of the atheromatic plaque is especially emphasized. These particles function as intracellular molecules and signaling intercellular proteins, and have the ability to initiate cytokine secretion and adhesion molecule expression in a cell. Cell adhesion molecules (CAMs) are involved in a vast away of processes including embryonic development, tissue building, inflammatory reactions, wound healing and metastasis. CAMs allow cells to interact with their environment and other cells; they determine the location of a cell in a tissue or organ. Adhesion molecules are divided into several categories: selectins, integrins, adhesins and a superfamily of immunoglobulins CAM (IgCAM) that includes: ICAM-1 and VCAM-1(Ptak & Ptak 1999). These adhesion molecules are released from damaged tissues due to shear forces of stress, subsequently reparatible the migration of inflammatory cells, within toward the site of injury and ultimately triggering the vessel wall.

Combining knowledge about the beneficial influence of estrogen on healthy endothelial cells, along with the inflammatory theory of atherosclerosis, we collected data which illustrate the influence of three different forms estrogen-progestin therapy on the bahaviour of serum ICAM-1 and VCAM-1 in healthy postmenopausal women with climacteric symptoms, and compared these results to the control group.

MATERIAL AND METHODS

Series description, inclusion criteria

The study was carried out with 120 women, who had been hospitalized or treated in the outpatient clinic at the Endocrinological Gynecology Department, at Jagiellonian University, between 2004 and 2009. All patients were of menopausal age (51±3 years), from southern Poland, with FSH levels above 30 mIU/ml, and complaining of menopausal symptoms that disturbed their normal daily activities. Each patient fulfilled the criteria for hormonal therapy as specified by the Standards of Polish Gynecological Society. The study was conducted over a period of 6 months, during which time no lifestyle changes were noted, and investigators observed whether quality of life improved (measured by Green Scale) and how changed the level of exam markers. No other drug use was reported, especially for chronic illnesses. Three groups of 40 randomized patients were selected. In the first group we introduced transdermal estrogen therapy in a 37.5 μg/24h dose combined with a 10 mg dose of dydrogesteron. In the second group we introduced transdermal estrogen therapy in a 50 μg/24h dose with 2.5 mg of oral medroxyprogesteron. In both these groups gestagens were administered continuously. In the third group we introduced continuous, oral, low-dose combined estrogen-gestagen therapy with 1mg of ethynyloestradiol and 0.5mg of noretisteron acetate.

Group I (n=20): 37.5 μg/24h of transdermal 17-β-estradiol, in combination with 10mg of dydrogesteron (commercial products: Oesclim 37.5 and Duphaston – tablets), abbreviation: 37.5+D

Group II (n=20): 50 μg/24h of transdermal 17-β-estradiol, in combination with 2.5 mg of medroxyprogesteron (commercial products: Oesclim 50 and Provera 5 mg – half tablet), abbreviation: 50+MPA

Group III (n=20): 1mg of 17-β-estradiol in combination with 0.5 mg noretisteron acetate as a continuous oral therapy (commercial product: Activelle), abbreviation: Activelle

Selection of above therapy schemas was suitable. Cho's *in vivo* data has shown that oral gestagen therapy enables smooth muscle relaxation by slackening calcium channels, independent of estrogen (Cho 2000).

The control group included 40 healthy volunteers from southern Poland, without chronic diseases, menopausal symptoms, or menstrual periods in the last 6 months. These subject were matched for height and weight, with FSH levels above 25 mIU/ml and normal TSH and prolactin values. All patients, in the treatment and control groups, were seronegative for Chlamydia pneumonia throughout the duration of the experiment.

Exclusion criteria.

In our study, candidates meeting the following criteria were excluded: taking hormone replacement therapy at the time of evaluation (medical history), suffering form unexplained uterine bleeding, reporting a history of endometriosis (transvaginal ultrasound), or uterine myoma(s), demonstrating arterial hypertension (daily blood pressure records – systolic pressure less then 140 mmHg and diastolic pressure less then 90 mmHg were accepted as normal), suffering from coronary artery disease (echocardiogram, ECG, cardiac stress test), serum level of triglycerides greater than 400 mg/dl, with diabetes mellitus (oral glucose tolerance test with 75 g of glucose), with an active or chronic inflammatory process (medical history, C-reactive protein >10 ng/dl, WBC>10 000/mm^3), autoimmune diseases, liver dysfunctions (liver function blood tests), or kidneys diseases (sodium, potassium, creatinine or blood urea level out of norm), reporting neoplasms in their medical history, thrombophlebitis or venous thromboembolism (medical history), suffered from cholelithiasis, but not after cholecystectomy (medical history), reporting seizures in the course of a disease including epilepsy (medical history), complaining of migraine (medical history), overweight or obese (BMI score equal to or more than 25 and with waist – hip ratio equal to or more than 0.78).

Smoking and alcohol consumption were also taken into consideration and excluded the patients from the study.

The following tests were performed on each patient:

- a detailed medical history including arterial hypertension, diabetes mellitus, smoking, inflammatory conditions, liver and kidneys diseases, neoplasms, and present pharmacological treatment
- physical examination with hemodynamic evaluation
- blood pressure measurement
- anthropometric measurements including body weight and height (BMI), and waist and hipline ratio (WHR)
- biochemical tests including morphology, hormone levels (FSH, E2, TSH, PRL), total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides
- serum adhesion molecules level (VCAM-1, ICAM-1)
- IgA antibodies against *Chlamydia pneumoniae*

All patients were informed about the medical study and joined willingly, giving their written consent. Our data has agreement from the Ethics Committe of Jagiellonian University.

Serum samples were collected and stored at –70 °C degree. All samples were tested at the same time. All hormones were determined by immunoassay "ECLIA". Total cholesterol and high density lipoprotein (HDL) levels were determined by enzymatic and colorimetric methods. Plasma triglyceride levels were determined with an enzymatic commercial kit (Abbot). Low density lipoprotein (LDL) levels were estimated according to the Friedwald equation. Serum vascular cell adhesion molecule-1 and serum intercellular adhesion molecule-1 were determined by an immunoenzymatic method using a commercial kit (R and D System, England). IgA antibodies against *Chlamydia pneumoniae* were also examined using an immunoenzymatic method (ANI Labsystem, Finland).

Statistial analysis

In the analysis conducted, nonparametric tests were used. Analysed parameters in treated groups were compared using the Mann-Whitney U test. Using the Wilcoxon test, the obtained results in examination 1 (before administering treatment) and examination 2 (six months after initiation of hormonal therapy) in each study group were compared. The results were analysed using the Kruskal-Wallis test (ANOVA), and in cases where significant changes were found between groups, a multiple comparison test was applied to detect the differences. R-Pearson's linear correlation ratio was exerted to estimate correlations between analyzed parameters and changes in these factors. The result $p<0.05$ was statistically significant. Lack of statistical significance was indicated by the abbreviation NS (non-statistically significant).

Results were shown in the form of tables and figures. Calculations and analysis were conducted by using STATISTICA 7.1.packet.

RESULTS

Characteristic of the study groups

The women in our study groups do not differ significantly based on age, anthropometric measurements, hormone levels (E2, FSH, PRL, and TSH), total cholesterol, LDL- and HDL-cholesterol and triglycerides.

There was no statistically significant difference in the blood level of VCAM-1 and ICAM-1 in the study groups.

The results for the 120 participants in the study and control group are shown in Tables 1,2 and Figures 1,2.

The sICAM Kruskal-Wallis test (ANOVA) (Figure 1) demonstrated statistically significant differences ($p<0.001$) between changes in results in control and study groups. To izolate these differences, a multiple comparisons test was performed, subsequently failing

Tab. 1. Level of sICAM-1 in examination 1 (before therapy) and examination 2 (six months after starting hormonal therapy) in examined groups.

Study groups	Test	sICAM-1 (ng/ml)					*p*-value
		x	SD	min	Me	max	
Activelle	1	266.8	73.3	176.4	277.6	378.0	0.005
	2	209.1	67.9	114.2	206.8	322.7	
50+MPA	1	256.1	43.3	200.5	244.7	324.1	0.005
	2	195.3	40.6	123.1	193.8	260.9	
37.5+D	1	280.8	58.6	197.0	272.8	355.1	0.005
	2	219.8	50.3	147.2	209.2	300.3	
Control group	1	305.8	67.3	190.6	301.5	387.9	0.009
	2	391.3	58.6	312.6	382.9	499.4	

Tab. 2. Level of sVCAM-1 in examination 1 (before therapy) and examination 2 (six months after starting hormonal therapy) in examined groups.

Study groups	Test	sVCAM-1 (ng/ml)					*p*-value
		x	SD	min	Me	max	
Activelle	1	599.2	199.2	254.8	555.3	987.5	0.005
	2	535.1	193.0	200.7	497.7	902.1	
50+MPA	1	669.7	108.0	587.3	632.1	953.8	0.05
	2	575.1	94.6	499.0	539.0	812.2	
37.5+D	1	718.9	164.2	499.1	734.7	932.1	0.007
	2	590.9	154.5	409.5	526.5	855.2	
Control group	1	688.6	159.9	499.2	678.5	987.3	0.05
	2	836.1	162.2	576.8	854.7	1099.5	

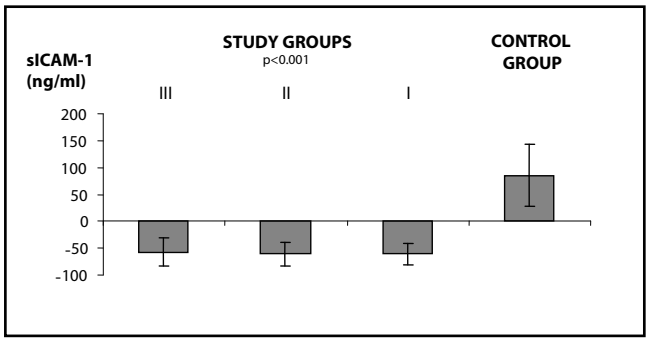

Fig. 1. Comparison of changes in levels of sICAM-1 between study groups in examined population.

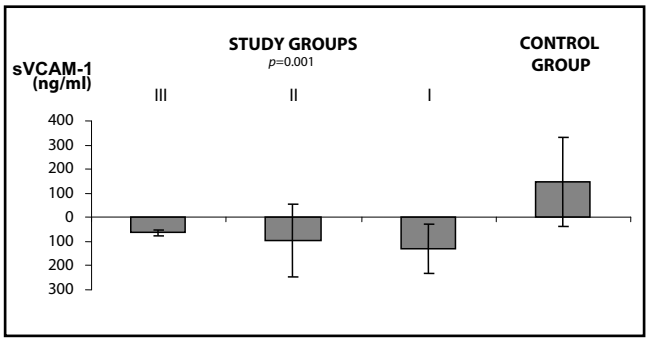

Fig. 2. The comparison of changes in levels of sVCAM-1 between study groups in examined population.

to detect any statistically significant differences. Clinically, this indicates that each dose of estrogen, made of administration, and type of oral gestagen has a positive effect on sICAM-1 levels. Analogical observation was done due to sVCAM-1 (Figure 2), but no statistically significant differences were detected in the control group after six months.

DISCUSSION

In current literature, the beneficial influence of hormonal therapy on bahaviour of sICAM-1 and sVCAM-1 is widely discussed.

The pathomechanism of this observation is still unclear, but there are many suggestions, including the theory that estrogen represses the expression of adhesion molecules' genes (Goudev *et al.* 2002; Lamon-Fava *et al.* 2003; Mueck *et al.* 2007; Ridker *et al.* 1998; Shifren *et al.* 2008). In our study, we proved that there is a beneficial effect on sICAM-1 and sVCAM-1 after hormonal treatment, as compared to the control group. However, we would like to emphasize that there is no statistically significant difference between the discussed groups, according to dose of estrogen, made of administration , or the type of gestagen given.

In our own data, a linear correlation was demonstrated between changes of sICAM-1, sVCAM-1 (r=0.6045, $p<0.001$; r=0.4434, $p=0.004$) and changes of FSH. An inversely proportional correlation between changes of sICAM-1, sVCAM-1, and changes of estradiol level was also detected (r=–0.64, $p<0.001$; r=–0.31, $p=0.049$).

Regression summary for sVCAM-1 and sICAM-1 reveals further information. A strong proportional correlation exists between adhesive molecules and fibrinogen, with estrogen mediating the relationships, despite a lack of its exact pathomechanism.

Av inverse correlation exists between sICAM-1, sVCAM-1 and antibodies IgA against Chlamydia pneumonia, further implicating the proportional dependence of this bacteria on estrogen. Literature has shown estrogens ability to stimulate the immune response and ward off infections potentially contributing to atherosclerosis. In world literature there are dates which present that estrogen may stimulate immune response to weak infections that causes that atherosclerosis develops slower (Goudev *et al.* 2002). Our data demonstrated a proportional correlation between estrogen and *Chlamydia pneumoniae* IgA antibodies (higher levels of estrogen corresponds higher levels of antibod-

ies of Chlamydia pneumonia which are still seronegative). It is worth noting that although increased level of antibodies IgA class of *Chlamydia pneumoniae* were observed, the results were negative for the entire duration of the experiment.

In conclusion, each of the investigated estrogen therapy schemas had a favorable impact on the blood level of sICAM-1 and sVCAM-1 in postmenopausal women without cardiovascular risk factors. The study strongly underline that estrogen application healthy women aged 50–59 prolong their life in good condition what is the main device of antiaging medicine.

An essential element in the prevention of endothelial dysfunction is to identify new biochemical or immunological markers including adhesive molecules. Adhesive molecules are markers of the formation and progression of endothelial dysfunction, but their clinical role warrants further investigation.

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