The effects of hormone therapy on ischemia modified albumin and soluble CD40 ligand levels in obese surgical menopausal women

M.A. Osmanağaoğlu[1], S.C. Karahan[2], T. Aran[1], S. Güven[1], A. Cora[1], M. Kopuz[2], H. Bozkaya[1]

[1]Karadeniz Technical University, Faculty of Medicine, Department of Obstetrics and Gynecology, Trabzon
[2]Karadeniz Technical University, Faculty of Medicine, Department of Biochemistry, Trabzon (Turkey)

Summary

Purpose: To determine the effects of hormone therapy (HT) on ischemia modified albumin (IMA) and soluble (s)CD40 ligand in obese surgical menopausal women. *Materials and Methods:* A total of 52 obese surgical menopausal women with a body mass index (BMI) > 30 kg/m^2 were admitted to the study. Twenty-seven women received estradiol hemihydrate two mg and 25 did not receive any menopausal therapy. At baseline and after three and six months of treatment, IMA and sCD40 ligand levels were measured. *Results:* There were no significant differences among the groups for any variables at baseline. No difference in change in the serum sCD40L levels was found in obese surgical menopausal women after three and six months of HT. Serum IMA levels were statistically lowered in obese women with HT after six months of treatment. *Conclusion:* HT may have a beneficial reduction in IMA levels in obese surgical menopausal women.

Key words: Hormone therapy; sCD40 ligand; Ischemia modified albumin; Body mass index; Surgical menopause.

Introduction

Cardiovascular disease (CVD) is the leading cause of death and morbidity in women aged 45 years and older [1]. The incidence of CVD and stroke increase after menopause because of the menopause transition is associated with an increased body weight [2], physical inactivity, high blood pressure, diabetes, and high cholesterol. In women with surgical menopause and who do not take estrogen, their risk for heart disease may also be higher. However, according to the Women's Health Initiative (WHI), randomized controlled trial, HT or estrogen therapy are not indicated for the prevention of coronary artery disease (CAD). In addition, the Heart and Estrogen/progestin Replacement Study (HERS) [3] showed that there was no beneficial reduction of CAD incidence in postmenopausal women with CAD who received HT. On the other hand, the Nurses' Health Study demonstrated an approximately 11% risk reduction for primary CVD in postmenopausal women using HT compared with women who had never used HT, irrespective of duration of use [4].

The ligand for CD40 (CD40L) is a membrane glycoprotein on activated T cells that induces B cell proliferation and immunoglobulin secretion. Activated platelets express CD40L on their plasma membrane and release the soluble fragment sCD40L. The interaction between platelet surface CD40L and endothelial cell CD40 leads to the activation of endothelium contributing to atherothrombosis [5]. Increased plasma levels of soluble CD40 ligand have been related with increased risk of unstable angina, myocardial infarction (MI), diabetes, CAD and atherothrombotic events [6].

Revised manuscript accepted for publication September 1, 2012

Ischemia modified albumin (IMA) is a sensitive and early biochemical marker of ischemia that is produced when circulating serum albumin contacts ischemic heart tissues. Postmenopausal obesity is associated with elevated serum IMA possibly due to obesity associated oxidative stress. IMA measurement could provide an assessment of atherosclerotic burden in postmenopausal women [7].

In this study, the authors' aim was to determine the serum concentrations of IMA and soluble (s)CD40 ligand in surgical menopausal women on HT with a body mass index (BMI) > 30 kg/m^2.

Materials and Methods

After following the process of screening, 59 subjects were randomized to the treatment and to a control group receiving no therapy (calcium 500 mg) according to a computer-generated randomization table. Seven patients (three women in the HT receiving group and four women in the placebo group) did not complete the study. The reasons for non-completion included generalized discomfort following medication at six months (n = 1), failure of compliance (n = 6). Therefore a total of 52 women aged 40-58 years, who were obese (BMI > 30 kg/m^2) and who had undergone total abdominal hysterectomy with bilateral salpingo-oopherectomy (surgical menopause) for one month previously due to benign gynecologic conditions, were included into this prospective, controlled clinical study. The patients had previously taken HT or treatment for cholesterol within the past year, women with diabetes, cancer, liver, renal or hematological disease, smokers, or other medical disorders were excluded. In summary, 27 women received estradiol hemihydrate two mg, q.d. and 25 women were not on HT.

Subjects underwent testing at baseline and after three and six months of therapy. Height (cm) and weight (kg) were measured to calculate BMI as weight (kg)/height (m^2). The study protocol was approved by the Medical Ethics Committee of this University and a written informed consent was obtained from each patient who

participated in this study. This work was supported by the Research Fund of Karadeniz Technical University, project number: 2009.114.001.2

Biochemical analysis was performed in the Department of Clinical Biochemistry. After obtaining blood samples in plain tubes containing separation gels, the samples was allowed to clot for 30 minutes and centrifuged before separating the serum. The samples were then immediately frozen and stored at –80 °C for assays IMA.

Levels of human Serum sCD40L were determined by enzyme-linked immunosorbent assay kit, according to the manufacturer's protocols. The absorbance of samples was measured at 450 nm using a tunable microplate reader. The results were expressed as ng/ml.

Reduced cobalt to albumin binding capacity (IMA level) was analyzed using the rapid and colorimetric method of Bar-Or *et al.* [8]. Two hundred μl of rat serum were placed into glass tubes and 50 μl of 0.1% cobalt chloride ($CoCl_2.6H_2O$) in H_2O was added. After gentle shaking, the solution was left for ten minutes to ensure sufficient cobalt albumin binding. Fifty microliters (μl) of dithiothreitol (DTT) (1.5 mg/ml H_2O) was added as a colorizing agent, and the reaction was quenched two min later by adding 1.0 ml of 0.9% NaCl. A colorimetric control was prepared for preoperative and postoperative serum samples. In the colorimetric control samples, 50 μl of distilled water was substituted for 50 μl of 1.5 mg/ml DTT. Specimen absorbance was analyzed at 470 nm by a spectrophotometer. The color of the DTT-containing specimens was compared with that of the colorimetric control tubes. The results were reported as absorbance units (ABSUs).

Statistical methods

Statistical analyses were performed by using Statistical Package for Social Sciences version 13.0.1. Results are expressed as means ± SD. The data were assessed for normal distribution by using Kolmogorov-Smirnov test. Mann-Whitney U-test was used to compare the variables obtained from patient and controls groups. Analysis of changes from baseline to six months was carried out with the t-test for paired samples. An overall analysis used a classification cut-off $p \leq 0.05$.

Results

Subject characteristics, biochemical measurements at inclusion time, and after three and six months of HT of obese women with surgical menopause are shown in Table 1. There were no significant differences between the pre-treatment groups for any variables shown. Compared with the untreated control group, although a small decreased values of sCD40L were found in the HT group after three and six months, no significantly difference was noted ($p > 0.05$, $p > 0.05$, respectively).

After three months of treatment, with respect to the measurement of IMA, there was no significant changes between HT and control groups ($p > 0.05$) (Table 2). After six months of treatment, IMA level was significantly increased in the untreated control group ($p < 0.05$) (Table 2). After three and six months of treatment, platelet counts were significantly decreased in the HT group ($p < 0.01$, $p < 0.01$, respectively) (Table 2).

Discussion

Results of the present study showed that plasma CD40L levels were not statistically decreased in obese surgical menopausal women after three and six months of hormone therapy. On the contrary, serum IMA levels in obese patients with HT were statistically lower than those of obese women without HT after six months of treatment.

Table 1. — *Baseline characteristics and biochemical measurements of obese surgical menopausal women before HT*.*

	Women not on HT (n = 25)	Women on HT (n = 27)
Age (years)	48 ± 4.74	49 ± 4.24
Time since menopause (years)	5.3 ± 1.51	5.9 ± 6.04
BMI (kg/m²)	33 ± 4.07	32 ± 3.17
Glucose (mg/dl)	87 ± 1.01	86 ± 1.86
Total cholesterol (mg/dl)	203 ± 40.05	198 ± 57.29
Triglycerides (mg/dl)	149 ± 76.80	143 ± 55.02
HDL (mg/dl)	49 ± 11.56	48 ± 9.43
LDL (mg/dl)	137 ± 34.99	151 ± 35.73
IMA	0.43 ± 0.08	0.49 ± 0.22
Soluble CD40 ligand (ng/ml)	5.15 ± 3.49	5.66 ± 2.85
Platelet (x103/l)	255,200.00 ± 533,44.00	277,925.93 ± 42,051.18

Values are mean ± SD and %.
HT: hormone therapy. IMA: ischemia modified albumin.
* There were no significant differences among treatment groups for any variables shown. BMI: body mass index. HDL: high-density lipoprotein. LDL: low-density lipoprotein.

Central obesity is associated with unfavorable changes in CVD risk factors (increasing coagulation, decreasing fibrinolytic factors, and insulin resistance), and with increased platelet activation leading to the release of proinflammatory mediators. Menopause is associated with an increased risk of obesity and a shift to an abdominal fat distribution with associated increase of cardiovascular risk, oxidative stress with the alterations in the metabolic and endocrine status.

It was estimated that > 95% of the circulating sCD40L is derived from platelets [9]. Some studies have shown a positive correlation between sCD40L and platelet counts [10], but others have demonstrated no relationship [11]. A role of platelets in the evolutionary phase of the atherosclerotic plaque can be related to the adhesion of exposed subendothelium after endothelial injury and to the releasing vasoactive substances that induce smooth muscle cell migration and proliferation [12] and can promote foam cell formation even in the absence of hyperlipidemia [13]. Obesity is characterized by the presence of a prothrombotic state with a combination of increased thrombin generation [14], platelet hyperactivity, and decreased fibrinolysis [15] suggesting that platelet activation plays a central role to accelerate atherothrombosis by an interaction with central obesity. However there is no direct clinical evidence that platelets contribute to coronary atherosclerosis [16]. sCD40L levels > 3.71 ng/ml were associated with a 2.8-fold increase and sCD40L levels > 5.54 ng/ml were associated with a 10.62-fold increase in cardiovascular risk in a nested case-control study in healthy, middle-aged women [17]. Since this study population was limited by the small number of cases, lack of statistical power may have limited the probability to detect any relationship. Secondly, it remains unclear whether HT-associated increases or de-

Table 2. — *Body mass index and hematological measurements at inclusion time and after three and six months of treatment in obese surgical menopausal women.*

	Time (months)	Women not on HT (n = 25)	Women on HT (n = 27)	*p*			
				p_a	p_b	p_c	p_d
BMI (kg/m^2)	0	33 ± 4.07	32 ± 3.17				
	3	33 ± 4.08	32 ± 3.20	> 0.05	> 0.05	> 0.05	> 0.05
	6	33 ± 4.14	32 ± 3.33				
IMA	0	0.43 ± 0.08	0.49 ± 0.22	> 0.05	< 0.05*	> 0.05	> 0.05
	3	0.44 ± 0.07	0.46 ± 0.16				
	6	0.49 ± 0.12	0.41 ± 0.16				
Soluble CD40 ligand (ng/ml)	0	5.15 ± 3.49	5.66 ± 2.85	> 0.05	> 0.05	> 0.05	> 0.05
	3	5.90 ± 3.43	5.29 ± 3.46				
	6	6.41 ± 2.64	4.81 ± 3.80				
Platelet (x103/l)	0	255,200.00 ± 53,344.00	277,925.93 ± 42,051.18	> 0.05	> 0.05	< 0.01*	< 0.01*
	3	274,240.00 ± 99,594.71	248,370.37 ± 46,469.57				
	6	278,240.00 ± 103,724.50	240,962.96 ± 53,088.56				

Values are presented as mean ± SD. *: Significant. HT: hormone therapy. BMI: body mass index. IMA: ischemia modified albumin
p_a: month 3 in comparison with 0 in surgical menopausal women without HT
p_b: month 6 in comparison with 0 in surgical menopausal women without HT
p_c: month 3 in comparison with 0 in surgical menopausal women with estradiol hemihydrate two mg
p_d: month 6 in comparison with 0 in surgical menopausal women with estradiol hemihydrate two mg
Student's paired t-test used.

creases in sCD40L levels can account for the adverse cardiovascular effects of HT in future. sCD40L is expressed in both adipocytes and stromal adipose fraction of obese patients. Adipocyte CD40 is biologically active, inducing adipocytokine secretion mediated by T-cell adipocyte interaction and T-cell CD40L [18]. As this study population was limited to obese surgical women, the authors speculated that the statistically mean difference of sCD40L could be documented if the study sample had also included non-obese women. Further work is necessary to determine the exact mechanism of sCD40L on platelet activation and arterial thrombosis between the processes of thrombosis and inflammation in obesity.

The determination of serum IMA levels may provide earlier information of the presence of CAD before high sensitivity C-reactive protein (hsCRP) elevation [19]. In the present study, focusing on surgical menopausal women, only serum IMA levels in obese patients with HT were statistically lower than those of obese women without HT after six months of treatment. Although there is no consensus on the impact of HT on CRP levels, it is well-recognized that women using HT are characterized by increased plasma CRP levels [20, 21]. The results in the present study suggest that there is an association of surgical menopausal obesity with high IMA levels, possibly due to obesity-associated oxidative stress and serum IMA levels that were statistically lower in obese patients with HT after six months of treatment. Despite the fact that oral estrogen therapy might be associated with increased CRP levels, the decreased IMA level after six months of HT in the present study may be explained with the association between HT use and elevated CRP likely involves first-pass hepatic up-regulation of CRP expression [22], rather than an inflammatory response as non-oral regimens do not appear to raise CRP [23]. Moreover, oral estrogen therapy increased CRP levels, while transdermal therapy did not modify CRP production [20, 24]. According to the present study results, it appears that HT benefit from the decreasing levels of IMA and may have a protective effect on CAD.

Based on the results of this study, including sCD40L was not associated with reduced levels of an inflammatory marker predictive of CVD in obese surgical menopausal women with HT, and consequently, serum IMA levels were statistically lower in obese women with HT after six months of treatment, it remains unclear whether HT-associated decreases in IMA can account for the protective cardiovascular effects of HT. In addition, the effect of HT on sCD40L and IMA levels might be changed if the exclusion criteria of not having any women with some risk factors associated with early cardiovascular events such as hereditary influence, diabetes, hypertension, and smoking could be included into the present study. Moreover, the results of HT on cardiovascular system are not uniform and this variability may be explained with single therapy with estrogen, route of medicaments, age of subjects, analysis of the overall sample (men and women), and the time of sampling. The complexity of the pathway of atherosclerosis and atherothrombosis process, and the fact that platelet activation and proinflammatory mediators, including sCD40L and IMA, sometimes correlate with each other, rendering it difficult to study the effects and determinants of each separate factor.

In conclusion, it is not clear whether HT confers coronary risk reduction through an inflammation-sensitive mechanism. It seems reasonable to consider that initiation and continuation of HT should be based on established non-coronary benefits and risks, and patient preference. However, based on the results of this study including serum IMA levels, were statistically lower in obese surgical menopausal women with HT after six months of treatment, and the authors also suggest that HT may be useful in obese surgical menopausal women. Larger prospective studies are needed to further investigate the effect of HT on serum markers of neutrophil and platelet activation.

References

[1] Hoerger T.J., Downs K.E., Lakshmanan M.C., Lindrooth R.C., Plouffe L. Jr., Wendling B. *et al.*: "Healthcare use among U.S. women aged 45 and older: total costs and costs for selected postmenopausal health risks". *J. Womens Health Gend Based Med.,* 1999, *8,* 1077.

[2] Gambacciani M., Ciaponi M., Cappagli B., Piaggesi L., De Simone L., Orlandi R. *et al.*: "Body weight, body fat distribution, and hormonal replacement therapy in early postmenopausal women". *J. Clin. Endocrinol. Metab.,* 1997, *82,* 414.

[3] Study Hulley S., Grady D., Bush T., Furberg C., Herrington D., Riggs B. *et al.*: "Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group". *JAMA,* 1998, *280,* 605.

[4] Grodstein F., Manson J.E., Colditz G.A., Willett W.C., Speizer F.E., Stampfer M.J.: "A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease". *Ann. Intern. Med.,* 2000, *133*, 933.

[5] Anand S.X., Viles-Gonzalez J.F., Badimon J.J., Cavusoglu E., Marmur J.D.: "Membrane-associated CD40L and sCD40L in atherothrombotic disease". *Thromb. Haemost.*, 2003, *90,* 377.

[6] Cipollone F., Ferri C., Desideri G., Paloscia L., Materazzo G., Mascellanti M. *et al.*: "Preprocedural level of soluble CD40L is predictive of enhanced inflammatory response and restenosis after coronary angioplasty". *Circulation,* 2003, *108*, 2776.

[7] Kazanis K., Dalamaga M., Kassi E., Nounopoulos C., Manolis A.S., Merantzi G. *et al.*: "Serum levels of ischemia modified albumin in overweight/obese postmenopausal women: a potential biomarker of atherosclerotic burden associated with oxidative stress". *Maturitas*., 2011, *70*, 182.

[8] Bar-Or D., Lau E., Winkler J.V.: "A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report". *J. Emerg. Med.*, 2000, *19*, 311.

[9] André P., Nannizzi-Alaimo L., Prasad S.K., Phillips D.R.: "Platelet-derived CD40L: the switch-hitting player of cardiovascular disease". *Circulation.*, 2002, *106,* 896.

[10] Viallard J.F., Solanilla A., Gauthier B., Contin C., Déchanet J., Grosset C. *et al.*: "Increased soluble and platelet-associated CD40 ligand in essential thrombocythemia and reactive thrombocytosis". *Blood.*, 2002, *99,* 2612.

[11] Inwald D.P., Faust S.N., Lister P., Peters M.J., Levin M., Heyderman R., Klein N.J.: "Platelet and soluble CD40L in meningococcal sepsis". *Intensive Care Med*., 2006, *32,* 1432.

[12] Hoak J.C.: "Platelets and atherosclerosis". *Semin. Thromb. Hemost.*, 1988, *14*, 202.

[13] Mendelsohn M.E., Loscalzo J.: "Role of platelets in cholesteryl ester formation by U-937 cells". *J. Clin. Invest.*, 1988, *81*, 62.

[14] Beijers H.J., Ferreira I., Spronk H.M., Bravenboer B., Dekker J.M., Nijpels G. *et al.*: "Body composition as determinant of thrombin generation in plasma: the Hoorn study". *Arterioscler. Thromb. Vasc. Biol.*, 2010, *30,* 2639.

[15] Mertens I., Van Gaal L.F.: "Obesity, haemostasis and the fibrinolytic system". *Obstet. Rev.*, 2002, *3,* 85.

[16] Elwood P.C., Renaud S., Beswick A.D., O'Brien J.R., Sweetnam P.M.: "Platelet aggregation and incident cardiovascular disease in the Caerphilly cohort". *Heart.*, 1998, *80,* 578.

[17] Schonbeck U., Varo N., Libby P., Buring J., Ridker P.M.: "Soluble CD40L and cardiovascular risk in women". *Circulation*, 2001, *104,* 2266.

[18] Poggi M., Jager J., Paulmyer-Lacroix O., Peiretti F., Gremeaux T., Verdier M. *et al*.: "The inflammatory receptor CD40 is expressed on human adipocytes: contribution to crosstalk between lymphocytes and adipocytes". *Diabetologia,* 2009, *52*, 1152.

[19] Kazanis K., Dalamaga M., Nounopoulos Ch., Manolis A.S., Sakellaris N., Jullien G., Dionyssiou-Asteriou A.: "Ischemia modified albumin, high sensitivity C-reactive protein and natriuretic peptide in patients with coronary atherosclerosis". *Clin. Chim. Acta,* 2009, *408,* 65.

[20] Eilertsen A.L., Høibraaten E., Os I., Andersen T.O., Sandvik L., Sandset P.M.: "The effects of oral and transdermal hormone replacement therapy on C-reactive protein levels and other inflammatory markers in women with high risk of thrombosis". *Maturitas.,* 2005, *52*, 111.

[21] Bowden D.W., Lohman K., Hsu F.C., Langefeld C.D., Carr J.J., Lenchik L. *et al.*: "Hormone replacement therapy is associated with increased C-reactive protein in women with Type 2 diabetes in the Diabetes Heart Study". *Diabet. Med.*, 2006, *23,* 763.

[22] Silvestri A., Gebara O., Vitale C., Wajngarten M., Leonardo F., Ramires J.A. *et al.:* "Increased levels of C-reactive protein after oral hormone replacement therapy may not be related to an increased inflammatory response". *Circulation*., 2003*, 107,* 3165.

[23] Langer R.D., Pradhan A.D., Lewis C.E., Manson J.E., Rossouw J.E., Hendrix S.L. *et al.:* "Baseline associations between postmenopausal hormone therapy and inflammatory, haemostatic, and lipid biomarkers of coronary heart disease. The Women's Health Initiative Observational Study". *Thromb. Haemost.,* 2005*, 93,* 1108.

[24] Menon D.V., Vongpatanasin W.: "Effects of transdermal estrogen replacement therapy on cardiovascular risk factors". *Treat. Endocrinol.*, 2006, *5,* 37.

Address reprint requests to:
M.A. OSMANAĞAOĞLU, M.D.
Karadeniz Technical University
Faculty of Medicine
Department of Obstetrics and Gynecology
61080 Trabzon (Turkey)
e-mail: drmaosmanaga@gmail.com).