CLINICAL STUDY

Effects of Hormone Replacement Therapy on Serum Homocysteine and C-Reactive Protein Levels in Postmenopausal Women

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Abstract

Objective: Elevated levels of homocysteine (Hcy) and C-reactive protein (CRP) are each independently associated with increased risk for cardiovascular events in women. We investigated the effects of hormone therapy (HT) on serum Hcy and CRP levels in postmenopausal women.
Materials and Methods: Postmenopausal women were treated with tibolone 2.5 mg daily (n=25), or with conjugated equine estrogens (CEE) 0.625 mg daily plus medroxyprogesterone acetate (MPA) 2.5 mg daily (n=24), or no treatment (controls, n=13). Blood samples were collected before therapy and after 6 months of therapy for serum homocysteine and C-reactive protein levels.
Results: After 6 months of treatment, tibolone and CEE plus MPA did not cause significant changes in serum Hcy levels in postmenopausal women (p=0.29, p=0.68, respectively). The median CRP levels increased by 105.7% in CEE+MPA group (p=0.017). Tibolone did not have a significant effect on CRP levels (p=0.85). No significant variation in CRP and Hcy levels was observed in the control group.
Conclusion: The both regimens are not likely to have a clinically relevant impact on serum Hcy levels in women with normal levels prior to therapy. HT does not seem to influence low-normal Hcy concentrations. The possible cardiovascular protective role of CEE+MPA is probably unrelated to its increasing effects on CRP levels in healthy postmenopausal women. Further studies are required to explore the clinical relevance of these observations.

Keywords: hormone replacement therapy, homocysteine, C-reactive protein, menopause

Özet

Postmenopozal Kadınlarda Hormon Tedavisinin Serum Homosistein ve C-reaktif Protein Düzeyleri Üzerine Etkisi

Amaç: Yüksek homosistein (Hcy) ve C-reaktif protein (CRP) düzeylerinin her biri, kadınlarda kardiyovasküler hastalıklar bakımından artmış riskle ilişkilidir. Çalışmamızda postmenopozal kadınlarda hormon tedavisinin serum Hcy ve CRP düzeyleri üzerine etkisini araştırdık.
Materyal ve Metot: Postmenopozal kadınların bir kısmı tibolon 2.5 mg/gün (n=25), veya konjuge equine östradiol (CEE) 0.625 mg/gün+medroksiprogesteron asetat (MPA) 2.5 mg/gün (n=24) tedavisine alınırken, bir grup tedavisiz olarak izlendi (kontrol grubu, n=13). Serum Hcy ve CRP düzeylerini ölçmek üzere çalışmanın başında ve tedavinin 6. ayında kan örnekleri toplandı.
Sonuçlar: Tedavinin 6. ayında tibolon ve CEE+MPA tedavisi, serum Hcy düzeylerinde anlamlı değişikliğe neden olmadı (sırasıyla p=0.29, p=0.68). CEE+MPA grubunda medyan CRP düzeylerinde %105.7 artış saptandı (p=0.017). Tibolon tedavisi öncesi ve sonrası CRP düzeyleri arasında anlamlı bir fark yoktu (p=0.85). Kontrol grubunda tedavi öncesi ve sonrası CRP ve Hcy düzeylerinde anlamlı bir farklılık gözlenmedi.

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Tartışma: Tedavi öncesiyle karşılaştırıldığında, normal Hcy düzeyi olan kadınlarda her iki rejim de klinik bakımdan önemli bir etki oluşturmadı. Hormon tedavisinin düşük ve normal Hcy konsantrasyonları olan kadınlar üzerinde etkisi görülmemiştir. Sağlıklı postmenopozal kadınlarda CEE+MPA tedavisinin kardiyovasküler sistem üzerindeki olası olumlu etkisi, CRP düzeylerini arttırıcı etkisiyle ilişkili değil gibi görünmektedir. Bu gözlemlerin klinik önemini ortaya koymak için yeni çalışmalara ihtiyaç vardır.

Anahtar sözcükler: hormon tedavisi, homosistein, C-reaktif protein, menopoz

Introduction

Cardiovascular disease is the principal cause of morbidity and mortality in developed countries. The incidence of coronary heart disease (CHD) increases after menopause (1). Several epidemiological studies have indicated that hormone therapy (HT) has a cardio protective effect in postmenopausal women (2-4). However, a large randomized clinical trial (Heart and Estrogen/progestin Replacement Study) showed that HT has no cardiovascular benefit which in postmenopausal women with coronary artery disease treated with conjugated equine estrogens (CEE) and medroxyprogesterone acetate (MPA) for 4.1 years (5).

Homocysteine (Hcy) is a sulfur-containing amino acid formed by demethylation of the essential amino acid methionine (6). Elevated Hcy levels have been established as an independent risk factor for coronary vascular disease, cerebrovascular disease, and peripheral vascular disease (7). *In vitro* studies have shown the toxic effect of Hcy on cells in culture and, in animal models, Hcy has been shown to cause vascular endothelial damage resulting in desquamation and atherosclerosis (8,9). Hcy levels are reported to be higher in postmenopausal women compared with premenopausal women (10). Genetic (e.g., methylen-tetrahydrofolate reductase deficiency) and/or nutritional (deficiencies of folate, vitamin B_6, and B_{12}) deficit may impair Hcy metabolism and result in elevated serum levels (11,12). Several studies suggested that HT reduces Hcy levels in postmenopausal women (13,14). Thus, the beneficial effects of HT on cardiovascular risk may, in part, be explained by a reduction in Hcy levels.

C-reactive protein (CRP) is a marker of inflammation and strongly predicts the occurence of cardiovascular events in healthy men and women (15,16). Several studies have shown that, oral combined HT was associated with increased serum CRP levels in postmenopausal women (17,18), which may explain the increased risk of cardiovascular events (5,19). It has been hypothesized that elevated CRP levels associated with HT, may have thrombotic effects and may increase plaque instability (20,21).

Tibolone is a synthetic steroid with mixed estrogenic, progestagenic, and androgenic activity used for postmenopausal HT. It has been shown to relieve climacteric symptoms and improve libido and abnormalities in bone density and lipid profiles (22,23). But, there are limited data on their effect on Hcy and CRP levels. On the other hand, the effect of conjugated equine estrogens plus medroxyprogesterone acetate (CEE+MPA) on these cardiovascular risk markers is controversial. Therefore, we investigated the effects of both regimens on serum Hcy and CRP levels in healthy postmenopausal women for 6 months.

Material and Methods

This trial was performed with local ethics approval and in accordance with the Declaration of Helsinki. Written informed consent was obtained from all women before participation. The study group consisted of 62 healthy postmenopausal women with an intact uterus. All participants were between 40-62 years old and normotensive. Menopause was determined by at least 6 months of amenorrhea and serum follicle stimulating hormone (FSH) concentration >30 mIU/mL. None of the women had received HT for at least 3 months before entering the study, and none took lipid lowering, antihypertensive drugs, vitamin B_6, vitamin B_{12}, or folate supplements at study entry or during the study period. Exclusion criteria included bilateral oophorectomy, undiagnosed vaginal bleeding, a history of cardiac, cerebrovascular, thromboembolic, hepatic or renal disease and cigarette consumption >10 cigarettes daily.

Sixty-two postmenopausal women were divided into three groups. One group (n=25) took oral tibolone, 2.5 mg/d (Livial 2.5 mg, Organon, Holland), and the other (n=24) received oral CEE, 0.625 mg/d, plus MPA, 2.5 mg/d (Premelle 2.5 mg, Wyeth, Philadelphia, U.S.A.). Remained 13 women were of no treatment (control group).

Blood samples were collected between 8:00 and 10:00 a.m. after at least 12 h fasting from a peripheral vein at study entry and after 6 months of therapy. All specimens were collected in Vacutainer (Becton-Dickinson, Franklin Lakes, NJ) blood-collecting tubes according to standard hospital guidelines for venipuncture and sample collection. Hcy specimens were placed on ice and all specimens were transported to the laboratory within 30 minutes of collection. Serum was obtained after centrifugation at 2.000 x g for 10 minutes, frozen, and stored at -20°C until analysis. Serum total Hcy concentrations were measured by using an IMX (Abbott Diagn. USA) Hcy assay. Assay is based on the fluorescence polarization immunoassay (FPIA) technology. CRP levels were evaluated by high sensitivity immunonephelometer test with commercially available test (Dade Behring, BM 100, Germany). The intra-assay coefficient of variation was < 5%.

Statistical Analysis

Nonparametric statistical methods were applied to continuous variables. For all measured parameters, statistical

analyses of between-group differences were performed by using Kruskal-Wallis variance analysis. Serum Hcy and CRP levels measured at baseline and after 6 months of treatment were compared using Wilcoxon's signed-rank test for each group. All data are given as means ± SD or as median values and range. A p value of less than 0.05 was considered statistically significant. All data were entered into and processed by SPSS 9.05 for Windows statistical package.

Results

Sixty-two participants were enrolled initially. During treatment, five women were excluded from the analysis. Two women receiving CEE+MPA complained of vaginal spotting and mastalgia. One woman receiving tibolone therapy was withdrawn due to vaginal spotting. Two women with CEE+MPA therapy left the study for reasons not related to the treatment. The remaining 57 patients completed the study. Basal clinical and laboratory parameters were similar in the groups (p>0.05) (Table 1). There were no statistically significant differences in the baseline levels of Hcy and CRP between the study groups (Table 2).

Neither tibolone nor CEE+MPA was not accompanied by any significant change in serum Hcy at the end of the therapy (p=0.29, p=0.68, respectively). Tibolone had no statistically significant effect on serum CRP levels (p=0.85) (Table 2). Percentage changes from baseline in serum median Hcy and CRP levels are given in Figure 1 and 2. The median CRP levels have significantly increased by 105.7% in the CEE+MPA group after 6 months of therapy (p=0.017) (Figu-

Table 1. Descriptive Characteristics at Baseline

Variable	Tibolone (n=24) mean ± SD	CEE+MPA (n=20) mean ± SD	Controls (n=13) mean ± SD	P value
Age (y)	50.9±3.5	50.6±4.8	50.5±5.5	NS
Body mass index (kg/m^2)	28.7±5.1	31.8±5.3	31±3.5	NS
Blood pressure (mmHg)				
Systolic	113.4±14.4	117.7±15.6	120±10	NS
Diastolic	73.4±9	76.6±11.1	81.1±10.5	NS
FSH (mIU/ml)	76.5±40.5	66.5±29.8	80.2±20.9	NS
Total Cholesterole (mg/dl)	220.6±37.1	211.9±37.1	228.2±44.4	NS
Triglyceride (mg/dl)	141.7±46.5	125.9±58.2	148.8±136.3	NS
LDL (mg/dl)	135±35.8	130.5±33.1	138.6±34.6	NS
HDL (mg/dl)	56.2±13.8	52.8±16.3	54.4±19.5	NS
Vitamin B12 (pg/ml)	233.9±112.4	266.6±96.7	255.3±91.4	NS
Folate (ng/ml)	5.5±1.9	4.4±0.5	5.3±1.7	NS

Note: Values are expressed as mean ± SD. All comparisons are nonsignificant.
CEE: conjugated equine estrogens; MPA: medroxyprogesterone acetate; FSH: follicle stimulating hormone; LDL: low-density lipoprotein; HDL: high-density lipoprotein; NS: not significant.

Table 2. Serum homocysteine and C-reactive protein levels at baseline and after 6 months

Variables	Group	Baseline		After 6 months		P value
		Mean ± SD	Median (range)	Mean ± SD	Median (range)	
Hcy (µmol/L)	Tibolone (n=24)	15.4±7.5	13.9 (6-45.8)	16.5±7.9	14.4 (9.9-50)	0.29
	CEE+MPA (n=20)	12.5±3	12.7 (6.8-17.4)	12.2±3.5	11.9 (7.2-22.3)	0.68
	Controls (n=13)	12.9±3.8	13.4 (8.3-21.7)	14.6±2.8	14.2 (8.5-19.7)	0.10
CRP (mg/dl)	Tibolone (n=24)	4.8±3.1	3.5 (1-13.3)	5.2±3.3	3.1 (3-16.2)	0.85
	CEE+MPA (n=20)	5.3±4.3	3.5 (3.1-20.6)	8.1±5.3	7.2 (3-19.9)	0.017
	Controls (n=13)	5.3±3	3.4 (3-11.5)	3.7±1.2	3.2 (3.1-6.8)	0.99

Values are given as mean ± SD (standart deviation) and median (range).
CEE: conjugated equine estrogens; MPA: medroxyprogesterone acetate; Hcy: homocysteine; CRP: C-reactive protein.

re 2). Serum Hcy and CRP levels were not altered in the control group after the therapy (p=0.10, p=0.99, respectively) (Table 2).

Discussion

We did not find any significant change in serum Hcy with tibolone after 6 months of treatment. Our results are in agreement with other studies investigating the effect of tibolone on Hcy levels (24,25). Celik et al found that tibolone had no significant effect on serum Hcy levels in postmenopausal women for 6 months (24). In the other study, serum Hcy levels did not show any significant change with tibolone at least during the first 18 months of therapy (25).

Tibolone is a synthetic steroid that combines estrogenic, progestogenic and androgenic activity. Its estrogenic potency is about 1/50 that of ethinyl-estradiol, the progestagenic potency is 1/8 that of norethisterone, and the androgenic potency is about 1/3 that of norethisterone (22). Norethisterone is an androgen derivative. Although several studies have reported that oral estrogen alone significantly decreases Hcy levels (13,14), such an effect may not be observed when combined with norethisterone in postmenopausal women. For example, Eviö et al. reported that neither oral nor transdermal combination of sequential estradiol and norethisterone acetate caused significant changes in plasma Hcy in postmenopausal women (26). Its estrogenic effect might have a partial protective role against the hyperhomocysteinemic effects of androgen in tibolone. Because, the data suggest that there is an association of hyperhomocysteinemia with high serum androgen levels. A sex difference in plasma Hcy levels has been found, with approximately 10-15% higher levels in men than in women (27). It has been recently shown that androgen administration in female-to-male transsexuals increased the plasma levels of Hcy by 17% (28). Our results suggest that the partial androgenic property of tibolone might hinder the decrease in serum Hcy levels.

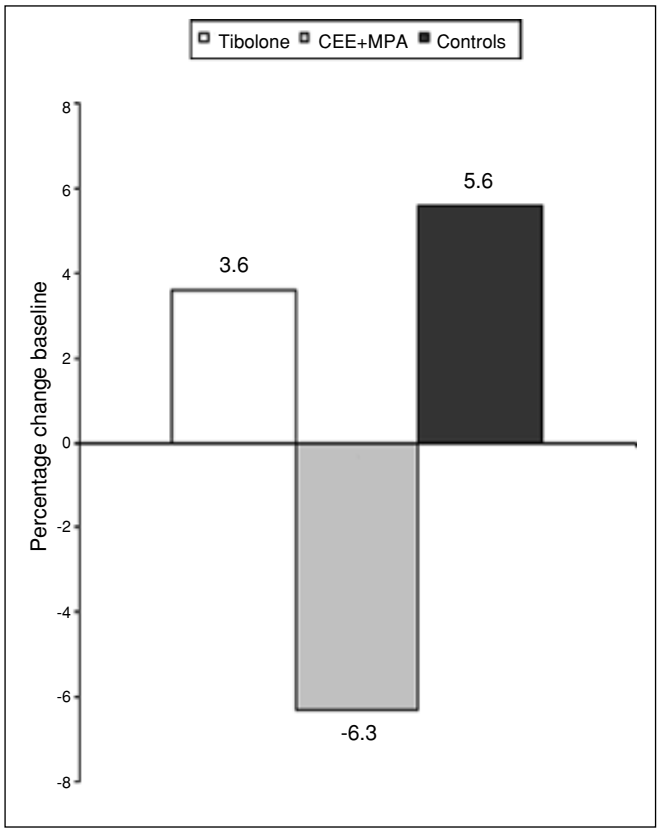

Figure 1. Neither tibolone nor CEE+MPA was accompained by any significant change in serum homocysteine at the end of the therapy.
CEE: conjugated equine estrogens; MPA: medroxyprogesterone acetate.

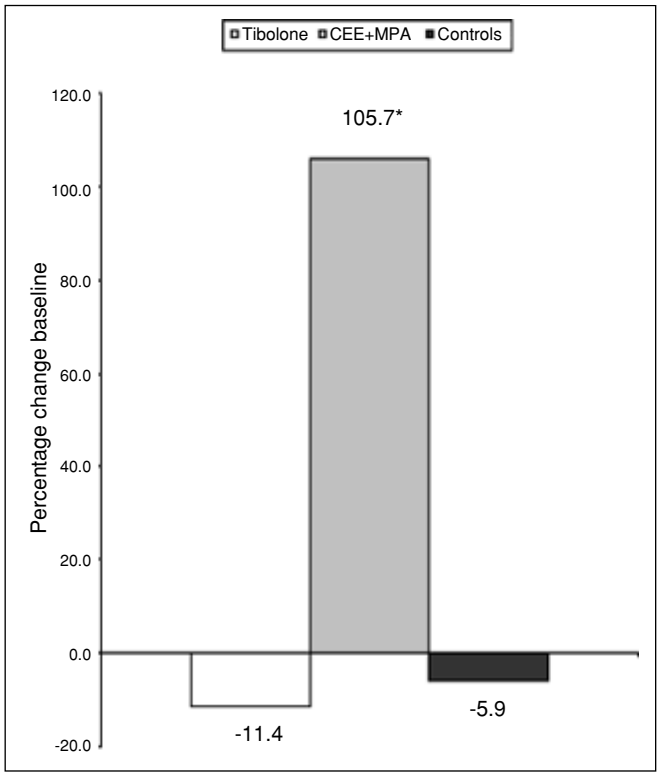

Figure 2. Median percentage changes in CRP levels at the end of study.
*p<0.05
CEE: conjugated equine estrogens; MPA: medroxyprogesterone acetate.

Although estrogen treatment has been shown to decrease serum Hcy levels in several studies (13,14), there is no consensus about the beneficial effects of its combination with a progestin on Hcy levels. Smolders et al. demonstrated that treatment with oral 17β-estradiol but not transdermal 17β-estradiol decreases fasting plasma concentration of Hcy and that addition of the progestogen gestodene attenuates the reduction induced by oral estradiol therapy (13). Christodoulakos et al. reported that continuous CEE+MPA administration resulted in a decrease of a lesser magnitude on Hcy levels compared with CEE (14). In our study, CEE+MPA therapy did not cause significant changes in serum Hcy levels in postmenopausal women.

Several studies reported that HT may primarily reduce elevated levels of Hcy (14,32). Our patients generally had normal levels of Hcy, and these levels might not be affected by therapy. Thus, this analysis does not address the effect of HT in women with hyperhomocysteinemia.

The effect of tibolone on serum CRP levels is unclear. In a randomized prospective study, Garnero et al. reported that tibolone and combined 17β-estradiol and norethisterone acetate increased serum CRP within 6 months in healthy postmenopausal women, and that this increase was sustained for up to 24 months (29). They hypothesized that it is possible that the effect of tibolone on serum CRP is due to a hepatic androgenic effect. On the other hand, in the other study the combination of 1 mg micronized 17β-estradiol and 0.5 mg norethisterone acetate did not have a significant effect on CRP levels (30). In our study, tibolone did not have a significant effect on serum CRP levels.

Most oral HT regimens increase levels of CRP in postmenopausal women who are given either estrogen alone (31,32) or estrogen in combination with a progestin (17,18). The recently published Women's Health Initiative (WHI) a double-blind, placebo-controlled trial studying the effects of continuous combined estrogen-progestin regimen (CEE 0.625 mg plus 2.5 mg MPA daily) reported that median baseline levels of CRP were significantly higher among combined HT users compared with controls (17). Plasma CRP levels were evaluated in a cross-sectional survey in healthy postmenopausal women. Overall, median CRP levels were two times higher among women taking HT (estrogen alone or estrogen plus progesterone) than among women not taking HT (18). Recent data have shown that higher levels of CRP predict future cardiovascular disease events in women (16,33). In the present study, CEE+MPA significantly increased serum CRP levels.

Despite the reported beneficial effect of combined HT on serum lipid profile (34), the possible cardiovascular protective role of CEE+MPA might be unrelated to its increasing effects on CRP levels in healthy postmenopausal women. By the positive changes in the lipid profile (35) and reducing leukocyte adhesion molecule expression on human endothelial cells (36), tibolone may have protective effects on the cardiovascular system. Besides, the fact that tibolone does not increase CRP levels also may contribute its cardio-protective effects.

The limitations of our study include the small sample size and a relatively short duration. There are still many obscurities and controversial observations concerning the influence of HT on Hcy and CRP levels in postmenopausal women. Further studies are required for determining the effects of tibolone on these cardiovascular risk markers in postmenopausal women.

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