Effect of short-term tibolone treatment on risk markers for cardiovascular disease in healthy postmenopausal women: a randomized controlled study

A. Traianos[1], D. Vavilis[1], A. Makedos[1], A. Karkanaki[2], K. Ravanos[2], N. Prapas[2], B.C. Tarlatzis[1]

[1]First Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, "Papageorghiou" General Hospital of Thessaloniki, Thessaloniki
[2]Third Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, "Ippokrateio" General Hospital of Thessaloniki, Thessaloniki (Greece)

Summary

Objective: The aim of this prospective randomized controlled cross sectional study was to evaluate the effect of a six month tibolone treatment in healthy postmenopausal women on biochemical CVD markers by calculating the changes of the blood serum levels of total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (Tg), high-sensitivity C-reactive protein (hsCRP), homocysteine (Hcy), and endothelin-1 (ET-1) at the beginning of the treatment and after six months. *Materials and Methods:* Fifty-two healthy postmenopausal women were enrolled in a prospective, randomized, case-controlled outpatient trial. Group 1 (n = 26) received 2,5 mg/d tibolone for six months, while Group 2 (n = 26) received no treatment. Serum levels of TC, LDL, HDL, Tg, hsCRP, Hcy, and ET-1 were evaluated at baseline and after six months. *Results:* The two groups did not statistically differ at baseline characteristics. In Group 1 tibolone treatment decreased significantly TC ($p = 0.01$), HDL ($p < 0.001$), and Tg ($p < 0.001$) serum levels while a significant increase of hsCRP ($p < 0.001$) was observed. Finally no changes were noticed on LDL, Hcy, and ET-1 serum levels. Regarding Group 2, no changes were observed. *Conclusion:* Short-term tibolone treatment in healthy postmenopausal women exerts a mixed action, acting beneficially in some markers (TC, LDL, Tg, Hcy, and ET-1) where as detrimentally in others (HDL, hsCRP).

Key words: Tibolone, cardiovascular disease; Risk markers; Postmenopausal women.

Introduction

Menopause is mainly connected to the gradual and massive reduction of the estrogen levels in women. This hormonal condition has different effect on various target organs such as the uterus, vaginal mucosa, skin, and endothelium. The protective role of estrogens on the endothelium has been proven by multiple studies and so menopause can induce endothelial dysfunction and lead to metabolic syndrome and cardiovascular disease (CVD), the first cause of death in women during the postmenopausal period [1, 2]. Several biochemical substances in the blood serum have been studied and used at present as valuable risk markers for CVD such as total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (Tg), high-sensitivity C-reactive protein (hsCRP), homocysteine (Hcy), endothelin-1 (ET-1), and many others both in men and women, mainly addressed to the endothelial function.

Hormone replacement therapy (HRT) in postmenopausal women had been welcomed with enthusiasm at the beginning, both by patients and clinicians due to the relief of the postmenopausal symptoms and the proven positive effect on the evolving osteoporosis during menopause and the positive effect on the prevention of CVD [3]. On the contrary the results of randomized-controlled studies showed that HRT has adverse effects on the cardiovascular system [4]. Further studies in the past decade has given more clarity in the safe length of HRT regimes and made patients less reluctant to the use of it [5].

Tibolone is a synthetic steroid with tissue-specific estrogenic, androgenic, and progestogenic properties. It mainly acts as an agonist at all Type I steroid hormone receptors [6]. It was primarily used against osteoporosis but nowadays is also used as an alternative to HRT for relief of menopausal symptoms. Though, acting as an estrogen, data suggest that tibolone may have cardio-protective role by acting positively on biochemical risk factors for CVD, when used in postmenopausal women [7]. The results among relevant studies on the topic are still conflicting.

Materials and Methods

Fifty-two Caucasian healthy postmenopausal women were enrolled in a prospective, randomized, case-controlled outpatient trial. All women presented at the Menopause Outpatient Clinic of the present university teaching hospital after referral for postmenopausal symptoms. After consultation the patients were randomized in two groups. Group 1 (n = 26) received 2.5 mg/d tibolone for six months, while Group 2 (n = 26) received no treatment.

Randomization was carried out by using sealed envelopes containing computer-generated randomization numbers. Informed consent was obtained from all women and the study was approved by the regional ethical committee.

Revised manuscript accepted for publication September 1, 2012

Inclusion criteria in the study were: the time interval since the last menstrual bleeding (MSM) more than 12 months; for surgical menopause time interval ≥ four months, and the body mass index (BMI) < 30 kg/m^2. In all patients follicular stimulating hormone (FSH) was > 40 IU/l. Moreover all patients were: healthy without taking any medication. Exclusion criteria were: medical history of thrombophilia, arterial hypertension, CVD, hepatic or kidney disease, thyroid disease, diabetes mellitus, use of HRT more than six months prior to the study, and any type of neoplasia. All women were requested to avoid any diet and lifestyle modifications or commence any long-term medication during the trial.

During the first visit in the clinic, medical history was taken and also clinical examination, transvaginal ultrasound (TVUS) of the internal genital organs, and smear test collection were performed. Further bone densitometry (DEXA) of the hip was ordered and performed by the radiology department of this hospital. At the same day blood sampling was performed after 12 hours of fasting for the evaluation of serum levels of TC, LDL, HDL, Tg, hsCRP, Hcy, and ET-1. Six months later, another blood sampling was performed under the same conditions for the evaluation of the same markers.

Assays

ET-1 serum levels were measured using ET-1 ELISA kits . The sensitivity of the kit is 0.064 pg/ml. ET-1 concentrations were found to be in the range 0.401-2.83 pg/ml. HsCRP serum levels were measured using Cardiphase hsCRP ELISA kits. Expected values for healthy individuals are typically ≤ 3 mg/l. The sensitivity of the method is 0.175 mg/l. Serum Hcy levels were measured by fluorescence polarization immunoassay. Within-assay and between-assay CV were 1.4 – 2.2% and 2.9 – 4.8%, respectively. TC serum levels were measured by enzymatic method. Expected values for normal individuals were < 200 mg/dl. Within-assay and between-assay CV were 0.8% and 1.7%, respectively. LDL serum levels were measured by enzymatic method. Expected values for normal individuals were < 100 mg/dl. Within-assay and between-assay CV were 0.71-0.81% and 1.16-1.2%, respectively. HDL serum levels were measured by enzymatic method. Expected values for normal individuals were ≥ 55 mg/dl. Within-assay and between-assay CV were 0.58-0.9% and 1.3-1.85%, respectively. Tg serum levels were measured by enzymatic method. Expected values for normal individuals were < 130 mg/dl. Within-assay and between-assay CV were 1.5% and 1.8%, respectively.

Statistics

Statistical analysis was conducted with the use of SPSS 17.0 and STATISTICA 8.0. The Kolmogorov-Smirnov test was used to check normality assumptions. All data are expressed as mean ± standard error of mean (SEM). Differences regarding measurements among groups were evaluated with t-test or Mann-Whitney U-test, where appropriate. A repeated measures ANOVA was used for the assessment of group differences over time. Fisher's post-hoc test was employed. All tests were performed at level a = 0.05. All values are expressed as mean ± SEM and statistical significance was set for confidence interval (CI) 95% ($p < 0.05$). In cases of $p > 0.05$, it was characterized as non-significant (NS).

Results

There was no statistical significant differences at the basic characteristics between the two groups (Group 1 vs Group 2) regarding the age (50.46 ± 0.52 vs 51.84 ± 0.54), BMI (25.44 ± 0.26 vs 24.84 ± 0.32), and MSM (16.8 ± 1.59 vs 18.81 ± 1.75) (Table 1).

Table 1. — *Baseline characteristics of the two groups. Data are given as mean ± SEM.*

	Group 1 n = 26	Group 2 n = 26	*p*
Age (years)	50.46 ± 0.52	51.84 ± 0.54	NS
BMI (kg/m^2)	25.44 ± 0.26	24.84 ± 0.32	NS
MSM (months)	16.8 ± 1.59	18.61 ± 1.75	NS

Table 2. — *Serum levels of TC, LDL, HDL, Tg, CRP, Hcy, and ET-1 in groups 1 and 2 at baseline, and at six months.*

	Group 1 n = 26	Group 2 n = 26
TC (mg/dl)		
Baseline	204.38 ± 4.33	210.57 ± 6.2
Six months	194.8 ± 4.58	206.34 ± 5.45
p	0.01	NS
LDL (mg/dl)		
Baseline	130.8 ± 4.33	134.46 ± 6.75
Six months	128.07 ± 5.06	132.61 ± 5.8
p	NS	NS
HDL (mg/dl)		
Baseline	52.65 ± 2.39	51.42 ± 2.38
Six months	49.07 ± 2.03	50.53 ± 2.3
p	< 0.001	NS
Tg (mg/dl)		
Baseline	106.08 ± 6.61	115.15 ± 5.06
Six months	84.26 ± 5.06	113.04 ± 5.72
p	< 0.001	NS
hsCRP (mg/l)		
Baseline	1.22 ± 0.15	1.23 ± 0.13
Six months	2.01 ± 0.16	1.08 ± 0.13
p	< 0.001	NS
Hcy (mmol/l)		
Baseline	10.26 ± 0.52	9.98 ± 0.41
Six months	10.15 ± 0.48	10.16 ± 0.4
p	NS	NS
ET-1 (pg/ml)		
Baseline	1.29 ± 0.11	1.03 ± 0.07
Six months	1.20 ± 0.11	0.98 ± 0.08
p	NS	NS

Data are given as mean ± SEM. $p < 0.05$ = statistically significant.

At baseline, no statistical significant difference was found between the two groups (Group 1 vs Group 2) regarding TC (204.38 ± 4.33 vs 210.57 ± 6.2 mg/dl), LDL (130.80 ± 4.33 vs 134.46 ± 6.75 mg/dl), HDL (52.65 ± 2.39 vs 51.42 ± 2.38 mg/dl), Tg (106.8 ± 6.61 vs 115.15 ± 5.06 mg/dl), hsCRP (1.22 ± 0.15 vs 1.23 ± 0.13 mg/l), Hcy (10.26 ± 0.52 vs 9.98 ± 0.41 mmol/l), and ET-1 (1.29 ± 0.11 vs 1.03 ± 0.07 pg/ml).

Within groups after six months treatment with tibolone, significant statistical decrease was found in TC (194.8 ± 4.33 mg/dl, p = 0.01), HDL (49.07 ± 2.03 mg/dl, $p < 0.001$), Tg (84.26 ± 5.06 mg/dl, $p < 0.001$), and significant increase in hsCRP (2.01 ± 0.16 mg/dl, $p < 0.001$) serum levels, while no change was noted in LDL (128.07 ± 5.06 mg/dl), Hcy (10.15 ± 0.48 mmol/l), and ET-1 (1.20 ± 0.11

pg/ml) levels. In the group of women who did not receive tibolone serum levels of TC (206.34 ± 5.45 mg/dl), LDL (132.61 ± 5.8 mg/dl), HDL (50.53 ± 2.3 mg/dl), Tg (113.04 ± 5.72 mg/dl), hsCRP (1.08 ± 0.13 mg/dl), Hcy (10.16 ± 0.4 mmol/l), and ET-1 (0.98 ± 0.08 pg/ml) remained unchanged (Table 2).

Regarding the menopausal symptoms, all patients reported improvement during the six month use of tibolone without mentioning any side-effects.

Discussion

The systematic study of the endothelial function through biochemistry has established several markers in the serum of the blood that can diagnose dysfunction and possible tendency for evolving CVD in these patients. There are many studies including women as patients that examine the short-term effect of different regimes such as oral contraceptives, HRT, tibolone, and others on these markers. In these studies, though that use the same medication, there is an obvious difference in the number of patients participating, the number of markers included, and the length of the study.

In the present study the authors examined the effect of tibolone for a six months period on the most important CVD markers in postmenopausal women. While patients were asked not to change any dietary habits, the results show that tibolone significantly decreased TC, Tg, and HDL serum levels, which is a finding in the majority of the related studies [8-12]. Very few studies did not show any effect on these markers [11, 13], but definitely did not prove that tibolone can cause an increase in a similar period of time.

HsCRP is an acute-phase protein and also a valuable marker of inflammation, but in low levels and without any symptomatic pathology, can be a marker of low-grade chronic inflammation, endothelial dysfunction, and an established CVD marker. In most of the studies where tibolone was used, there was a significant increase of hsCRP serum levels [14-18] and very few studies showed that serum levels remained unchanged during similar time interval [19, 20]. From the present results, the authors agree that tibolone may increase hsCRP serum levels in postmenopausal women during a six-month period course, but not above the physiological range.

The non-significant impact of tibolone on LDL, Hcy, and ET-1 is at least favorable for the endothelial function of postmenopausal women and these results come into agreement with the existing literature regarding LDL [8, 12, 21] and Hcy [15, 22, 23]. Concerning ET-1, tibolone is known to lower the ET-1 levels from the limited existing literature [24, 25].

Limitations of the study can be considered the short-term interval of tibolone use (six months) and also the inclusion of both women with surgical and natural menopause, with different time-interval since the last menstrual period, taking though into consideration that it is still unclear if the CVD risk factors are age or estrogen-related [26]. The authors believe that similar studies only with patients shortly after surgical menopause will give stronger evidence on the subject.

Conclusion

The results of the present study suggest that the use of tibolone in postmenopausal women for six months may have a favorable effect on the endothelial function or at least not negatively affect other CVD markers, excluding HDL and hsCRP. After the safe length of tibolone is established, it would be valuable that more studies with further follow-up of these specific patients be announced in the future, with further biochemical and clinical follow-up and definitely, as in all clinical trials, a proper meta-analysis with adequate number of studies that will further clarify the effect of tibolone on CVD markers in postmenopausal women.

References

[1] Rosano G.M., Vitale C., Marazzi G., Volterani M.: "Menopause and cardiovascular disease: the evidence". *Climacteric.,* 2007, *10* (suppl. 1), 19.
[2] Barrett-Connor E., Laughlin G.A.: "Hormone therapy and coronary artery calcification in asymptomatic postmenopausal women: The rancho bernardo study". *Menopause,* 2005, *12,* 40.
[3] Hulley S., Grady D., Bush T., Furberg C., Herrington D., Riggs B., Vittinghoff E.: "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] Writing Group for the Women's Health Initiative Investigators. Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women. Principal Results From the Women's Health Initiative Randomized Controlled Trial. *JAMA*, 2002, *288*, 321.
[5] Beral V., Million Women Study Collaborators: "Breast cancer and hormone-replacement therapy in the Million Women Study". *Lancet*, 2003*, 362*, 419.
[6] Escande A., Servant N., Rabenoelina F., Auzou G., Kloosterboer H., Cavaillès V. *et al.*: "Regulation of activities of steroid hormone receptors by tibolone and its primary metabolites". *J. Steroid Bioch. Mol. Biol.,* 2009, *116*, 8.
[7] Genazzani A.R., Gambacciani M.; International Menopause Society: "Controversial issues in climacteric medicine I. Cardiovascular disease and hormone replacement therapy". International Menopause Society Expert Workshop. 13-16 October 2000, royal society of medicine, London, UK. *Climacteric.* 2000, *3,* 233.
[8] Koh K.K., Han S.H., Shin M.S., Ahn J.Y., Lee Y., Sheen E.K.: "Significant differential effects of lower doses of hormone therapy or tibolone on markers of cardiovascular disease in post-menopausal women: a randomized, double-blind, crossover study". *Eur. Heart J*., 2005, *26,* 1362.
[9] Stuckey B.G., Barrett P.H., Wagner J.M., Hampton R.A., Chan D.C., Brown S.J., Watts G.F.: "The effect of fenofibrate on HDL cholesterol and HDL particle concentration in postmenopausal women on tibolone therapy". *Clin. Endocrinol. (Oxf.)*, 2010, *73,* 497.
[10] Skouby S.O., Sidelmann J.J., Nilas L., Gram J., Jespersen J.: "The effect of continuous combined conjugated equine estrogen plus medroxyprogesterone acetate and tibolone on cardiovascular metabolic risk factors". *Climacteric.*, 2008, *11,* 489.
[11] Vassalle C., Cicinelli E., Lello S., Mercuri A., Battaglia D., Maffei S.: "Effects of menopause and tibolone on different cardiovascular biomarkers in healthy women". *Gynecol. Endocrinol.,* 2011, *27,* 163.
[12] Creatsas G., Christodoulakos G., Lambrinoudaki I., Panoulis C., Chondros C., Patramanis P.: "Serum lipids and apolipoproteins in Greek postmenopausal women: association with estrogen, estrogen-progestin, tibolone and raloxifene therapy". *J. Endocrinol. Invest.*, 2003, *26,* 545.
[13] Hudita D., Posea C., Ceausu I., Rusu M.: "Efficacy and safety of oral tibolone 1.25 or 2.5 mg/day vs placebo in postmenopausal women". *Eur. Rev. Med. Pharmacol. Sci.,* 2003, *7,* 117.
[14] Garnero P., Jamin C., Benhamou C.L., Pelissier C., Roux C.: "Effects of tibolone and combined 17beta-estradiol and norethisterone acetate on serum C-reactive protein in healthy post-menopausal women: a randomized trial". *Hum. Reprod*., 2002, *17*, 2748.

[15] Barnes J.F., Farish E., Rankin M., Hart D.M.: "Effects of two continuous hormone therapy regimens on C-reactive protein and homocysteine". *Menopause,* 2005, *12*, 92.
[16] Todorova M., Kamenov Z., Christov V., Baleva M.: "Inflammatory activity and anticardiolipin antibodies during tibolone treatment of healthy postmenopausal women". *Methods Find Exp. Clin. Pharmacol.*, 2006, *28,* 147.
[17] Engin-Ustün Y., Ustün Y., Türkcüoglu I., Mutlu Meydanli M., Kafkasli A., Yetkin G.: "Short-term effect of tibolone on C-reactive protein in hypertensive postmenopausal women". *Arch. Gynecol. Obstet.*, 2009, *279,* 305.
[18] Eilersten A.L., Sandvik L., Steinsvik B., Sandset P.M.: "Differential impact of conventional-dose and low-dose postmenopausal hormone therapy, tibolone and raloxifene on C-reactive protein and other inflammatory markers". *J. Thromb. Haemost.,* 2008, *6,* 928.
[19] Ostberg J.E., Damjanovic T., Dimkovic N., Byrne D., Mikhailidis D.P., Prelevic G.M.: "Effect of tibolone on markers of cardiovascular disease risk in postmenopausal women undergoing hemodialysis: a pilot study". *Fertil. Steril.*, 2004, *81,* 1624.
[20] Kiran H., Kiran G.: "Short-term effects of hormone therapy on serum C-reactive protein levels in postmenopausal women". *Arch. Gynecol. Obstet.*, 2006, *274*, 9.
[21] Prelevic G.M., Kwong P., Byrne D.J., Jagroop I.A., Ginsburg J., Mikhailidis D.P.: "A cross-sectional study of the effects of hormon replacement therapy on the cardiovascular disease risk profile in healthy postmenopausal women". *Fertil. Steril*., 2002, *77*, 945.
[22] Bayram M., Ozer G., Kalender H., Kabakci N., Kisa U., Ozkan Y.: "The effects of raloxifene and tibolone on homocysteine and vascular histopathological changes". *Clin. Exp. Med.*, 2007, *7,* 149.
[23] Christodoulakos G.E., Panoulis C.P., Lambrinoudaki I.V., Dendrinos S.G., Rizos D.A., Creatsas G.C.: "Effect of hormone replacement therapy and tibolone on serum total homocysteine levels in postmenopausal women". *Eur. J. Obstet. Gynecol. Reprod. Biol.,* 2004, *15,* 112.
[24] Register T.C., Wagner J.D., Zhang L., Hall J., Clarkson T.B.: "Effects of tibolone and conventional hormone replacement therapies on arterial and hepatic cholesterol accumulation and on circulating endothelin-1, vascular cell adhesion molecule-1, and E-selectin in surgically menopausal monkeys". *Menopause*, 2002, *9,* 411.
[25] Haenggi W., Bersinger N.A., Mueller M.D., Birkhaeuser M.H.: "Decrease of serum endothelin levels with postmenopausal hormone replacement therapy or tibolone". *Gynecol. Endocrinol.,* 1999, *13,* 202.
[26] Antonicelli R., Olivieri F., Morichi V., Urbani E., Mais V.: "Prevention of cardiovascular events in early menopause: possible role for hormone replacement therapy". *Int. J. Cardiol.*, 2008, *130,* 140.

Address reprint requests to:
A. MAKEDOS, M.D.
3 Patriarchou Ioakim street
546 22 Thessaloniki (Greece)
e-mail: anmakedos@yahoo.gr