Menopause: The Journal of The North American Menopause Society
Vol. 17, No. 6, pp. 1188-1193
DOI: 10.1097/gme.0b013e3181e04a18

Effects of raloxifene on serum macrophage colony-stimulating factor and interleukin-18 levels in postmenopausal women younger than 60 years

Efser Oztas, MD,[1] and Gulay Kurtay, MD[2]

Abstract

Objective: Macrophage colony-stimulating factor (M-CSF) and interleukin-18 (IL-18) are cytokines expressed predominantly in atheromatous plaque, and overproduction of these has been found to be associated with coronary artery disease. The aim of this study was to investigate the effect of raloxifene, a selective estrogen receptor modulator, on serum M-CSF and IL-18 levels, cytokines that are presumably involved in the pathogenesis of atherosclerosis.

Methods: A total of 70 postmenopausal women (age, 56.45 ± 1.52 y) without previously confirmed cardiovascular disease were enrolled in a 6-month prospective, randomized, controlled study. Women were randomly assigned to two groups: 35 women received oral administration of 60 mg/day raloxifene for 6 months and 35 were in the control group and received no medications. Serum lipid concentrations and high-sensitivity C-reactive protein (hs-CRP), M-CSF, and IL-18 levels were measured at baseline and at the sixth month in both groups.

Results: Compared with the control group, the raloxifene group had a significant decrease in serum IL-18 concentrations and a 25.29% reduction in serum hs-CRP concentrations. M-CSF levels were reduced by 5.94% in the raloxifene group, but the difference was not statistically significant. At the sixth month, 60 mg/day of raloxifene significantly decreased the median serum total cholesterol and low-density lipoprotein cholesterol levels when compared with the baseline levels.

Conclusions: Raloxifene reduces serum total cholesterol, low-density lipoprotein cholesterol, hs-CRP, and IL-18 levels. According to the results of our study, it is suggested that raloxifene may have a favorable effect on the prevention of cardiovascular disease in healthy postmenopausal women younger than 60 years.

Key Words: Raloxifene – Macrophage colony-stimulating factor – Interleukin-18.

Atherosclerosis, the main etiological factor for cardiovascular disease (CVD), is not only a degenerative condition as previously assumed but is also now considered to be a chronic inflammatory process. It is now well established that from the earliest lesion of atherosclerosis to the plaque formation, various cellular and molecular inflammatory mediators participate in the disease process.[1] Overproduction of interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-α (TNF-α) has been found to be associated with CVD.[2]

Interleukins are considered to be key players in the chronic vascular inflammatory response that is typical for atherosclerosis. Recently, a review of studies about the relationship of cytokines with atherosclerosis in mice pointed out that there are sufficient consistent data allowing the classification of only a few cytokines as typically proatherogenic: IL-1, IL-12, IL-18, macrophage migration inhibitory factor, interferon-γ, TNF-α, and macrophage colony-stimulating factor (M-CSF).[3] M-CSF is known to promote atherogenesis by inducing monocyte-macrophage activation, foam cell formation, and the release of other cytokines such as IL-1β, monocyte chemotactic protein 1, and IL-6 by vascular cells, which lead to hepatic C-reactive protein (CRP) production.[4] IL-18, also known as IF-γ inducing factor, is responsible for the upregulation of adhesion molecules, as well as other inflammatory cytokines like IL-1β, IL-8, and TNF-α; inhibits collagen synthesis by smooth muscle cells; and promotes T helper 1 responses that dominate during human atherogenesis.[5] Furthermore, the beneficial effect of inhibiting IL-18 on plaque progression and composition has been shown in animal models.[6] Therefore, increased M-CSF and IL-18 levels are thought to be representative of other proinflammatory cytokines. In addition, an increasing number of investigations on humans have demonstrated that M-CSF and IL-18, cytokines expressed predominantly in atheromatous plaque, play an important role in atherogenesis and contribute to plaque instability, thrombosis, and acute coronary syndrome.[7-9]

Received March 1, 2010; revised and accepted March 29, 2010.

From the [1]Department of Obstetrics and Gynecology, Ufuk University Faculty of Medicine, Ankara, Turkey; and [2]Department of Obstetrics and Gynecology, Ankara University Faculty of Medicine, Ankara, Turkey.

Financial disclosure/conflicts of interest: None reported.

Address correspondence to: Efser Oztas, MD, Mevlana Bulvarı No. 86-88 Konya yolu, Balgat, Ankara, Turkey 06590. E-mail: efseroztas@yahoo.com

Raloxifene, a nonsteroidal benzothiophene selective estrogen receptor modulator, has beneficial effects on the arterial system by decreasing total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and the levels of some inflammatory markers such as CRP and homocysteine[10-12] and induces endothelial-dependent vasodilatation due to a reduction in the risk factors causing endothelial damage.[13] In addition, recently, some studies have suggested that raloxifene has effects on circulating levels of IL-4, IL-6, IL-7, and TNF-α, but the results were discrepant.[14-18] Furthermore, the results of two randomized, placebo-controlled studies seem to be conflicting, because these suggested that raloxifene did not affect the risk of CVD.[19,20] The Multiple Outcomes of Raloxifene Evaluation (MORE) trial investigated the effect of raloxifene on cardiovascular events as a secondary endpoint and reported that raloxifene therapy for 4 years did not significantly affect the overall risk of cardiovascular events but did significantly reduce cardiovascular events in women with increased risk for cardiovascular events.[19] In addition, according to the results of the Raloxifene Use for the Heart (RUTH) study, raloxifene did not significantly affect the risk of coronary heart disease, but when the incidence of coronary events among subgroups of participants were evaluated, it was realized that in women younger than 60 years, the incidence of coronary events was significantly lower in those assigned to raloxifene compared with those assigned to placebo.[20,21] Therefore, it is unclear whether raloxifene plays a role in protection against atherosclerosis and CVDs. In addition, there are insufficient data about the effects of raloxifene on other cytokines that are proved to be proatherogenic.

The aim of the current clinical study was to evaluate the effects of raloxifene on serum M-CSF and IL-18 levels in relation to changes in serum lipids and high-sensitivity CRP (hs-CRP) levels in healthy postmenopausal women younger than 60 years.

METHODS

Participants

This prospective randomized open-label study was conducted at the University of Ankara, School of Medicine. A total of 70 postmenopausal women with an intact uterus between 55 and 59 years of age recruited from the patients referred to the menopause clinic were eligible to participate in the study. The range of years past menopause was 5 to 10 years. Postmenopause status was confirmed by measurement of serum follicle-stimulating hormone levels greater than 40 IU/L and serum estradiol levels less than 20 pg/mL. All participants had amenorrhea for at least 12 months. Body mass index was required to be between 18 and 31 kg/m^2. All women underwent gynecological examination and transvaginal ultrasonography before recruitment. Exclusion criteria in the study were smoking and having any history of hypertension, venous thromboembolism, cerebral or coronary events, any endocrinopathy, impaired renal or liver function, breast cancer, gynecological malignancy, or chronic inflammatory disease. Women treated with hypolipidemic drugs, systemic corticosteroids, and nonsteroidal antiinflammatory drugs within 6 months of entry and those who received hormone therapy or raloxifene at any time in the past were also excluded.

The procedures used in this study were in accordance with the guidelines of the Declaration of Helsinki on human experimentation. The study was approved by the ethical review board of the University of Ankara. The purpose of the study protocol was explained to the women before they entered the study, and their informed consent was obtained.

Study design

This prospective, randomized, controlled study was conducted at the University of Ankara, School of Medicine. A total of 70 eligible participants were randomly assigned to two groups: 35 women received oral administration of 60 mg raloxifene HCl (Evista, Eli Lilly) daily for 6 months, and 35 were in the control group and received no medications. Randomization was performed using a computer-generated random number table. During the follow-up period, it was formally assessed that none of the women started any kind of medications including hormones, lipid-altering therapy, and analgesics.

After the women fasted for 12 hours, venous blood samples were collected at the baseline and at the sixth month of raloxifene treatment. Samples were obtained, centrifuged within 30 minutes of collection at 3,000*g* for 5 minutes, and stored at −70°C until assayed.

Analytical techniques

At baseline and after 6 months, we measured serum TC, high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels with the enzymatic color test method. LDL-C was calculated by the Friedewald formula. Serum CRP levels were measured by a nephelometric method using the Beckman Coulter Immage Immunochemistry System. The sensitivity level of the CRP kits was 0.002 mg/dL. Concentrations of M-CSF and IL-18 in serum samples were measured using respective human enzyme-linked immunosorbent assay (ELISA) kits. The sensitivity level of the M-CSF ELISA kits (R&D Systems, Minneapolis, MN) was 9 pg/mL, and that of the IL-18 ELISA kits (MBL, Nagoya, Japan) was 12.5 pg/mL. Moreover, according to the manufacturer guidelines, assay characteristics for the measurement of hs-CRP, IL-18, and M-CSF were as follows: intra-assay coefficients of variation, 5%, 4.3%, and 3.4%, respectively; and interassay coefficients of variation, 7.5%, 5.21%, and 3.1%, respectively.

Statistical analysis

Evaluation of the data was performed using SPSS v 11.5 (SPSS Inc., Chicago, IL). The results were expressed as means and medians. To determine whether any changes are present in the parameters at baseline and at the sixth month, a paired *t* test was used when the parameters are homogenically distributed and the Wilcoxon signed-rank test was used when the parameters are nonhomogenically distributed. Comparisons between the raloxifene and the control groups were

performed using an unpaired t test or the Mann-Whitney U test as appropriate. The differences regarding primary outcomes between groups were evaluated by both intention-to-treat analysis and per protocol analysis. $P < 0.05$ was considered to be statistically significant.

RESULTS

Background characteristics

A total of 70 women were originally enrolled in the study and were randomized to treatment. The intent-to-treat population consisted of 70 women (35 in the raloxifene group and 35 in the control group), 90% (n = 63) of whom completed the 6-month follow-up period. One of the 35 women who received raloxifene dropped out of the study at 2 months because of increased hot flushes as an adverse effect. Six women in the control group dropped out of the study because of the following reasons: four received medications including lipid-lowering drugs and bisphosphonates, and two withdrew for personal reasons. Of the 63 women who completed the study, 34 were in the raloxifene group (60 mg raloxifene daily) and 29 were in the control group (received no medications).

Baseline demographic characteristics according to assignment are presented in Table 1. There were no significant differences among the two groups for age, duration of the postmenopausal period, family history of CVD, body mass index, bone mineral density, and endometrial thickness. In addition, baseline lipid profiles and levels of serum M-CSF, IL-18, and hs-CRP were not significantly different in women in both groups who completed the study (Table 2).

After a 6-month follow-up period, serum TC, LDL-C, TG, HDL-C, M-CSF, IL-18, and hs-CRP concentrations were reevaluated.

Changes in serum concentrations of lipids

At the sixth month, 60 mg of daily raloxifene decreased the median serum TC levels by 12 mg/dL ($P < 0.0001$) and the median LDL-C levels by 9 mg/dL ($P = 0.025$) when compared with the baseline levels (Table 3). At the sixth month of the follow-up period, we found that, compared with the control group, the raloxifene group had significantly decreased serum TC, TG, and LDL-C levels ($P < 0.0001$, $P = 0.015$, and $P = 0.009$, respectively) and significantly increased serum HDL-C levels ($P = 0.009$; Table 4).

TABLE 1. *Baseline characteristics of the intent-to-treat population*

Characteristic[a]	Raloxifene	Control
No. of participants (n = 70)	35	35
Age, y		
Mean ± SD	56.51 ± 1.48	56.4 ± 1.59
Range	55-59	54-59
Postmenopausal period, y		
Mean ± SD	6.45 ± 0.91	6.77 ± 1.23
Range	5-10	5-9
Family history of CVD, %	25.7	28.5
BMI, kg/m^2		
Mean ± SD	27.17 ± 2.81	28.04 ± 2.27
Range	20.43-30.80	23.80-30.80
BMD (T score)		
Mean ± SD	−2.89 ± 0.40	−2.87 ± 0.36
Range	−3.79 to −2.50	−3.79 to −2.50
Endometrial thickness, mm		
Mean ± SD	2.75 ± 0.9	2.67 ± 0.85
Range	1.1-4.3	1-4.5

CVD, cardiovascular disease; BMI, body mass index; BMD, bone mineral density.
[a]No statistically significant difference with respect to baseline characteristics among the groups ($P > 0.05$).

TABLE 2. *Baseline lipid, M-CSF, IL-18, and hs-CRP levels of the women enrolled in the study*

	Raloxifene (n = 34)	Control (n = 29)	P^a
TC, mg/dL			
Mean ± SD	203.64 ± 28.95	199.20 ± 31.73	
Median (range)	202 (136-268)	201 (148-279)	0.564
LDL-C, mg/dL			
Mean ± SD	122.29 ± 26.02	114.06 ± 24.76	
Median (range)	121 (82-196)	115 (56-164)	0.206
Triglyceride, mg/dL			
Mean ± SD	158.11 ± 51.38	154.75 ± 45.23	
Median (range)	153 (81-294)	160 (75-234)	0.786
HDL-C, mg/dL			
Mean ± SD	47.23 ± 10.54	49.51± 10.15	
Median (range)	46.00 (24-72)	49 (27-70)	0.387
M-CSF, pg/mL			
Mean ± SD	377.67 ± 517.84	376.12 ± 279.41	
Median (range)	247.52 (44.15-2,895.45)	249.65 (30.15-1,007.40)	0.486
IL-18, pg/mL			
Mean ± SD	222.57 ± 195.51	198.73 ± 230.10	
Median (range)	206.60 (0-877.10)	115.45 (0-911.80)	0.339
CRP, mg/L			
Mean ± SD	3.48 ± 2.15	3.19 ± 2.88	
Median (range)	3.18 (0.54-10.40)	2.7 (0.4-14.20)	0.270

M-CSF, macrophage colony-stimulating factor; IL-18, interleukin-18; hs-CRP, high-sensitivity C-reactive protein; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.
[a]No statistically significant difference with respect to baseline characteristics among the groups ($P > 0.05$).

TABLE 3. *Comparison of baseline and sixth-month values in the raloxifene group (n = 34)*

	Baseline	Sixth month	P^a
TC, mg/dL			
Mean ± SD	203.64 ± 28.95	188.88 ± 31.36	
Median	202 (136-268)	190 (124-248)	<0.001
LDL-C, mg/dL			
Mean ± SD	122.29 ± 26.02	113.94 ± 24.84	
Median	121 (82-196)	112 (71-181)	0.041
Triglyceride, mg/dL			
Mean ± SD	158.11 ± 51.38	151.67 ± 44.27	
Median	153 (81-294)	144 (90-261)	0.193
HDL-C, mg/dL			
Mean ± SD	47.23 ± 10.54	48.52 ± 9.994	
Median	46.00 (24-72)	48.00 (26-74)	0.188
M-CSF, pg/mL			
Mean ± SD	377.67 ± 517.84	250.90 ± 126.54	
Median	247.52 (44.15-2,895.45)	247.00 (0-497.75)	0.48
IL-18, pg/mL			
Mean ± SD	222.57 ± 195.51	184.40 ± 195.15	
Median	206.60 (0-877.10)	130.76 (0-846.25)	0.176
CRP, mg/L			
Mean ± SD	3.48 ± 2.15	2.53 ± 1.49	
Median	3.18 (0.54-10.40)	2.57 (0.48-7.08)	0.009

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; M-CSF, macrophage colony-stimulating factor; IL-18, interleukin-18; CRP, C-reactive protein.
[a]When $P < 0.05$, the result is statistically significant.

TABLE 4. *Comparison of baseline and sixth-month values in the control group (n = 29)*

	Baseline	Sixth month	P^a
TC, mg/dL			
Mean ± SD	199.20 ± 31.73	204.89 ± 35.62	
Median	201 (148-279)	197 (143-291)	0.31
LDL-C, mg/dL			
Mean ± SD	114.06 ± 24.76	112.48 ± 28.23	
Median	115 (56-164)	119 (52-162)	0.65
Triglyceride, mg/dL			
Mean ± SD	154.75 ± 45.23	162.86 ± 43.33	
Median	160 (75-234)	168 (76-241)	0.074
HDL-C, mg/dL			
Mean ± SD	49.51± 10.15	46.82 ± 9.69	
Median	49 (27-70)	47 (25-76)	0.063
M-CSF, pg/mL			
Mean ± SD	376.12 ± 279.41	386.87 ± 256.52	
Median	249.65 (30.15-1,007.40)	301.00 (11.45-1,171.05)	0.38
IL-18, pg/mL			
Mean ± SD	198.73 ± 230.10	272.71 ± 367.68	
Median	115.45 (0-911.80)	171.35 (0-1,675.40)	0.013
CRP, mg/L			
Mean ± SD	3.19 ± 2.88	4.19 ± 3.47	
Median	2.7 (0.4-14.20)	3.9 (0.75-14.90)	0.114

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; M-CSF, macrophage colony-stimulating factor; IL-18, interleukin-18; CRP, C-reactive protein.
[a]When $P < 0.05$, the result is statistically significant.

Changes in serum concentrations of hs-CRP, M-CSF, and IL-18

The serum hs-CRP levels were unchanged in the control group after 6 months (Table 4). In contrast, raloxifene significantly decreased hs-CRP levels when compared with the baseline levels ($P = 0.009$) (Table 3). In the raloxifene group, the median value of hs-CRP was decreased from 3.18 to 2.57 mg/L, and when compared with the control group, the difference was statistically significant ($P = 0.001$; Table 5).

As can be seen in Table 5, raloxifene induced a significant decrease in serum IL-18 levels when compared with the control group ($P = 0.005$). Median percentage changes in serum M-CSF concentrations were –5.94% in the raloxifene group and 44.75% in the control group at the sixth month of the therapy when compared with the baseline levels. However, this difference was not statistically significant ($P = 0.083$; Table 5). Among the 63 women who participated in the study, serum levels of IL-18 in 13 women and M-CSF in 1 woman were less than the sensitivity levels of the assay.

DISCUSSION

In the present study, the group given 60 mg/day of raloxifene had significantly decreased serum levels of IL-18 and hs-CRP when compared with the control group over a 6-month treatment period. However, M-CSF levels tended to decrease in the raloxifene group, but the difference was not statistically significant. To the best of our knowledge, there has been no report evaluating the effects of raloxifene on serum M-CSF and IL-18 concentrations in healthy postmenopausal women younger than 60 years.

According to the MORE study, 60 mg/day of raloxifene did not differ from a dose of 120 mg/day when compared for changes in lipid concentrations, risk of cardiovascular events, and cumulative risk of new vertebral fractures.[19] In addition, women who participated in the RUTH study were assigned to 60 mg of raloxifene daily or placebo.[20] Therefore, we preferred a dose of 60 mg/day of raloxifene to compare our results efficiently with those RUTH, a randomized study investigating the effect of raloxifene on coronary heart disease as a primary outcome.

The results of the current study showed that 60 mg/day of raloxifene reduces serum concentrations of LDL-C, TG, and TC in healthy postmenopausal women. These results are in accordance with the previous reports.[11,22-24]

hs-CRP is an independent marker for the risk of CVD in postmenopausal women without clinically evident coronary artery disease (CAD).[25] Previous studies regarding the effect of raloxifene on hs-CRP were discrepant. Some of them found no or little effect on CRP in postmenopausal women, whereas others showed that raloxifene exerted favorable effects by decreasing CRP levels.[26-30] On the basis of data from the study of Ridker et al reported in 2002, in which 27,939 initially healthy women participated, women with a CRP level of 1 to 3 mg/L were established as at average risk, and those with a CRP level greater than 3 mg/L were established as at high risk for a first cardiovascular event.[31] When the results of Ridker et al are considered, it is evident that the raloxifene group in our study had been in the high-risk group for CVD at baseline and became included in the average-risk group at the sixth month (median hs-CRP levels at the baseline and at the sixth month were 3.18 and 2.57 mg/L,

TABLE 5. *Comparison of the median baseline, median change, and median percent change values of the raloxifene and control groups in the intent-to-treat population*

	Raloxifene (n = 35)	Control (n = 35)	P^a
TC			
Median change, mg/dL	−12	5.68	<0.0001
Median % change	−6.7	3.65	<0.0001
LDL-C			
Median change, mg/dL	−7	−1.58	0.009
Median % change	−5.78	−1.05	0.017
Triglyceride			
Median change, mg/dL	−3.0	7.0	0.015
Median % change	−1.63	3.22	0.02
HDL-C			
Median change, mg/dL	1.0	−2.68	0.009
Median % change	2.32	−4.28	0.017
M-CSF			
Median change, pg/mL	−26.75	10.74	0.315
Median % change	−5.94	44.75	0.083
IL-18			
Median change, pg/mL	−33.17	73.98	<0.0001
Median % change	−17.0	42.56	0.005
CRP			
Median change, mg/L	−0.84	0.99	0.001
Median % change	−25.29	57.14	0.001

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; M-CSF, macrophage colony-stimulating factor; IL-18, interleukin-18; CRP, C-reactive protein.
[a]When $P < 0.05$, the result is statistically significant.

respectively). In the study mentioned above, it is suggested that CRP was superior to LDL-C in predicting the risk of first CVD. The results of the current study show that raloxifene causes a significant reduction in both CRP and LDL-C levels; thus, in agreement with the study of Ridker et al, we propose that raloxifene decreases the most important cardiovascular risk predictors.

CVD is the most common cause of death in postmenopausal women, and the risk of CAD is increased markedly in women older than 50 years, which coincides with the appearance of menopause. During the menopausal transition, changes in cytokines and inflammatory markers, as well as changes in serum lipid levels are known to be involved in the pathogenesis, development, and progression of postmenopausal atherosclerosis. Welsh et al[32] demonstrated that postmenopausal women not taking hormone therapy had very significantly higher levels of IL-18. In addition, Yasui et al[33] showed a significant increase in serum concentrations of IL-2, IL-4, granulocyte-macrophage CSF, and granulocyte CSF in postmenopausal women. These alterations might be responsible for the increased cardiovascular events after menopause in women. Recently, a review of studies on the relationship of cytokines with atherosclerosis in mice pointed out that there are sufficient consistent data allowing the classification of only a few cytokines as typically proatherogenic: IL-1, IL-12, IL-18, macrophage migration inhibitory factor, interferon-γ, TNF-α, and M-CSF.[3]

We first found that raloxifene reduced serum IL-18 levels when compared with the control group. IL-18, also known as IF-γ inducing factor, is a member of the IL-1 family, like IL-1β. IL-18 has been found to up-regulate the expression of intercellular adhesion molecule 1 and other cytokines including IL-1β, IL-6, and IL-8,[34] and it induces the production of vascular cell adhesion molecule 1.[35] Indeed, Mallat et al[6] demonstrated IL-18 in atherosclerotic plaques in human carotids. In addition, serum levels of IL-18 have been identified as a strong predictor of cardiovascular death in stable and unstable angina.[8] The only available report examining the effect of raloxifene on serum IL-18 levels associated with osteoporosis is by Maugeri et al[36] and suggested that raloxifene has been found to have minimal effects on IL-18; however, the patient population was small and the study included women older than 60 years.

M-CSF, also known as CSF-1, is the primary regulator of the survival, proliferation, and differentiation of mononuclear phagocytes. Plasma levels of M-CSF have been found to be elevated in patients with chronic CAD, unstable angina, and myocardial infarction.[37] Furthermore, circulating levels of M-CSF were found to be an independent predictor of cardiac events[38,39] and are associated with the process of atherosclerosis in hemodialysis patients.[40] We could not find any reports investigating the effects of raloxifene on serum M-CSF levels. However, an in vitro study by Devaraj et al[41] suggested that when human aortic endothelial cells are incubated with CRP and M-CSF release is examined, CRP increased M-CSF mRNA in a significant and dose-dependent manner. In the current study, the reduction in M-CSF levels was not statistically significant, but both M-CSF and hs-CRP levels were reduced in the raloxifene group, whereas these were increased in the control group. This condition suggests that in a larger study population and also for a longer treatment period, the difference may tend to be statistically significant.

However, the results of two randomized, prospective, controlled studies[19,20] seemed to be conflicting because they suggested that raloxifene did not affect the risk of CVD. But when the secondary outcomes of the MORE trial were evaluated, it is evident that raloxifene did significantly reduce the cardiovascular events in a subset of women with increased cardiovascular risk. But the results of the RUTH trial did not support this hypothesis. In addition, when the results are examined in detail, it can be seen that the baseline characteristics of the groups in the RUTH trial were not similar because the raloxifene group had a higher cardiovascular risk score and the number of women with established CAD were also higher in the raloxifene group than those in the placebo group. In our study, the mean age of the participants was 57.66 years, whereas in the MORE and RUTH trials mean age was approximately 67 years; in other words, participants were already at increased risk for CVD. This may be another reason leading to the different results. On the other hand, when the incidence of coronary events among subgroups of participants was evaluated, it was realized that in women older than 60 years, the incidence of coronary events was significantly lower in those assigned to raloxifene compared with those assigned to placebo.[20,21] This is in accordance with our results suggesting that raloxifene may have a favorable effect on cardiovascular events only when used in the early postmenopausal stage.

CONCLUSIONS

In our study, circulating levels of IL-18 and hs-CRP were reduced in postmenopausal women younger than 60 years who received raloxifene. These results are consistent with those of the subgroup analysis of the RUTH study. In conclusion, we suggest that raloxifene may decrease the risk of cardiovascular events in younger postmenopausal women. Physicians should evaluate postmenopausal osteoporotic women, considering the individual risks and benefits, and should be aware of the fact that raloxifene treatment may be suitable, especially for women younger than 60 years. Further randomized, placebo-controlled, multicenter studies are required to arrive at a final decision.

REFERENCES

1. Von der Thusen JH, Kuiper J, Van Berkel TJ, Biessen EA. Interleukins in atherosclerosis: molecular pathways and therapeutic potential. *Pharmacol Rev* 2003;55:133-166.
2. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* 2006;86:515-581.
3. Kleemann R, Zadelaar S, Kooistra T. Cytokines and atherosclerosis: a comprehensive review of studies in mice. *Cardiovasc Res* 2008;79: 360-376.

4. Ikonomidis I, Stamatelopoulos K, Lekakis J, Vamvakou GD, Kremastinos DT. Inflammatory and non-invasive vascular markers: the multimarker approach for risk stratification in coronary artery disease. *Atherosclerosis* 2008;199:3-11.
5. Gerdes N, Sukhova GK, Libby P, et al. Expression of interleukin-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis. *J Exp Med* 2002;195:245-257.
6. Mallat Z, Corbaz A, Scoazec A, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. *Circulation* 2001;104:1598-1603.
7. Seshiah PN, Kereiakes DJ, Vasudevan SS, et al. Activated monocytes induce smooth muscle cell death. Role of macrophage colony-stimulating factor and cell contact. *Circulation* 2002;105:174-180.
8. Blankeberg S, Tiret L, Bickel C, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. *Circulation* 2002;106:24-30.
9. Haraguchi K, Kubo M, Saito T, et al. Serum level of macrophage colony-stimulating factor and atherosclerosis in hemodialysis patients. *Nephron Clin Pract* 2006;102:14-20.
10. Blumenthal RS, Baranowski B, Dowsett SA. Cardiovascular effects of raloxifene: the arterial and venous systems. *Am Heart J* 2004;147: 783-789.
11. Walsh BW, Kuller LH, Wild RA, et al. Effects of raloxifene on serum lipids and coagulation factors in healthy postmenopausal women. *JAMA* 1998;279:1445-1451.
12. Saitta A, Morabito N, Frisina N. Cardiovascular effects of raloxifene hydrochloride. *Cardiovasc Drug Rev* 2001;19:57-74.
13. Colacurci N, Manzella D, Fornaro F, Carbonella M, Paolisso G. Endothelial function and menopause: effects of raloxifene administration. *J Clin Endocrinol Metab* 2003;88:2135-2140.
14. Gianni W, Ricci A, Gazzaniga P, et al. Raloxifene modulates interleukin-6 and tumor necrosis-α synthesis in vivo: results from a pilot clinical study. *J Clin Endocrinol Metab* 2004;89:6097-6099.
15. Kumru S, Yildiz FM, Godekmerdan A, Kutlu S, Yilmaz B, Gurates B. Effects of raloxifene and hormone replacement therapy on serum T_H2 and T_H3 type cytokine concentrations in healthy postmenopausal women: a randomised controlled trial. *Arch Gynecol Obstet* 2008;277:489-493.
16. Ozmen B, Kirmaz C, Aydin K, Kafesciler SO, Guclu F, Hekimsoy Z. Influence of the selective estrogen receptor modulator (raloxifene hydrochloride) on IL-6, TNF-α, TGF-β1 and bone turnover markers in the treatment of postmenopausal osteoporosis. *Eur Cytokine Netw* 2007; 18:148-153.
17. Yasui T, Uemura H, Hyodo S, et al. Raloxifene reduces circulating levels of interleukin-7 and monocyte chemoattractant protein-1 in postmenopausal women. *Atherosclerosis* 2009;204:471-475.
18. Walsh BW, Cox DA, Sashegyi A, Dean RA, Tracy RP, Anderson PW. Role of tumor necrosis factor-α and interleukin-6 in the effects of hormone replacement therapy and raloxifene on C-reactive protein in postmenopausal women. *Am J Cardiol* 2001;88:825-828.
19. Barrett-Connor E, Grady D, Sashegyi A, et al. Raloxifene and cardiovascular events in osteoporotic postmenopausal women: four-year results from the MORE (Multiple Outcomes of Raloxifene Evaluation) randomized trial. *JAMA* 2002;287:847-857.
20. Barrett-Connor E, Mosca L, Collins P, et al. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. *N Engl J Med* 2006;355:125-137.
21. Collins P, Mosca L, Geiger MJ, et al. Effects of selective estrogen receptor modulator raloxifene on coronary outcomes in the raloxifene use for the heart trial: results of subgroup analyses by age and other factors. *Circulation* 2009;119:922-930.
22. Griffiths KA, Sader MA, Skilton MR, Harmer JA, Celermajer DS. Effects of raloxifene on endothelium-dependent dilation, lipoproteins, and markers of vascular function in postmenopausal women with coronary artery disease. *J Am Coll Cardiol* 2003;42:698-704.
23. De Leo W, la Marca A, Morgante G, Lanzetta D, Setacci C, Petraglia F. Randomized control study of the effects of raloxifene on serum lipids and homocysteine in older women. *Am J Obstet Gynecol* 2001;184:350-353.
24. Delmas PD, Bjarnason NH, Mitlak BH, et al. Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. *N Engl J Med* 1997;337:1641-1647.
25. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998;98:731-733.
26. Herrington DM, Brosnihan KB, Pusser BE, et al. Differential effects of E and droloxifene on C-reactive protein and other markers of inflammation in healthy postmenopausal women. *J Clin Endocrinol Metab* 2001;86:4216-4222.
27. Walsh BW, Paul S, Wild RA, et al. The effects of hormone replacement therapy and raloxifene on C-reactive protein and homocysteine in healthy postmenopausal women: a randomized, controlled trial. *J Clin Endocrinol Metab* 2000;85:214-218.
28. Blum A, Cannon RO. Selective estrogen receptor modulator effects on serum lipoproteins and vascular function in postmenopausal women and in hypercholesterolemic men. *Ann N Y Acad Sci* 2001;949:168-174.
29. de Valk-de Roo GW, Stehouwer CD, Meijer P, et al. Both raloxifene and estrogen reduce major cardiovascular risk factors in healthy postmenopausal women: a 2-year, placebo-controlled study. *Arterioscler Thromb Vasc Biol* 1999;19:2993-3000.
30. Eilertsen AL, Sandvik L, Steinsvik B, Sandset PM. 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;6:928-934.
31. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2000;347: 1557-1565.
32. Welsh P, Woodward M, Rumley A, Lowe G. Associations of plasma pro-inflammatory cytokines, fibrinogen, viscosity and C-reactive protein with cardiovascular risk factors and social deprivation: the fourth Glasgow MONICA study. *Br J Haematol* 2008;141:852-861.
33. Yasui T, Maegawa M, Tomita J, et al. Changes in serum cytokine concentrations during the menopausal transition. *Maturitas* 2007;56:396-403.
34. Dinarello CA. IL-18: a T_H1-inducing, proinflammatory cytokine and new member of the IL-1 family. *J Allergy Clin Immunol* 1999;103:11-24.
35. Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, et al. IL-18 regulates IL-1β-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc Natl Acad Sci U S A* 2000;97:734-739.
36. Maugeri D, Mamazza C, Lo Giudice F, et al. Interleukin-18 (IL-18) and matrix metalloproteinase-9 (MMP-9) in post-menopausal osteoporosis. *Arch Gerontol Geriatr* 2005;40:299-305.
37. Ikonomidis I, Andreotti F, Economou E. Increased proinflammatory cytokines in patients with chronic stable angina and their reduction by aspirin. *Circulation* 1999;100:793-798.
38. Rallidis LS, Zolidaki MG, Manioudaki HS, Laoutaris NP, Velissaridou AH, Papasteriadis EG. Prognostic value of C-reactive protein, fibrinogen, interleukin 6 and macrophage colony stimulating factor in severe unstable angina. *Clin Cardiol* 2002;25:461-466.
39. Ikonomidis I, Lekakis J, Revela I, Andreotti F, Nihoyannopoulos P. Increased circulating C-reactive protein and macrophage-colony stimulating factor are complementary predictors of long-term outcome in patients with chronic coronary artery disease. *Eur Heart J* 2005;26: 1618-1624.
40. Kihara T, Miyata Y, Furukawa M, et al. Predictive value of serum macrophage colony-stimulating factor for development of aortic calcification in haemodialysis patients: a 6 year longitudinal study. *Nephrol Dial Transplant* 2005;20:1647-1652.
41. Devaraj S, Yun JM, Duncan-Staley C, Jialal I. C-reactive protein induces M-CSF release and macrophage proliferation. *J Leukoc Biol* 2009;85: 262-267.