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Effects of raloxifene on serum malondialdehyde, erythrocyte superoxide dismutase, and erythrocyte glutathione peroxidase levels in healthy postmenopausal women

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Abstract

Objective: To investigate the relationship between raloxifene administration and serum malondialdehyde (MDA), erythrocyte superoxide dismutase (SOD), erythrocyte glutathione peroxidase (GPx) levels in healthy postmenopausal women. *Methods:* In a randomized and placebo-controlled design, 80 women received either 60 mg/day raloxifene or placebo for 24 weeks. MDA, SOD, and GPx levels were assessed at 0,4,12, and 24 weeks. Wilcoxon signed-rank test and Mann-Whitney U test were used for comparisons. *Results:* Six women in the treatment arm and eight women in the placebo group discontinued the study. Mean serum MDA levels were significantly ($p = 0.001$) decreased from 11.4 nmol/ml at baseline to 8.9 nmol/ml at week 12 with raloxifene treatment. Mean erythrocyte SOD activity was significantly ($p = 0.02$) reduced from 1472 U/g Hb at baseline to 1173 U/g Hb at week 12 following raloxifene administration. Lowered serum MDA and erythrocyte SOD levels persisted during treatment. On contrary, erythrocyte GPx levels did not change significantly with raloxifene administration. *Conclusions:* Raloxifene (60 mg/day) lowers serum MDA levels and erythrocyte SOD activity in postmenopausal women after 12 weeks of treatment. The clinical implications of these findings need to be determined.

Keywords: Raloxifene; Malondialdehyde; Superoxide dismutase; Glutathione peroxidase; Antioxidant

1. Introduction

Cardiovascular morbidity and mortality rates increase sharply following menopause [1]. Some investigations in the past decade [2–4] have shown therapeutic benefits of hormone replacement therapy for prevention of adverse cardiovascular events. However, recent large-scale studies [5,6] did not confirm these findings leading to controversy as to the potential cardioprotective effects of hormone replacement therapy in postmenopausal women.

There has been intense interest in the development of new molecules, which lack the limitation of estrogen replacement therapy but bear positive effects on bone, serum lipids, and menopausal symptoms.

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Selective estrogen receptor modulators have been investigated within this context and were found to act as estrogen receptor antagonists in the breast and the uterus, probably omitting the harmful effects of estrogens, but presumably preserving the beneficial effects of estrogens on bone, serum lipids, and the cardiovascular system [7]. Raloxifene is a benzothiophene derivative that binds to estrogen receptors and acts as a selective estrogen receptor modulator [8].

The oxidation of low-density lipoprotein (LDL) is an important factor in the development of atherosclerosis. The antioxidant activity of some compounds buffers the free radicals generated either endogenously or exogenously, thus decreasing the potential damage mediated by oxidation [9]. Animal studies and in vitro investigations have shown antioxidative and vasoprotective effects of raloxifene [9–14]. However, there is scarce data on raloxifene and oxidative damage in human subjects. We were able to identify only one in vivo study investigating the relationship between raloxifene and free radical damage [15]. In the present study, we investigated the relationship between use of raloxifene and serum malondialdehyde (MDA) levels, erythrocyte superoxide dismutase (SOD), and erythrocyte glutathione peroxidase (GPx) activities in postmenopausal women through a 24-week period.

2. Methods

Healthy nonsmoker postmenopausal women (follicle-stimulating hormone level >30 mIU/ml) were eligible for participation. Subjects were excluded if they were prescribed any hormone replacement therapy or vitamin preparations within 6 months of entry; had any history of diabetes, hypertension, impaired renal or hepatic function, deep venous thrombosis, thromboembolic disorders, coronary heart disease, or any other endocrinopathy requiring medical treatment. Initially, data regarding age, duration of menopause, weight, and height were collected. Venous blood samples for follicle-stimulating hormone (FSH), estradiol, MDA, SOD, and GPx assays were also taken.

The trial utilized a randomized, placebo-controlled design. Study protocol was reviewed and approved by the ethical committee of our hospital. All procedures were conducted in accordance with the Helsinki Declaration. Women were randomly assigned, using sealed opaque envelopes in computer-generated random sequence into two groups: (1) raloxifene hydrochloride 60 mg/day, and (2) placebo tablets identical in color and shape to original medication. Women were seen every 4 weeks and were instructed to return the package of tablets each time they came for a control visit. The compliance criteria were taking more than 75% ($\geq$21 tablets/28 tablet pack) of the expected study medication. Blood was sampled at weeks 4, 12, and 24. A questionnaire about the exclusion criteria was also completed at 4-week intervals. Eighty women were randomly assigned to therapy ($n = 40$) and placebo ($n = 40$). Six women in the treatment group and eight women in the placebo group were excluded. Reasons of exclusion were: (1) lost to follow-up ($n = 9$), (2) non-compliance to medication ($n = 3$), and (3) acquiring one of the exclusion criteria during the study period ($n = 2$). The number of women discontinuing the study was not significantly different among groups.

Blood was drawn on EDTA as anticoagulant from the antecubital vein after an overnight fast. Samples were immediately centrifuged at 1500 $\times$ g for 10 min. After separation of plasma, the packed erythrocytes were washed three times with normal saline and hemolyzed with ice-cold water. Erythrocyte SOD and GPx activities were determined, using Randox kits (Antrim, United Kingdom). Briefly, the determination of SOD activity was based on the production of O_2^- anions by the xanthine/xanthine oxidase system. GPx was catalyzed by the oxidation of reduced glutathione in the presence of cumene hydroperoxide. The results were expressed as U/g hemoglobin. The hemoglobin values were measured by Drabkin's method [16,17]. For measurement of MDA, 0.25 ml of serum was treated to 2.5 ml of 20% trichloroacetic acid, and then 1 ml of 0.67% thiobarbituric acid. The mixture was incubated at 100 °C for 30 min. After cooling, the sample was extracted with 4 ml *n*-butanol and centrifuged at 3000 rpm for 20 min. The absorbances of extract were measured at 535 nm, and the results were expressed as nmol/ml. MDA standards were prepared from 1,1,3,3-tetraethoxypropane [18]. All the calorimetric measurements were carried out, using UV-1601 spectrophotometer with CPS-Controller 240A temperature control attachment (Shimadzu, Japan).

MDA, SOD and GPx levels at 0, 4, 12, and 24 weeks of treatment were compared with each other using

Wilcoxon signed-rank test, as parametric assumptions were violated. Comparisons across groups were performed by Mann–Whitney *U*-test for continuous and chi-square or Fisher's exact test for discrete data. $P < 0.05$ was considered significant in statistical analyses.

3. Results

The mean age of all the women ($n = 66$) was 56.8 ± 6.5 years (range, 46–77 years) with a mean postmenopausal duration of 10.8 ± 6.1 years (range, 1–30 years). The mean body mass index (BMI) of the study group was 27.0 ± 6.4 kg/m^2 (range, 18–42 kg/m^2). Baseline characteristics were similar among groups (Table 1). Initial serum MDA, erythrocyte SOD and erythrocyte GPx values were not significantly different across raloxifene and placebo groups (Table 1).

Table 1
Comparisons of baseline characteristics[a] among groups

Characteristics	Raloxifene ($n = 34$)	Placebo ($n = 32$)
Age (years)	57.7 ± 6.7	55.8 ± 6.3
Body mass index (kg/m^2)	25.4 ± 5.0	28.3 ± 7.3
Time since last menstrual period (years)	11.4 ± 6.7	10.2 ± 5.4
Hysterectomy	2 (11.8%)	3 (18.8%)
FSH (mIU/ml)	75.2 ± 18.4	71.8 ± 21.2
Estradiol (pg/ml)	12.3 ± 4.2	10.9 ± 5.1
Serum MDA (nmol/ml)	11.4 ± 2.4	10.1 ± 4.1
Erythrocyte SOD (U/g Hb)	1472 ± 458	1375 ± 625
Erythrocyte GPx (U/g Hb)	47.6 ± 20.1	46.8 ± 29.9

[a] No significant differences ($P > 0.05$) across groups for any characteristic. Values are mean ± standard deviation, except for hysterectomy (frequency and percentage within parentheses).

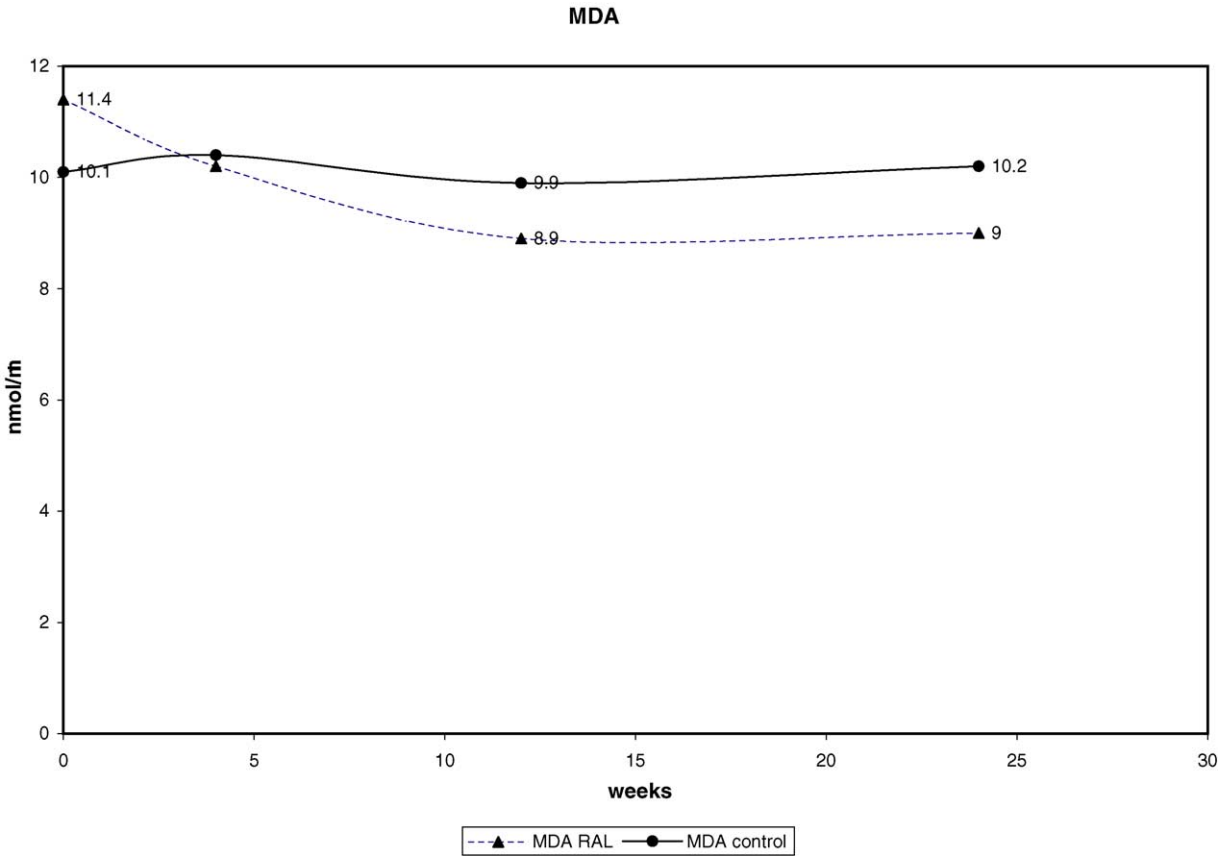

Fig. 1. Mean serum MDA levels at baseline and weeks 4, 12, and 24 according to treatment groups.

Table 2
Serum MDA, erythrocyte SOD, and erythrocyte GPx activities at 4, 12, and 24 weeks

		Week 4	Week 12	Week 24
MDA (nmol/ml)	Raloxifene	10.2 ± 3.0	8.9 ± 2.3[a]	9.0 ± 2.4
	Placebo	10.4 ± 4.3	9.9 ± 4.2	10.2 ± 3.9
SOD (U/g Hb)	Raloxifene	1324.2 ± 420.7	1173.0 ± 439.5[b]	1094.4 ± 672.3
	Placebo	1545.2 ± 552.9	1365.7 ± 653.9	1386.7 ± 594.3
GPx (U/g Hb)	Raloxifene[c]	55.1 ± 21.4	41.8 ± 15.1	48.0 ± 20.1
	Placebo[c]	43.3 ± 24.1	49.1 ± 31.1	46.6 ± 18.9

[a] $P = 0.001$ (serum MDA level decreased significantly following 12 weeks of therapy with raloxifene).
[b] $P = 0.02$ (erythrocyte SOD activity decreased significantly following 12 weeks of therapy with raloxifene).
[c] $P > 0.05$ (erythrocyte GPx levels at 4, 12, and 24 weeks were unchanged by placebo and raloxifene treatment).

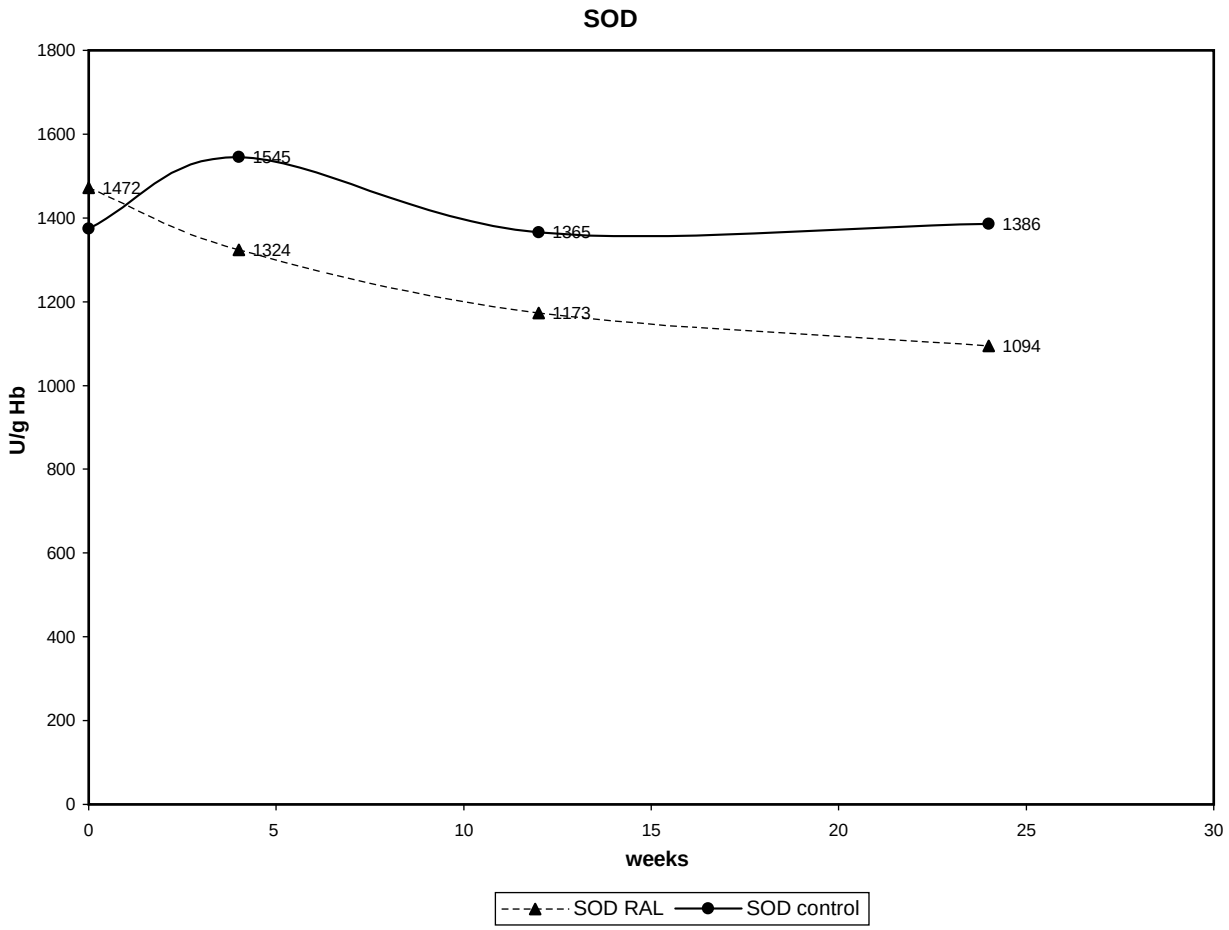

Fig. 2. Mean erythrocyte SOD activities at baseline and weeks 4, 12, and 24 according to treatment.

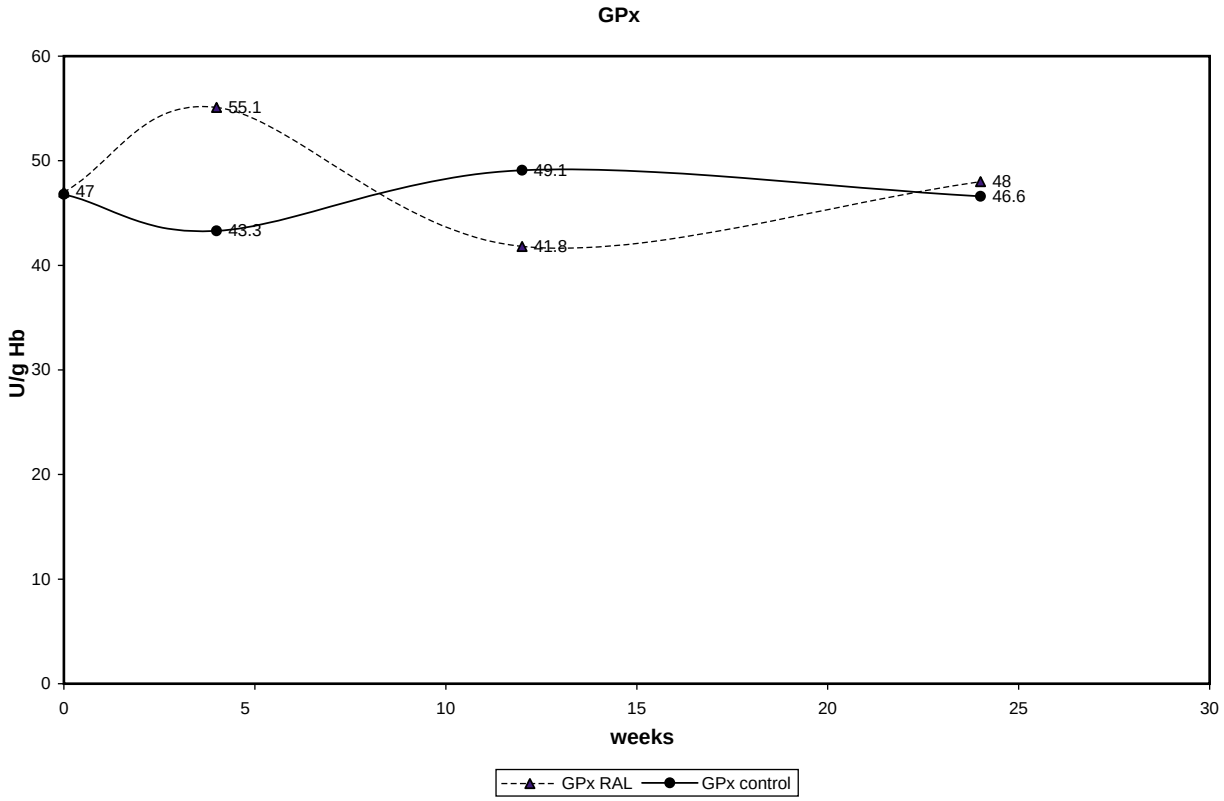

Fig. 3. Mean erythrocyte GPx activities at baseline and weeks 4, 12, and 24 according to treatment.

Means (± standard deviations) of MDA, SOD, and GPx levels in treatment and placebo groups are given in Table 2. In both the treatment and placebo groups serum MDA, erythrocyte SOD, and erythrocyte GPx activities at 4 weeks were not significantly different (for all comparisons, $P > 0.05$) from corresponding baseline values (Table 2, Figs. 1–3). Serum MDA and erythrocyte SOD levels decreased significantly ($P < 0.01$ and $P < 0.05$, respectively) following 12 weeks of therapy with raloxifene. However, erythrocyte GPx activity at 4, 12, and 24 weeks were unchanged by placebo and raloxifene treatment (for all comparisons, $P > 0.05$) (Table 2, Fig. 3). At 24 weeks of raloxifene treatment, serum MDA and erythrocyte SOD levels were not significantly ($P > 0.05$) different from levels at 12 weeks, but were still significantly ($P < 0.01$ and $P < 0.01$, respectively) lower than baseline values (Table 2, Figs. 1 and 2). Serum MDA and erythrocyte SOD levels did not change at 4, 12, and 24 weeks in the placebo group (for all comparisons, $P > 0.05$).

4. Discussion

It has been known that oxidative process is related to inflammation and specifically the chronic inflammatory changes. Inflammatory processes stimulated by oxidized LDL are consistent with the progression of an atherosclerotic lesion [19,20]. The antioxidant activity of estrogen is secondary to the presence of two phenolic groups. Phenolic structures exhibit this activity through binding iron and by reducing peroxyl or alkoxyl radicals [20,21]. Estrogens have antioxidant properties not only because of their hydroxyphenolic structure but probably also because of their influence on antioxidant enzyme activity. Raloxifene carries two phenolic structures similar to estrogens. This moiety

led to research investigating the antioxidant properties of raloxifene, and its antioxidant activity as an in vitro free radical scavenger has been demonstrated in some animal studies [10–14,21]. However, few human data on various antioxidant markers during raloxifene administration is available.

In a study that included male spontaneously hypertensive rats [10], raloxifene treatment at a dose of 10 mg/kg/day was reported to improve hypertension-induced endothelial dysfunction by increasing activity of endothelial nitric oxide. Raloxifene was also found to inhibit angiotensin-II-induced reactive oxygen species production dependent on estrogen receptor activation in rat aortic vascular smooth muscle cells [10]. Another study [22] investigated the effects of estradiol and raloxifene on levels of nitric oxide and antioxidant enzymes in brain cortex of ovariectomized rats. At the end of the 8-week treatment period with raloxifene, total nitrite–nitrate levels were decreased, but no significant effects on SOD and catalase activities were observed.

Arteaga et al. [9] compared the in vitro antioxidant activity of raloxifene, tamoxifene, and estradiol in the plasma of 12 healthy, untreated postmenopausal women. They used MDA as a marker of LDL oxidation, but antioxidant capacity was not studied. They concluded that in vitro antioxidant activity of raloxifene was superior to tamoxifene and estradiol. Colocurci et al. [15] studied the effects of raloxifene on endothelial regulation on 60 healthy postmenopausal women. Serum thiobarbituricacid-reactive substance (TBARS) levels were shown to decrease following 4-months of treatment with raloxifene. They also reported a decrease in serum lipid levels and an increase in plasma Trolox equivalent antioxidant capacity. These two papers [9,15] reported decreasing MDA levels with raloxifene administration supporting our findings. However, our study differed from these two investigations with some respects. First, we evaluated our parameters at weeks 4, 12, and 24 of treatment. Multiple measurements helped us to observe the changes in serum concentrations with time. Secondly, we measured erythrocyte SOD and GPx activities in addition to serum MDA concentrations. We found a significant decrease in serum MDA levels and erythrocyte SOD activity at week 12 following treatment with raloxifene. These lowered levels persisted until the end of our follow-up period at 24 weeks. On contrary, erythrocyte GPx levels did not change in time with raloxifene treatment.

Both SOD and GPx are antioxidant enzymes in red blood cells. We are not able to explain lowered erythrocyte SOD levels with raloxifene administration with our current information. A possible explanation might be different biochemical pathways of antioxidant mechanism for GPx and SOD enzymes. Because, we could not locate any investigations in the English literature about in vivo erythrocyte SOD levels during raloxifene treatment, further comparison and discussion is not possible at this point.

In conclusion, raloxifene administration at a daily dosage of 60 mg significantly lowers serum MDA levels in postmenopausal women. This effect probably begins following 12 weeks and persists during treatment with raloxifene. Raloxifene seems to have no effect on erythrocyte GPx activity. Effects of raloxifene on in vivo erythrocyte SOD activity merit further investigation.

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