JOURNAL OF BONE AND MINERAL RESEARCH
Volume 11, Number 6, 1996
Blackwell Science, Inc.

A Controlled Trial of Raloxifene (LY139481) HCl: Impact on Bone Turnover and Serum Lipid Profile in Healthy Postmenopausal Women*

MICHAEL W. DRAPER,[1] DAVID E. FLOWERS,[2] WILLIAM J. HUSTER,[1] JULIE A. NEILD,[1] KRISTINE D. HARPER,[3] and CLAUDE ARNAUD[4]

ABSTRACT

This randomized, double-blind, placebo-controlled, multicenter, 8-week study evaluated short-term effects of raloxifene on bone turnover, serum lipids, and endometrium in healthy, postmenopausal women. A total of 251 women received either placebo, raloxifene HCl 200 or 600 mg/day, or conjugated estrogens (Premarin, 0.625 mg/day). Bone turnover (serum alkaline phosphatase, serum osteocalcin, urinary pyridinoline cross-links, urinary calcium excretion, urinary hydroxyproline) and serum lipids (total serum cholesterol, high- and low-density lipoprotein cholesterol [HDL-C and LDL-C]) were evaluated at weeks 0, 2, 4, and 8. Endometrial biopsies were performed at weeks 0 and 8. Treatment groups were compared for each parameter for baseline-to-endpoint changes. The estrogen and raloxifene groups experienced similar decreases in serum alkaline phosphatase (range 10–11%), serum osteocalcin (range 21–26%), urinary pyridinoline cross-links (range 20–26%), and urinary calcium excretion (range 45–72%). These decreases differed significantly compared with placebo-treated subjects for all markers except serum osteocalcin, the raloxifene HCl 200 mg group. LDL-C decreased significantly in the estrogen and both raloxifene groups (range 5–9%) compared with placebo-treated subjects. HDL-C increased significantly in the estrogen group (16%) but was unchanged in the raloxifene groups. HDL-C:LDL-C ratios increased significantly in the estrogen and raloxifene groups (range 9–29%). Serum cholesterol decreased significantly in both raloxifene groups (range 4–8%) but was unchanged in the estrogen group. Uterine biopsies of raloxifene-treated subjects showed no change in the endometrium during this short-term treatment. Biopsies of the estrogen group showed significant endometrial stimulation. The only adverse event possibly related to raloxifene was vasodilatation (hot flashes) which was most common in the raloxifene HCl 600 mg group. Study results indicate that raloxifene may provide beneficial effects to bone and serum lipids in humans without uterine stimulatory effects. (J Bone Miner Res 1996;11:835–842)

INTRODUCTION

ESTROGEN REPLACEMENT THERAPY (ERT) is often prescribed to postmenopausal women to diminish the symptoms that accompany the decrease of circulating estrogen during the postmenopausal years.[1–4] This therapy has also been shown to prevent bone loss and to counteract the development of coronary artery disease, at least partially, via alterations in high- and low-density lipoprotein cholesterol (HDL-C and LDL-C).[4–10] These favorable effects, however, are offset by symptoms that are undesirable to many women, including uterine bleeding (even a resumption of menses), breast tenderness, enlargement of benign tumors of the uterus, water retention, and an in-

*Preliminary results from this study were presented at the Fourth International Symposium on Osteoporosis, 27 March–2 April, 1993, Hong Kong.

[1]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, U.S.A.
[2]MedE America, Twinsburg, Ohio, U.S.A.
[3]Center for Bone Diseases, Durham, North Carolina, U.S.A.
[4]Program in Osteoporosis and Bone Biology, San Francisco, California, U.S.A.

crease in the incidence of uterine cancer and possibly of breast cancer.[4] As a result, a major research effort has been targeted at finding a therapy that has the positive skeletal and cardiovascular effects of estrogen without the potentially negative effects on reproductive tissues.

Preclinical data indicate that raloxifene hydrochloride may have the combination of therapeutic effects sought for this "ideal" profile. Raloxifene (previously studied under the name keoxifene) is a compound derived from a benzothiophene series of antiestrogens[11] and can be classified as a selective estrogen receptor modulator (SERM).[12] Raloxifene blocks uterotropic action in response to estrogen in the rat[13] and has a greater binding affinity for estrogen receptors in the uterus and breast than estrogen.[13,14] In an ovariectomized rat model, raloxifene displays beneficial bone and cardiovascular effects without significant uterine effects.[15]

The purpose of this study was to document the short-term effects of raloxifene (Eli Lilly and Company, Indianapolis, IN, U.S.A.), compared with estrogen and placebo, on biochemical markers of bone turnover, serum lipids, and the uterine endometrium in healthy postmenopausal women. The currently recommended dosage of conjugated estrogens (Premarin, Wyeth-Ayerst Laboratories, Philadelphia, PA, U.S.A.) was used as the positive control in this short-term study. Preliminary results from this study were presented at the Fourth International Symposium on Osteoporosis.[16]

MATERIALS AND METHODS

Subject population

A total of 251 healthy postmenopausal women were recruited from the community in 11 study centers throughout the U.S.A.[16] After thorough explanation of the study, which was approved by the appropriate institutional review boards, the subjects were invited to sign a written informed consent document.

Subjects were eligible for the study if they were in good health and free of any serious acute or chronic medical condition, were 45–60 years of age, had an intact uterus, and had been postmenopausal for more than 6 months but less than 6 years before beginning the treatment phase of the study. Subjects were ineligible for the study if they had been treated with estrogen within the 3 months before the study, had ever been treated with fluoride, calcitonin, or bisphosphonate, or were not eligible for therapy according to the Premarin package insert. The date of menopause was defined as the subject's recall of the date of her last menstrual period. Postmenopausal status was confirmed by postmenopausal levels of serum estradiol (<120 pmol/l) and follicle-stimulating hormone (FSH > 30 IU/l).

Study design

This was a Phase 2, randomized, controlled, double-blind study consisting of three phases: a screening phase, an 8-week double-blind treatment phase, and a 12-day post-treatment phase. Subjects were assessed during the screening phase to determine their eligibility to enter the study. Qualified subjects who consented were assigned by blocked random allocation stratified by clinical center to one of four treatment groups during the double-blind treatment phase: placebo, raloxifene HCl 200 mg once daily, raloxifene HCl 600 mg once daily, or conjugated estrogens (Premarin) 0.625 mg once daily. Once treatment was completed, each subject received medroxyprogesterone acetate (Provera, The Upjohn Company, Kalamazoo, MI, U.S.A.) 5 mg/day during the post-treatment phase.

To ensure adequate calcium intake, all subjects also received daily oral calcium carbonate supplements (520 mg/day of elemental calcium) throughout the study from the screening phase to the post-treatment phase. Calcium consumption from dietary sources was not recorded, and no dietary restrictions or changes were implemented during the study. To further minimize confounding variables, subjects were instructed on how to maintain consistent intake of dietary phosphate, magnesium, hydroxyproline, and protein throughout the study. All medications and supplements were taken daily in the morning.

Sample size

The sample size of 251 subjects provides more than 80% power to detect pairwise differences between treatment groups with respect to serum osteocalcin (difference 1.2 ng/ml) and urinary hydroxyproline (difference 0.003 mg/mg of creatinine). Sample size and power calculations were based on variability estimates observed in a similar population and an expected between-treatment difference of 50% of previously observed within-subjects differences.[17]

Serum and urine measurements

Fasting blood and urine samples (second void) were collected at baseline and at weeks 2, 4, and 8 of treatment. Treatment effects on the following biochemical parameters were evaluated: five markers of bone turnover (serum alkaline phosphatase, serum osteocalcin, urinary pyridinoline cross-links, urinary calcium excretion, and urinary hydroxyproline) and three serum lipid parameters (total serum cholesterol, LDL-C, and HDL-C). A central laboratory was used to evaluate the tests performed at each visit.[18–26]

Endometrial biopsies

To evaluate subtle morphologic estrogenic effects on the endometrium, a uterine biopsy was performed at the baseline visit and after 8 weeks of treatment. Endometrial biopsy samples were collected using a Pipelle (Unimar, Wilton, CT, U.S.A.) catheter and were graded based on a system of standard criteria for estrogen-induced proliferation. Biopsy slides were read independently and in a blinded manner by two pathologists. Scores were assigned according to the "estrogenicity" of the biopsy samples. The weighted sum of these scores resulted in an estrogen effect grade of 0 to 3, which was assigned to each sample.[27] A separate manuscript detailing the methodology of the estrogenicity scoring system is being prepared for publication.

TABLE 1. BASELINE DEMOGRAPHICS

Characteristic	Placebo (n = 64)	Raloxifene HCl 200 mg (n = 60)	Raloxifene HCl 600 mg (n = 63)	Total (n = 251)	Estrogen (N = 64)
Race, % caucasian	93.8	95.0	95.2	93.8	94.4
Mean age (years)	53.6 (3.4)	52.8 (3.3)	53.2 (3.0)	53.1 (3.4)	53.2 (3.3)
age range	46–60	45–61	46–61	45–60	45–61
Mean weight (kg)	73.9 (15.8)	72.0 (14.7)	69.6 (12.9)	74.1 (17.8)	72.4 (15.5)
weight range	43–126	46–122	49–118	50–129	43–129
Mean height (cm)	163 (7.1)	164 (7.0)	163 (6.2)	164 (6.2)	164 (6.6)
height range	136–178	140–178	150–175	152–180	136–180
Median time from LMP (months)	40.4	32.8	33.7	35.0	35.5
LMP range	8–87	6–69	7–75	8–73	6–87
FSH (U/l)	104 (43)	103 (41)	106 (45)	101 (33)	104 (40)
FSH range	31–235	31–208	6–213	48–184	6–235
Estradiol (pmol/l)	29.4 (29.5)	21.3 (22.2)	22.2 (18.6)	30.5 (39.7)	25.9 (28.9)
estradiol range	0–131	0–85	0–105	0–233	0–233

Abbreviations: FSH = follicle-stimulating hormone; LMP = last menstrual period.

Evaluation of adverse events

At the time of each return visit, the subject was questioned regarding the occurrence and nature of any adverse events. All adverse events that occurred during the study were recorded, and their causes were evaluated. Actual adverse event terms were coded using preferred terms from Coding Symbol and Thesaurus for Adverse Reaction Terminology (COSTART). In this manuscript, we present the COSTART term followed by examples of actual terms that would code to the COSTART term, e.g., vasodilatation (hot flashes, hot flushes, feeling of warmth).

Statistical analysis

The four treatment groups were compared for each parameter with respect to the baseline-to-endpoint change, where the endpoint was week 8 ($n = 237$) or week 4 if no data were available from week 8 ($n = 14$). Overall treatment comparisons were conducted by an analysis of variance (ANOVA) on the ranks of the data. If there was overall treatment significance, paired comparisons were conducted (Fisher's least-significant-difference multiple comparison procedure).[28] The primary comparisons were between the placebo group and each of the raloxifene or estrogen groups. Results from the parametric ANOVA of the actual change values gave results similar to the ranked analysis; however, the ranked analysis is presented due to the non-normality of the residuals from the parametric analysis. Pearson correlations were calculated to assess concomitant changes in the bone turnover markers. The incidence of adverse events was analyzed using a two-tailed Fisher's exact test for each pairwise comparison. Statistical significance was defined as a two-sided p-value < 0.05.

RESULTS

Subject characteristics

The demographic characteristics (race, age, height, and weight) of the subjects at study entry did not differ significantly between the four treatment groups (Table 1). About 94% of the subjects enrolled in the four treatment groups were white; the majority of the remaining subjects were black. The mean age of subjects was 53 years (range 45–61 years), mean body weight was 72 kg, and mean height was 164 cm. The groups were balanced in terms of the number of months postmenopause and the entry FSH and estradiol levels.

Bone turnover

Over the treatment course, the estrogen treatment group and both raloxifene treatment groups demonstrated decreases in four of the five markers of bone turnover (serum alkaline phosphatase, serum osteocalcin, urinary pyridinoline cross-links, and urinary calcium excretion) (Table 2). These decreases were significantly different ($p < 0.05$), compared with placebo-treated subjects, for all markers except serum osteocalcin in the raloxifene HCl 200 mg group. Significant differences in urinary hydroxyproline were not demonstrated in any of the treatment groups. Changes in markers of bone turnover in the raloxifene treatment groups were not significantly different from those in the estrogen treatment group.

A statistically significant positive correlation between osteocalcin and alkaline phosphatase (range 0.15 to 0.22) and between pyridinoline cross-links and hydroxyproline (range 0.41 to 0.53) was observed at weeks 0 and 8 (visits 2 and 5) as well as for the baseline-to-endpoint changes when all treatment groups were combined. No other significant correlations were observed.

TABLE 2. BASELINE VALUES AND MEAN (±SEM) GROUP CHANGES FROM BASELINE TO ENDPOINT IN MARKERS OF BONE TURNOVER

Marker	Placebo (n = 64)	Raloxifene HCl 200 mg (n = 60)	Raloxifene HCl 600 mg (n = 63)	Estrogen (n = 64)
Serum alkaline phosphatase (U/l)				
baseline	75.7 (±2.6)	75.6 (±2.5)	71.8 (±2.7)	74.3 (±2.5)
change	0.7 (±1.5)	−5.7* (±1.1)	−7.6* (±1.2)	−4.7* (±1.4)
% change	3.2 (±2.5)	−6.8* (±1.4)	−8.3* (±1.9)	−5.9* (±1.8)
Serum osteocalcin (ng/ml)				
baseline	3.6 (±0.2)	3.7 (±0.2)	3.2 (±0.2)	3.3 (±0.2)
change	0.3 (±0.2)	−0.2 (±0.2)	−0.5* (±0.2)	−0.5* (±0.2)
% change	23.6 (±10.2)	14.6 (±10.6)	−2.4* (±7.4)	2.6* (±10.7)
Urinary pyridinoline cross-links (pyridinoline:creatinine ratio)				
baseline	61.8 (±3.2)	68.2 (±4.4)	65.9 (±3.2)	65.2 (±2.3)
change	3.4 (±6.2)	−14.7* (±4.9)	−10.4* (±7.4)	−9.6*(±4.7)
% change	12.5 (±9.1)	−14.9* (±6.2)	−7.6* (±11.0)	−11.5 (±6.7)
Urinary calcium excretion (calcium:creatinine ratio)				
baseline	0.26 (±0.02)	0.34 (±0.03)	0.35 (±0.03)	0.34 (±0.03)
change	−0.03 (±0.03)	−0.13* (±0.03)	−0.17* (±0.03)	−0.15* (±0.04)
% change	33.5 (±20.5)	−13.5 (±11.8)	−38.0* (±10.1)	−11.9* (±18.6)
Urinary hydroxyproline (hydroxyproline:creatinine ratio)				
baseline	33.7 (±2.4)	37.1 (±2.3)	31.6 (±2.2)	35.1 (±2.5)
change	0.6 (±4.0)	−3.6 (±3.8)	1.4 (±3.7)	−7.1 (±2.9)
% change	16.2 (±10.3)	13.2 (±12.5)	21.5 (±14.2)	−8.0 (±8.9)

Note: n = greatest number of subjects tested for any one marker; SEM = standard error of the mean.
* Statistically significantly ($p < 0.05$) different from placebo (two-tailed comparison).

Serum lipids

Serum lipids were affected by both estrogen and raloxifene (Table 3). Statistically significant decreases in LDL-C were observed in the estrogen treatment group and in both raloxifene treatment groups when compared with the placebo treatment group. HDL-C increased significantly in the estrogen treatment group compared with the placebo and both raloxifene treatment groups. Compared with the placebo treatment group, the HDL-C:LDL-C ratios increased significantly in the estrogen treatment group and in both raloxifene treatment groups. The HDL-C:LDL-C ratios were significantly increased in the estrogen group compared with both raloxifene treatment groups. Both raloxifene treatment groups had significant decreases in serum cholesterol compared with the placebo and the estrogen treatment groups, but no significant change in total cholesterol was observed in the estrogen treatment group.

Uterine effects

Uterine biopsies of subjects treated with raloxifene HCl 200 and 600 mg showed no change in the estrogen effect grade, indicating no change in the endometrium during treatment.[27] The placebo group showed a statistically significant increase in the estrogen effect grade compared with both raloxifene groups. Stimulation of the endometrium was indicated in the estrogen group based on a significant increase in the estrogen effect grade from baseline to endpoint. This increase was statistically significantly different from placebo and each raloxifene group.

Safety assessments

The safety analysis showed no significant findings related to raloxifene except for vasodilatation (hot flashes, hot flushes, feeling of warmth) which was most common in the raloxifene HCl 600 mg group. The overall discontinuation rate was low (14 of 251 or 6%). Half of the discontinuations (seven) were in the estrogen treatment group. Three subjects discontinued in the raloxifene HCl 600 mg treatment group, and two subjects discontinued in the raloxifene HCl 200 mg treatment group. None of the raloxifene-treated subjects discontinued the study for reasons attributed to the drug.

No statistically significant treatment differences were observed in the proportions of subjects reporting at least one treatment-emergent adverse event (adverse events that first occurred or worsened after initiation of therapy). When comparisons of specific adverse events were performed between each dose of raloxifene, estrogen, and placebo, only

TABLE 3. BASELINE VALUES AND MEAN (±SEM) GROUP CHANGES FROM BASELINE TO ENDPOINT IN SERUM LIPIDS

		Raloxifene HCl		
Variable	*Placebo (n = 64)*	*200 mg (n = 60)*	*600 mg (n = 63)*	*Estrogen (n = 64)*
LDL-C (mmol/l)				
baseline	3.98 (±0.13)	3.61* (±0.12)	3.80 (±0.1)	3.63* (±0.12)
change	−0.17 (±0.06)	−0.38* (±0.08)	−0.55* (±0.09)	−0.45* (±0.07)
% change	−4.0 (±1.5)	−9.5* (±1.9)	−12.6* (±2.0)	−11.2* (±2.0)
HDL-C (mmol/l)				
baseline	1.42 (±0.05)	1.44 (±0.06)	1.46 (±0.05)	1.45 (±0.05)
change	0.04 (±0.03)	0.02† (±0.04)	0.00† (±0.03)	0.27* (±0.03)
% change	4.4 (±2.9)	5.7† (±4.0)	1.5† (±2.3)	20.9* (±2.6)
HDL-C:LDL-C ratio				
baseline	0.39 (±0.02)	0.44 (±0.03)	0.43 (±0.03)	0.44 (±0.03)
change	0.03 (±0.01)	0.06*† (±0.02)	0.06*† (±0.02)	0.15* (±0.02)
% change	10.9 (±4.0)	19.6*† (±4.8)	21.3*† (±5.9)	39.7* (±4.6)
Total cholesterol (mmol/l)				
baseline	6.12 (±0.13)	5.77* (±0.16)	5.93 (±0.15)	5.7* (±0.13)
change	−0.10 (±0.07)	−0.36* (±0.10)	−0.63*† (±0.10)	−0.12 (±0.08)
% change	−1.4 (±1.1)	−5.3* (±1.4)	−9.7*† (±1.4)	−1.4 (±1.4)

Note: LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; *n* = greatest number of subjects tested for any one marker; SEM = standard error of the mean.

* Statistically significantly ($p < 0.05$) different from placebo (two-tailed comparison).

† Statistically significantly ($p < 0.05$) different from estrogen (two-tailed comparison).

TABLE 4. INCIDENCE OF ADVERSE EVENTS BY TREATMENT GROUP*

		Raloxifene HCl		
Adverse event	*Placebo (N = 64)* n *(%)*	*200 mg (N = 60)* n *(%)*	*600 mg (N = 63)* n *(%)*	*Estrogen (N = 64)* n *(%)*
Breast pain	5 (7.8)	0 †	1 (1.6)†	10 (15.6)
Back pain	7 (10.9)	1 (1.7)	2 (3.2)	0 (0.0)‡
Vaginitis	2 (3.1)	1 (1.7)†	3 (4.8)	8 (12.5)
Vasodilatation	7 (10.9)	7 (11.7)	14 (22.2)†	2 (3.1)

Note: *N* = number of subjects randomly assigned to a particular treatment group; *n* = number of subjects reporting a particular adverse event during the cource of the study.

* Includes adverse events for which there was at least one statistically significant ($p < 0.05$) pairwise treatment comparison.

† Statistically significantly different from estrogen ($p < 0.05$).

‡ Statistically significantly different from placebo ($p < 0.05$).

four adverse events were statistically significantly different (Table 4). Statistically significant treatment differences were observed for breast pain (breast soreness or tenderness), back pain (back discomfort, lumbago), vaginitis (dry vagina, vaginal discharge, or infection), and vasodilatation. More estrogen-treated than raloxifene HCl 200 mg– and 600 mg–treated subjects reported breast pain ($p = 0.001$ and $p = 0.009$, respectively). Significantly fewer estrogen-treated subjects reported back pain compared with placebo-treated subjects ($p = 0.013$). Fewer subjects treated with raloxifene HCl 200 mg reported back pain compared with placebo-treated subjects ($p = 0.063$). Significantly more raloxifene HCl 600 mg-treated subjects reported vasodilatation than estrogen-treated subjects ($p < 0.001$). Vasodilatation was also reported more frequently in raloxifene HCl 200 mg–treated subjects compared with estrogen-treated subjects ($p = 0.088$). Vasodilatation was generally mild in severity. No subject discontinued the study because of vasodilatation. Vaginitis was reported more frequently in the estrogen-treated subjects than in the raloxifene HCl 200 mg–treated or placebo-treated subjects ($p = 0.034$ and $p = 0.096$, respectively).

DISCUSSION

The results of this study show that the short-term effects of raloxifene observed in animal models are also observed in postmenopausal women. Raloxifene appears to exert an estrogen-like positive effect on several markers of skeletal turnover.[29–31] Serum alkaline phosphatase and serum osteocalcin (formation-based markers of bone turnover) were suppressed by raloxifene treatment as well as by estrogen treatment. The magnitude of this suppression was similar. Since urinary pyridinoline cross-links measurement is becoming the assay of choice for assessing bone resorption because of high specificity and sensitivity, it is particularly meaningful that pyridinoline cross-links excretion decreased significantly in the estrogen treatment group and both raloxifene treatment groups. In this study, we were not able to show significant changes in hydroxyproline excretion (another resorption-based assay) in any of the treatment groups, possibly because of the sensitivity or specificity problems (subjects not restricted to a hydroxyproline-free diet) commonly encountered with this assay.[29] Of particular interest were the significant decreases in urinary calcium excretion (expressed as a ratio to creatinine excretion) in the estrogen treatment group and both raloxifene treatment groups; they occurred in the setting of supplemental oral calcium given to all subjects in all groups.

Results of this study also confirmed that raloxifene, like estrogen, exerts a positive effect on serum lipids. LDL-C was significantly decreased in the estrogen treatment group and in both raloxifene treatment groups. Estrogen treatment resulted in an elevated HDL-C, while raloxifene had no effect. Estrogen and both doses of raloxifene positively affected the HDL-C:LDL-C ratios. (Dietary control was not strict enough to permit accurate assessment of triglyceride results.) Similar results have been observed with the antiestrogen tamoxifen.[32,33] The long-term significance of these short-term changes has not been established with certainty.[9] Current assessments attribute only a part of the positive cardiovascular effect of estrogen therapy to changes in serum lipids.[8] The implications of any particular pattern of changes, particularly over a short time frame, is not known.

Though the endometrial biopsies provided only limited data in this short-term study, these data support the hypothesis that raloxifene does not stimulate the endometrium in humans.[27] The significant stimulatory effect of conjugated estrogens on the endometrium of subjects during this 8-week study was expected. Conversely, neither dose of raloxifene showed any stimulatory effect on the uterus. The estrogenicity scoring system used in this study is being used in longer term studies to evaluate more extensively the effect of raloxifene on the human endometrium. A separate manuscript detailing the results of the raloxifene on the endometrium has been submitted for publication.

The effect of raloxifene on the uterus is particularly meaningful when placed in context with similar compounds such as tamoxifen. Like raloxifene, tamoxifen has both antiestrogen and estrogen-agonist activities and has a favorable effect on bone markers and serum lipid profiles.[33,34] Unlike raloxifene, tamoxifen seems to have a partial estrogen-agonist effect on endometrial tissue which may be significant with respect to the occurrence of nonfatal endometrial cancers in women undergoing long-term treatment with tamoxifen in at least one study.[35] However, this study of raloxifene in postmenopausal women was short-term; the more conclusive long-term effects of raloxifene on the uterus remain to be determined.

Despite the many potential benefits of ERT, many women experience side effects with estrogen therapy and discontinue the use of estrogen early in the course of treatment.[4] These side effects include breakthrough bleeding, breast tenderness or enlargement, enlargement of benign tumors of the uterus, and water retention. In addition, subjects may be reluctant to use ERT because of anticipation of these side effects. In this present study, the incidence of breast pain and vaginitis was similar in raloxifene- and placebo-treated subjects and was lower than in estrogen-treated subjects. There was an increase in vasodilatation in the high-dose (raloxifene HCl 600 mg) treatment group. Since raloxifene appears to have significant activity at much lower doses,[36] vasodilatory effects will probably not seriously impact the tolerability of raloxifene in many postmenopausal women. However, this observation indicates that the raloxifene effect on postmenopausal symptoms may be antiestrogenic, at least at very high doses.

Although the subjects in the placebo group had discontinued previous estrogen therapy significantly earlier than subjects in the raloxifene HCl 200 mg and estrogen groups, this baseline characteristic is unlikely to affect study results. The time since discontinuation of estrogen therapy was comparable among the active treatment groups. Also women were ineligible for the study if they had been treated with estrogen within 3 months of the start of the study.

Because this was an 8-week study and serum and urine markers of skeletal processes may evolve slowly over a period of months, the ultimate effects of raloxifene treatment on the skeleton remain to be established.[17] However, studies with estrogen have shown that suppression of bone turnover in the short-term correlates with positive effects on bone mineral density and ultimately on the fracture rates in long-term studies.[5,6,37–41] Only long-term studies, currently underway with raloxifene, will ultimately demonstrate the clinically significant effects of this agent on the human skeleton and uterus.

In summary, the results of this clinical study are consistent with those observed in preclinical studies in the ovariectomized rat model. Raloxifene appears to be as effective as estrogen in short-term suppression of markers of bone turnover. Raloxifene also significantly lowers total serum cholesterol and LDL-C and significantly increases the HDL-C:LDL-C ratio. In humans, raloxifene displays beneficial bone and lipid effects without uterine stimulatory effects.

REFERENCES

1. American College of Physicians 1992 Guidelines for counseling postmenopausal women about preventive hormone therapy. Ann Intern Med **12:**1038–1041.

2. Greendale GA, Judd HL 1993 The menopause: Health implications and clinical management. J Am Geriatr Soc **41:**426–436.
3. Lomax L, Schönbaum E 1993 Postmenopausal hot flushes and their management. Pharmacol Ther **57:**347–358.
4. Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, Ernster VL, Cummings S 1992 Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med **117:**1016–1037.
5. Lindsay R 1993 Hormone replacement therapy for prevention and treatment of osteoporosis. Am J Med **95** (Suppl 5A):37–39 (abstract).
6. Field CS, Ory SJ, Wahner HW, Herrmann RR, Judd HL, Riggs BL 1993 Preventive effects of transdermal 17β-estradiol on osteoporotic changes after surgical menopause: A two-year placebo-controlled trial. Am J Obstet Gynecol **168:**114–121.
7. Harris ST, Genant HK, Baylink DJ, Gallagher C, Karp SH, McConnell MA, Green EM, Stoll RW 1991 The effects of estrone (Ogen) on spinal bone density of postmenopausal women. Arch Intern Med **151:**1980–1984.
8. Stampfer MJ, Colditz GA, Willett WC, Manson JE, Rosner B, Speizer FE, Hennekens CH 1991 Postmenopausal estrogen therapy and cardiovascular disease. New Engl J Med **325:**756–762.
9. Lobo RA 1991 Clinical Review 27. Effects of hormonal replacement on lipids and lipoproteins in postmenopausal women. J Clin Endocrinol Metab **73:**925–930.
10. Barrett-Connor E, Buch TL 1991 Estrogen and coronary heart disease in women. JAMA **265:**1861–1867.
11. Jones CD, Jevnikar MG, Pike AJ, Peters MK, Black LJ, Thompson AR, Falcone JF, Clemens JA 1984 Antiestrogens. 2. structure-activity studies in a series of 3-aroyl-2-arylbenzo-[*b*]thiophene derivatives leading to [6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]-phenyl] methanone hydrochloride (LY156758), a remarkably effective estrogen antagonist with only minimal intrinsic estrogenicity. J Med Chem **27:**1057–1066.
12. Draper MW, Flowers DE, Neild JA, Huster WJ, Zerbe RL 1995 Antiestrogenic properties of raloxifene. Pharmacology **50:**209–217.
13. Black LJ, Jones CD, Falcone JF 1983 Antagonism of estrogen action with a new benzothiophene derived antiestrogen. Life Sci **32:**1031–1036.
14. Miller MA, Katzenellenbogen BS 1983 Characterization and quantitation of antiestrogen binding sites in estrogen receptor-positive and -negative human breast cancer cell lines. Cancer Res **43:**3094–3100.
15. Black LJ, Sato M, Rowley ER, Magee DE, Dekele A, Williams DC, Cullinan GJ, Bendele R, Kauffman RF, Bensch WR, Frolik CA, Termine JD, Bryant HU 1994 Raloxifene (LY139481 HCl) prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats. J Clin Invest **93:**63–69.
16. Draper MW, Flowers DE, Huster WJ, Neild JA 1993 Effects of raloxifene (LY139481 HCl) on biochemical markers of bone and lipid metabolism in healthy postmenopausal women. In: Christiansen C, Riis B (eds.) Proceedings 1993, Fourth International Symposium on Osteoporosis and Consensus Development Conference. Aalborg, Denmark: Handelstrykkeriet Aalborg ApS, pp. 119–121. The principal investigators participating in this study are acknowledged in this Proceedings.
17. Stock JL, Coderre JA, Mallette LE 1985 Effects of a short course of estrogen on mineral metabolism in postmenopausal women. J Clin Endocrinol Metab **61:**595–600.
18. Hill CS, Wolfert RL 1989 The preparation of monoclonal antibodies which react preferentially with human bone alkaline phosphatase and not liver alkaline phosphatase. Clin Chim Acta **186:**315–320.
19. Gundberg CM, Wilson PS, Gallop PM, Parfitt AM 1985 Determination of osteocalcin in human serum: Results with two kits compared with those by a well-characterized assay. Clin Chem **31:**1720–1723.
20. Eastell R, Hampton L, Colwell A 1990 Urinary collagen crosslinks are highly correlated with radioisotopic measurements of bone resorption. Presented at the Third International Symposium on Osteoporosis. Copenhagen, Denmark, October 1990.
21. Tietz NW 1983 Clinical Guide to Laboratory Tests. W.B. Saunders, Philadelphia, Pennsylvania, U.S.A. pp. 152–154.
22. Baginski ES, Marie SS, Clark WL, Zak B 1973 Direct microdetermination of serum calcium. Clin Chim Acta **46:**46–54.
23. Palmerini CA, Fini C, Floridi A, Morelli A, Vedovelli A 1985 High-performance liquid chromatography analysis of free hydroxyproline and proline in blood plasma and of free and peptide-bound hydroxyproline in urine. J Chromatog **339:**285–292.
24. Yaegaki K, Tonzetich J, Ng ASK 1986 Improved high-performance liquid chromatography method for quantitation of proline and hydroxyproline in biological materials. J Chromatog **356:**163–170.
25. Friedewald WT, Levy RI, Fredrickson DS 1972 Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. Clin Chem **18:**499–502.
26. Warnick GR, Benderson J, Albers JJ 1982 Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density lipoprotein cholesterol. Clin Chem **28:**1379–1388.
27. Glant M, Eisenhut C, Boss S, Draper M 1995 A scale for assessing postmenopausal endometrial proliferation in response to potential or known selective estrogen respector modulators. Am J Clin Path **104:**341–342.
28. D'Agostino RB, Massaro J, Kwan H, Cabral H 1993 Strategies for dealing with multiple treatment comparisons in confirmatory clinical trials. Drug Info J **27:**625–641.
29. Delmas PD 1993 Biochemical markers of bone turnover for the clinical investigation of osteoporosis. Osteoporos Int **3:**81–86.
30. Eastell R, Robins SP, Colwell T, Assiri AMA, Riggs BL, Russell RGG 1993 Evaluation of bone turnover in type-I osteoporosis using biochemical markers specific for both bone-formation and bone-resorption. Osteoporos Int **3:**255–260.
31. Risteli L, Risteli J 1993 Biochemical markers of bone metabolism. Ann Med **25:**385–393.
32. Grey AB, Stapleton JP, Evans MC, Reid IR 1995 The effect of the anti-estrogen tamoxifen on cardiovascular risk factors in normal postmenopausal women. J Clin Endo Metab **80:**3191–3195.
33. Love RR, Wiebe DA, Newcomb PA, Cameron L, Leventhal H, Jordan VC, Feyzi J, DeMets DL 1991 Effects of tamoxifen on cardiovascular risk factors in postmenopausal women. Ann Intern Med **115:**860–864.
34. Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC, Carbone PP, DeMets DL 1992 Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. New Engl J Med **326:**852–856.
35. Fornander T, Cedermark B, Mattsson A, Skoog L, Theve T, Askergren J, Rutqvist LE, Glas U, Silfverswärd C, Somell A, Wilking N, Hjalmar M-L 1989 Adjuvant tamoxifen in early breast cancer: Occurrence of new primary cancers. Lancet **1:**117–120.
36. Draper MW, Boss SM, Huster WJ, Neild JA 1994 Effects of raloxifene hydrochloride on serum markers of bone and lipid

metabolism—Dose-response relationships. Calcif Tissue Int **54:**339 (abstract).
37. Lufkin EG, Wahner HW, O'Fallon WM, Hodgson SF, Kotowicz MA, Lane AW, Judd HL, Caplan RH, Riggs BL 1992 Treatment of postmenopausal osteoporosis with transdermal estrogen. Ann Intern Med **117:**1–9.
38. Weiss NS, Ure CL, Ballard JH, Williams AR, Daling JR 1980 Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. New Engl J Med **303:**1195–1198.
39. Genant HK, Cann CE, Ettinger B, Gordan GS 1982 Quantitative Computed Tomography of Vertebral Spongiosa: A sensitive method for detecting early bone loss after oophorectomy. Ann Intern Med **97:**699–705.
40. Ettinger B, Genant HK, Cann CE 1985 Long-term estrogen replacement therapy prevents bone loss and fractures. Ann Intern Med **102:**219–324.
41. Lindsay R, Aitken JM, Anderson JB, Hart DM, MacDonald EB, Clarke AC 1976 Long-term prevention of postmenopausal osteoporosis by estrogen. Lancet **1:**1038–1041.

Address reprint requests to:
Dr. Michael W. Draper
Lilly Research Laboratories (LRL)
Eli Lilly and Company
Lilly Corporate Center 2244
Indianapolis, IN 46285 U.S.A.

Received in original form June 10, 1995; in revised form January 27, 1996; accepted February 5, 1996.