Cardiovascular Effects of Droloxifene, a New Selective Estrogen Receptor Modulator, in Healthy Postmenopausal Women

David M. Herrington, Benjamin E. Pusser, Ward A. Riley, Tom Y. Thuren, K. Bridget Brosnihan, Eliot A. Brinton, David B. MacLean

Abstract—Selective estrogen receptor modulators, like tamoxifen and related compounds, have mixed estrogen agonistic/antagonistic effects. Tamoxifen may confer significant cardiovascular benefits without the estrogen-associated risks of endometrial and breast cancer. Droloxifene, a structural analogue of tamoxifen, has estrogen agonistic effects on bone and antagonistic effects on endometrial and breast tissue. Its cardiovascular effects in women are unknown. We enrolled 24 healthy postmenopausal women in a randomized, double-blind, 2-period crossover trial comparing the effects of droloxifene (60 mg/d) with conjugated estrogen (0.625 mg/d). Plasma lipids, coagulation and fibrinolytic factors, and brachial flow-mediated vasodilator responses were measured at the beginning and end of each treatment period. Droloxifene and estrogen resulted in 16.6% and 12.0% reductions, respectively, in low density lipoprotein cholesterol (P<0.001) and 13.2% and 9.5% reductions, respectively, in lipoprotein(a) (P<0.05). In contrast, estrogen, but not droloxifene, increased high density lipoprotein (18.5%, P<0.001). Droloxifene also reduced fibrinogen by 17.8% versus a 7.3% reduction with estrogen (P=0.004) but produced no estrogen-like changes in plasminogen, plasminogen activator inhibitor-1, or tissue plasminogen activator. Droloxifene and estrogen produced 36.4% and 27.3% increases, respectively, in flow-mediated vasodilation (percent change from baseline, P<0.05 for both). Droloxifene has estrogen agonistic properties regarding low density lipoprotein and lipoprotein(a) metabolism, certain coagulation factors, and endothelium-dependent vasodilation but, unlike estrogen, has no effect on high density lipoprotein/triglyceride metabolism and the fibrinolytic cascade. It remains unknown whether droloxifene can confer a true cardiovascular benefit. (Arterioscler Thromb Vasc Biol. 2000;20:1606-1612.)

Key words: droloxifene ■ hormone replacement therapy ■ women ■ estrogen ■ cardiovascular disease

Estrogen replacement therapy favorably influences several domains of vascular health, including lipid metabolism, endothelial function, and aspects of hemostasis. However, estrogen replacement also carries an increased risk of endometrial and possibly breast carcinoma. The recent Heart and Estrogen/Progestin Study trial also raises questions about the favorable effects of estrogen on the cardiovascular system may be offset by other, previously unrecognized, adverse cardiovascular effects, resulting in no overall clinical cardiovascular benefit.

Selective estrogen receptor modulators (SERMs), like tamoxifen, are compounds with mixed estrogen agonistic/antagonistic effects. Other benzothiophene derivatives, such as raloxifene, and other structurally unrelated compounds, such as soy phytoestrogens and tibolone, have variable degrees of estrogen agonistic and antagonistic effects. Tamoxifen lowers cholesterol, and in trials in women with breast cancer, it was associated with 15% to 63% fewer cardiovascular events. However, tamoxifen also increases the risk for endometrial hyperplasia/carcinoma and is therefore not an ideal cardioprotective agent. Raloxifene is a related compound with tamoxifen-like LDL-lowering effects. However, the chemical structure of raloxifene differs considerably from that of tamoxifen, and the effects of raloxifene on atherosclerosis are uncertain.

Droloxifene is a structural analogue of tamoxifen with tissue-specific estrogen agonistic/antagonistic effects. In ovariectomized rodents, it prevents bone loss and lowers cholesterol without causing uterine hypertrophy. Preliminary data suggest that it may be useful in advanced breast cancer. The cardiovascular effects of droloxifene in women are unknown. The purpose of the present study was to compare the effects of droloxifene with the effects of conventional estrogen replacement on measures of plasma lipids, coagulation and fibrinolytic factors, and brachial artery flow-mediated vasodilation in healthy postmenopausal women.
Methods

Study Subjects

Eligible subjects were healthy postmenopausal women aged 50 to 65 years. Postmenopausal status was defined as cessation of menses for ≥1 year or self-report of hysterectomy/oophorectomy with estradiol concentrations <25 pg/mL and follicle-stimulating hormone concentrations ≥40 IU/L. Women were ineligible if they had a fasting LDL cholesterol >200 mg/dL or a fasting triglyceride level >400 mg/dL, if they were receiving medical therapy for diabetes mellitus, or if their body mass index was >35 or <18 kg/m². Any woman with recurrent deep venous thrombosis or who had taken anticoagulant therapy for thromboembolic disease within 2 years was excluded. No women were currently taking angiotensin-converting enzyme inhibitors, anticoagulants (except aspirin), calcium channel blockers, nitrates, or anticonvulsants. Subjects could not have taken conjugated estrogen or another hormone replacement regimen within 3 months.

We recruited 25 women, and they gave written informed consent to participate. One woman withdrew after the first treatment period because of an exacerbation of previously diagnosed chronic hepatitis. We report results from the 24 women who completed the trial. The study was approved by our institution’s Clinical Research Practices Committee.

Study Design

Participants received oral conjugated equine estrogens (0.625 mg/d Premarin, Wyeth-Ayerst) or oral droloxifene (60 mg/d, Pfizer) during 2 randomly ordered 6-week treatment periods, separated by a 4-week washout (Figure 1). Investigators and participants were blinded to treatment assignments. Subjects took 3 tablets and 1 capsule every morning. The tablets contained either droloxifene (one 40-mg and two 10-mg tablets) or placebo, and the capsule contained either conjugated equine estrogen or placebo. Each subject received a 6-week supply of study medications for 1 regimen. After completing the first treatment period and the washout, each woman then received a 6-week pill supply for the other regimen. Compliance was ascertained by pill count at the end of each treatment period. Overall mean compliance by pill count was 97.8%. When the second 6-week treatment period was finished, all subjects received a 12-day supply of study medications for 1 regimen. After completion of each treatment period, subjects returned to the laboratory for additional measurements.

Plasma Lipid and Hemostatic Measurements

Fasting plasma specimens for lipoprotein determinations were obtained twice (on separate days) at the beginning and twice at the end of each treatment period. Results were averaged to improve statistical precision. Cholesterol and triglyceride analyses were performed on a Technicon RA-1000 autoanalyzer as described in the Technicon technical manual for cholesterol (SM4-0139A85) and for triglyceride (SM4-0189K87, glycerol phosphate-oxidase blank method). The cholesterol method is based on the enzymatic cholesterol procedures of Allain et al.18 and Roeschelau et al.17 and the peroxidase/4-aminophenazone system of Trinder.19 The triglyceride method was described by Fossati and Prencipe.20 Total glyceride levels in plasma were quantified. HDL cholesterol was measured by use of the heparin-manganese precipitation procedure.21,22 LDL cholesterol was calculated by using the Friedewald formula. Lp(a) measurements were made on a COBAS FarA II centrifugal autoanalyzer.22

Fibrinolytic and coagulation elements were measured by MDS Clinical Trial Laboratories. Fibrinogen,23 plasminogen,24 tissue plasminogen activator (tPA),25 antithrombin III,26,27 and plasminogen activator inhibitor (PAI-1)28 were measured by using previously described techniques.

Measurement of Brachial Artery Flow-Mediated Dilation

Details of the procedure have been published previously.29 Once the transducer position was established over the left brachial artery, baseline images were obtained for 2 minutes. The brachial artery was continuously imaged for the 4 minutes of blood pressure cuff occlusion and for 2 minutes immediately after cuff release. Nitroglycerin was not administered to avoid headaches and hypotension.

Ultrasound images were analyzed by using an automated analysis system30 that determines changes in brachial artery diameter for 2 minutes after flow stimulus (Figure 2). Diameter is defined as the average distance between the medial-adventitial boundaries for the segment of interest. Baseline and maximum diameter and the area under the diameter versus time curve (AUC) are automatically determined and stored in a database for further analyses. Reproducibility was previously determined to be R²=0.80, 0.84, and 0.78 for percent change, absolute change, and AUC, respectively.29

Statistical Analysis

Data are expressed as mean±SD. Repeated measures ANOVA models found no evidence of an order effect for baseline or change in any variable. Therefore, simple paired t tests were used to compare baseline versus follow-up values for each treatment. ANOVA was used to compare the effect of droloxifene versus estrogen within the same subjects. ANCOVA models were used to determine whether treatment-associated changes in brachial responses were independent of treatment-associated changes in plasma lipids or hemostatic factors. The primary outcome was prospectively defined as percent change in brachial diameter after the flow stimulus. On the basis of previous similar studies,29 sample size was estimated to detect at least a 30% treatment effect with 80% power at the 2-sided 0.05 level of significance.

Results

Lipids

Droloxifene and estrogen produced significant reductions in LDL cholesterol (16.6% and 12.0%, respectively; P<0.001 for each; Table 1, Figure 3). In contrast, estrogen produced a 18.5% increase in HDL cholesterol (P<0.001), whereas droloxifene had no effect. In addition, estrogen, but not droloxifene, produced a 22% increase in triglyceride levels (P=NS). The combined effects of estrogen on LDL and HDL resulted in a 24.3% reduction in the LDL/HDL ratio versus a 15.8% reduction with droloxifene (P<0.001 for both). The pattern of LDL reduction with no offsetting increase in triglycerides resulted in a 11.1% reduction in total cholesterol with droloxifene (P<0.001). Estrogen had no overall effect on total cholesterol. Droloxifene and estrogen each produced significant reductions in Lp(a) (13.2% and 9.5%, respectively; P<0.05 for both).

Hemostatic Factors

Droloxifene and estrogen significantly lowered fibrinogen and antithrombin III (P<0.01 for both factors and both treatments, Table 1). However, for fibrinogen, the droloxifene-associated reduction was greater than with the reduction with estrogen (17.8% versus 7.3%, P=0.004). In contrast, droloxifene had no effect on fibrinolytic elements, whereas estrogen significantly increased circulating plasminogen levels (P<0.001) and lowered circulating levels of PAI-1 (P=0.008) and tPA (P=0.02).
Brachial Flow-Mediated Vasodilator Responses
Droloxifene produced a 36.4% increase in response to flow stimulus (percent change, Figure 4) and a 42.9% increase in absolute change in diameter ($P<0.05$ for each, Table 2), whereas estrogen produced 27.3% and 29.4% improvements, respectively ($P=0.001$ for each). Two women showed no improvement with either regimen. Identical patterns were observed when the vasodilator response was quantified by using AUC. There were no statistical differences between any improvements realized with estrogen and droloxifene.

Correlation Between Vasodilator Responses and Other Outcomes
To determine whether treatment-associated improvements in brachial vasodilator responses were attributable to changes in plasma lipids or hemostatic factors, we examined the correlations between changes in brachial responses and other variables and developed ANCOVA models of treatment effects after adjusting for changes in plasma lipoproteins and hemostatic factors. There were no significant correlations between treatment-associated changes in any variables, and

<table>
<thead>
<tr>
<th>TABLE 1. Plasma Lipids and Hemostatic Factors Before and After 6 wk of Droloxifene or Estrogen (n=24)</th>
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<tbody>
<tr>
<td>Lipoproteins, mg/dL</td>
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<tr>
<td>Total cholesterol</td>
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<tr>
<td>LDL cholesterol</td>
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<td>HDL cholesterol</td>
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<tr>
<td>Triglycerides</td>
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<td>LDL/HDL ratio</td>
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<tr>
<td>Lp(a)</td>
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<tr>
<td>Hemostatic factors</td>
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<tr>
<td>Fibrinogen, g/L</td>
</tr>
<tr>
<td>Antithrombin III, U/mL</td>
</tr>
<tr>
<td>Plasminogen, U/mL</td>
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<tr>
<td>tPA-1, U/mL</td>
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<tr>
<td>tPA, ng/mL</td>
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</tbody>
</table>

Values are mean±SD. NS indicates not significant.
*Paired t test.
HDL and triglycerides. Clinical studies of raloxifene have sometimes accompanied by estrogen-like increases in plasma levels of HDL cholesterol or triglycerides. The modest LDL-lowering effect of droloxifene and other SERMs, and the absence of an HDL-elevating effect, may limit the lipid-mediated benefit of SERMs on cardiovascular risk. However, the lack of effect on triglyceride levels could be a benefit of SERMs versus estrogen, because elevated triglyceride levels are an important independent cardiovascular risk factor, especially in women.

The present study reveals a significant estrogen agonistic effect of droloxifene on Lp(a). Previous studies of tamoxifen and raloxifene have also shown an Lp(a)-lowering effect in healthy normal women. Lp(a) levels are related to angiographically defined coronary atherosclerosis, risk for myocardial infarction, and cerebrovascular disease. Elevated levels of Lp(a) may lead to atherosclerotic disease by interfering with LDL catabolism, promoting vascular smooth muscle cell proliferation, and attenuating plasmin formation. Thus, the ability of estrogen and certain SERMs, including droloxifene, to lower Lp(a) could be an important cardiovascular benefit.

**Effects of SERMs on Hemostasis**

Like estrogen replacement, tamoxifen and raloxifene lower fibrinogen by 10% to 20%, an effect that we confirmed for droloxifene. Fibrinogen levels are independently associated with coronary disease risk, perhaps because of its role in the coagulation cascade and acute coronary thrombosis. However, the Bezafibrate Infarction Prevention Study showed no effect of bezafibrate on cardiovascular mortality despite an 11.8% reduction in fibrinogen. Furthermore, lowering fibrinogen levels via drugs may not have the same effect as naturally low levels. van Baal et al observed an estrogen-associated 12% reduction in clottable fibrinogen but no change in fibrinogen antigen, suggesting that estrogen may act on fibrinogen clotting, not synthesis.

In ovariectomized rats (2.5 to 10 mg kg$^{-1}$ d$^{-1}$), droloxifene caused 65% to 70% reductions in total cholesterol, LDL cholesterol with little or no effect on HDL cholesterol or triglycerides. Clinical studies of raloxifene have demonstrated 5% to 14% reductions in LDL cholesterol with no effect on HDL cholesterol or triglycerides.

In ovariectomized rats, droloxifene caused 65% to 70% reductions in total cholesterol, LDL cholesterol with little or no effect on HDL cholesterol or triglycerides. The present study confirms a hypolipidemic effect in healthy postmenopausal women, and its magnitude (17% reduction in LDL cholesterol) is similar to that with tamoxifen and raloxifene. Like raloxifene, droloxifene also leaves plasma levels of HDL cholesterol or triglycerides unchanged. The modest LDL-lowering effect of droloxifene and other SERMs, and the absence of an HDL-elevating effect, may limit the lipid-mediated benefit of SERMs on cardiovascular risk. However, the lack of effect on triglyceride levels could be a benefit of SERMs versus estrogen, because elevated triglyceride levels are an important independent cardiovascular risk factor, especially in women.

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Effects of SERMs on Endothelial Function

The favorable effects of acute and chronic estrogen therapy on endothelium-dependent vasodilator responses are well documented in monkeys and postmenopausal women. In vitro, estrogen causes endothelial cell release of NO. Clinical studies have confirmed that the favorable effect of estrogen on brachial flow-mediated dilation is accompanied by increased NO production and release and can be inhibited by N\textsuperscript{\textbf{G}}-monomethyl-L-arginine, an NO synthase inhibitor.

Far fewer data are available regarding SERMs and endothelium-dependent vasodilator responses. In in vitro studies, tamoxifen and raloxifene have vasorelaxant effects similar to those of 17\textbeta-estradiol in comparable doses. These effects are inhibited by endothelium denudation and by coadministration of N\textsuperscript{\textbf{G}}-nitro-L-arginine methyl ester, another competitive NO synthase inhibitor, and by ICI 182,780, an estrogen receptor-\alpha antagonist. In contrast, in ovariectomized atherosclerotic monkeys, tamoxifen had no effects on coronary vasomotor responses to acetylcholine, whereas conjugated estrogen produced significant improvements.

In the present study, 60 mg droloxifene daily improved flow-stimulated brachial dilation in healthy women as much as 0.625 mg conjugated estrogens daily. Numerous studies have documented that flow-stimulated vasodilation is an endothelium-dependent response mediated in part by NO production. The roughly 30\% improvement in brachial response with droloxifene and estrogen is comparable to improvements with estrogen in studies using a variety of techniques. The fact that our subjects were healthy, the treatment period was brief, and there was a lack of correlation between vasodilation and plasma lipids suggests a direct effect of the vessel wall, not an effect secondary to lipid-mediated changes in atherosclerosis.

A randomized, blind, crossover study is an efficient means to observe the effects of multiple therapies within the same subjects. Nonetheless, the relatively few subjects hampers statistical inferences concerning subtle differences between the 2 hormone regimens. In addition, on the basis of this short-term study, we cannot say whether the effects of droloxifene will persist, increase, or decrease over time. Finally, we did not comprehensively evaluate the effects of droloxifene on hemostasis or vasomotor tone. For example, we did not evaluate the effects of droloxifene on endothelium-independent vasodilation or metabolism of other endothelial modulators of vascular tone, eg, endothelin or prostaglandins. In addition, we did not determine whether droloxifene or estrogen altered the magnitude of the flow stimulus that was due to distal hyperemia, although several other investigators have failed to observe such an effect.

In summary, droloxifene has estrogen agonistic properties on LDL and Lp(a) metabolism, certain coagulation factors, and endothelium-dependent vasodilation. This is the first evidence in women of an estrogen agonistic effect on endothelium-directed vasodilation with a SERM. However, droloxifene had no estrogen agonistic activity on HDL or triglyceride metabolism or on the expression of key elements of the fibrinolytic cascade. It remains unknown whether the selective profile of cardiovascular effects from this or other SERMs will translate into fewer cardiovascular events. Moreover, preliminary data (Pfizer Central Research, unpublished data, 2000) suggest that droloxifene, like raloxifene and estrogen, may also increase the risk for venous thromboembolic events. Thus, the efficacy and safety of droloxifene and other SERMs should be evaluated in clinical trials before recommendations can be made concerning their use for the prevention of heart disease.

Acknowledgments

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References


TABLE 2. Brachial Flow-Mediated Vasodilator Responses Before and After 6 wk of Droloxifene or Estrogen (n=24)

<table>
<thead>
<tr>
<th>Vasodilator Response</th>
<th>Droloxifene Baseline</th>
<th>Follow-Up</th>
<th>P*</th>
<th>Estrogen Baseline</th>
<th>Follow-Up</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting diameter, mm</td>
<td>3.71±0.50</td>
<td>3.72±0.51</td>
<td>NS</td>
<td>3.72±0.49</td>
<td>3.71±0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Absolute diameter change, mm</td>
<td>0.14±0.08</td>
<td>0.20±0.11</td>
<td>0.04</td>
<td>0.17±0.06</td>
<td>0.22±0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>Diameter change, %</td>
<td>3.98±2.30</td>
<td>5.43±2.89</td>
<td>0.03</td>
<td>4.58±2.01</td>
<td>5.83±1.97</td>
<td>0.01</td>
</tr>
<tr>
<td>AUC, mm·s</td>
<td>25.49±19.26</td>
<td>37.85±22.09</td>
<td>0.03</td>
<td>30.80±18.04</td>
<td>42.60±19.36</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are mean±SD. *Paired t test (baseline vs follow-up).


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