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/Progesterone Study trial also raises questions about whether 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.
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

\begin{table}
\centering
\begin{tabular}{lccc}
\hline
 & Droloxifene & & Estrogen & \\
 & Baseline & Follow-Up & $P^*$ & Baseline & Follow-Up & $P^*$ \\
\hline
Lipoproteins, mg/dL & & & & & \\
Total cholesterol & 208.1±35.4 & 184.4±29.6 & <0.001 & 204.8±31.0 & 200.4±26.7 & NS \\
LDL cholesterol & 131.3±26.8 & 109.3±24.3 & <0.001 & 126.8±25.5 & 110.3±19.4 & <0.001 \\
HDL cholesterol & 52.3±8.9 & 51.2±8.5 & NS & 51.8±9.8 & 60.6±10.3 & <0.001 \\
Triglycerides & 122.8±68.2 & 119.7±62.4 & NS & 131.0±84.0 & 144.6±60.7 & NS \\
LDL/HDL ratio & 2.60±0.78 & 2.20±0.69 & <0.001 & 2.55±0.74 & 1.87±0.45 & <0.001 \\
Lp(a) & 27.2±30.3 & 23.6±23.9 & 0.033 & 26.3±27.8 & 23.8±27.5 & 0.014 \\
Hemostatic factors & & & & & \\
Fibrinogen, g/L & 350.4±62.0 & 287.9±57.9 & <0.001 & 355.8±55.9 & 330.0±60.4 & 0.017 \\
Antithrombin III, U/mL & 1.05±0.13 & 0.95±0.15 & 0.003 & 1.05±0.14 & 0.98±0.13 & 0.011 \\
Plasminogen, U/mL & 1.03±0.16 & 1.01±0.14 & NS & 1.03±0.14 & 1.14±0.21 & <0.001 \\
PAI-1, U/mL & 9.3±3.1 & 9.4±2.9 & NS & 10.7±3.0 & 8.8±4.6 & 0.008 \\
TPIA, ng/mL & 12.5±10.8 & 9.1±5.3 & NS & 13.4±11.5 & 7.5±6.3 & 0.02 \\
\hline
\end{tabular}
\caption{Plasma Lipids and Hemostatic Factors Before and After 6 wk of Droloxifene or Estrogen (n=24)}
\end{table}

Values are mean±SD. NS indicates not significant.
*Paired t test.
the statistically significant effects of each regimen on brachial artery flow-mediated responses remained.

**Discussion**

Droloxifene has several important estrogen agonistic effects on the cardiovascular system in healthy postmenopausal women, including effects on LDL metabolism, Lp(a), and coagulation factors, but little or no effect on HDL and triglyceride metabolism or regulation of the fibrinolytic cascade. In addition, its estrogen agonistic effects on endothelium-dependent vasodilation suggest that droloxifene may favorably influence endothelial cell nitric oxide (NO) metabolism, thereby adding to its prospects as a cardioprotective agent. However, it remains unknown whether these effects will translate into a cardiovascular benefit.

**Lipid Effects of SERMs**

Observational studies and clinical trials have demonstrated modest LDL-lowering effects of tamoxifen in normal postmenopausal women, including effects on LDL metabolism, Lp(a), and coagulation factors, but little or no effect on HDL and triglyceride metabolism or regulation of the fibrinolytic cascade. In addition, its estrogen agonistic effects on endothelium-dependent vasodilation suggest that droloxifene may favorably influence endothelial cell nitric oxide (NO) metabolism, thereby adding to its prospects as a cardioprotective agent. However, it remains unknown whether these effects will translate into a 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 clotable fibrinogen but no change in fibrinogen antigen, suggesting that estrogen may act on fibrinogen clotting, not synthesis.

However, in some, but not all, studies, estrogen replacement, tamoxifen, tamoxifen, and raloxifene also reduced antithrombin III levels, potentially a prothrombotic effect. Only limited data are available concerning the effects of estrogen or SERMs on the activation of the coagulation cascade. Caine et al reported significant increases, and Walsh et al reported nonsignificant trends toward higher levels of prothrombin fragments 1 and 2 and fibrinopeptide A with estrogen replacement therapy. In the present study, droloxifene and estrogen lowered antithrombin III levels by 7% and 10%, respectively, but also lowered fibrinogen 18% and 7%, respectively. On the basis of these limited data, the net effect on risk for arterial thrombosis is unknown.

In contrast, droloxifene had no estrogen agonistic effect on fibrinolytic factors. In the present and other studies, estrogen replacement raised plasminogen and lowered PAI-1 levels. This may occur directly via PAI-1 gene transcription or indirectly through an angiotensin-converting enzyme-inhibiting effect of estrogen, which lowers angiotensin II, a known potent stimulus for PAI-1 synthesis. Estrogen also lowers tPA levels; however, because most tPA is complexed to PAI-1, this reduction in tPA likely reflects lower PAI-1 levels and not a reduced capacity for plasminogen activation. Thus, by increasing plasminogen and enhancing its conversion to plasmin, estrogen could promote fibrinolysis. If these estrogenic effects on the fibrinolytic system are important modulators of heart disease risk, they are apparently not shared with droloxifene and other tamoxifen analogues.
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 Nω-nitro-arginine methyl ester, another competitive 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 17β-estradiol in comparable doses. These effects are inhibited by endothelium denudation and by coadministration of Nω-nitro-arginine methyl ester, another competitive NO synthase inhibitor, and by IC182,780, an estrogen receptor 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 on 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 hemostasis or vasomotor tone. 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.

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Herrington et al. Cardiovascular Effects of Droloxifene 1611


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