Comparison of Tibolone and Conjugated Equine Estrogens Effects on Carotid Artery Atherosclerosis of Postmenopausal Monkeys

Thomas B. Clarkson, DVM; Mary S. Anthony, PhD; Tomi S. Mikkola, MD, PhD; Richard W. St Clair, PhD

Background and Purpose—Tibolone is a tissue-specific compound that has favorable effects on bone and menopausal symptoms without stimulating endometrium or breast, but lowers concentrations of plasma high-density lipoprotein (HDL) cholesterol (HDLC). This study was designed to determine whether the HDL lowering with tibolone exacerbated common or internal carotid artery atherosclerosis and to evaluate tibolone treatment relative to conjugated equine estrogens (CEE) alone or in combination with medroxyprogesterone acetate (MPA).

Methods—Carotid artery atherosclerosis was compared in groups of surgically postmenopausal cynomolgus monkeys treated with CEE, CEE+MPA, or either of 2 doses of tibolone versus untreated monkeys.

Results—Despite a 30% to 52% lowering of HDLC with tibolone, there was no significant effect on carotid artery atherosclerosis. CEE and CEE+MPA, however, inhibited carotid artery atherosclerosis by ≈60%.

Conclusions—In surgically postmenopausal cynomolgus monkeys, CEE and CEE+MPA inhibited common and internal carotid artery atherosclerosis. Despite the potentially adverse effects of tibolone on HDLC, tibolone did not exacerbate atherosclerosis. (Stroke. 2002;33:2700-2703.)

Key Words: carotid atherosclerosis ■ conjugated equine estrogens ■ hormone replacement therapy ■ tibolone ■ monkeys

Ischemic stroke among middle-aged and older women is an important public health concern.1 The exact pathogenesis of these events remains uncertain; however, positive associations with increasing atherosclerosis extent and inverse associations with concentrations of plasma high-density lipoprotein (HDL) cholesterol (HDLCH) seem clear.2-4 Although the effect of traditional hormone replacement therapy on stroke prevalence is controversial,5-8 it is important to know whether newer menopausal therapies have either adverse or beneficial effects on atherosclerosis of common and internal carotid arteries.

Tibolone, used widely in several countries for the treatment of menopausal symptoms9,10 and for the prevention of postmenopausal osteoporosis,11,12 is being considered for use in the United States. Tibolone is metabolized into 3 biologically active metabolites; the 3-β hydroxy metabolite and the 3-α hydroxy metabolite have estrogen agonist properties, whereas the δ-4 ketoisomer has progestogenic and androgenic effects. The δ-4 isomer, produced primarily within the endometrium, protects the endometrium from the agonist effects of the 2 estrogenic metabolites,13,14

Tibolone treatment of postmenopausal women has some beneficial effects on plasma lipid/lipoprotein concentrations (reductions in plasma triglyceride15,16 and lipoprotein[a] concentrations)17; however, concern has arisen about its “cardiovascular safety” because of reductions in HDLC.15,18

We have reported the long-term effects of tibolone on coronary artery atherosclerosis of surgically postmenopausal cynomolgus monkeys relative to the effects of conjugated equine estrogen (CEE) treatment and treatment with CEE plus medroxyprogesterone (MPA) administered continuously.19 Reported herein are the common carotid and internal carotid artery atherosclerosis results from that long-term study. Specifically, we investigated whether the large decreases in HDLC associated with tibolone treatment would influence the progression of common carotid and internal carotid artery atherosclerosis and evaluated the effects of widely used postmenopausal hormone replacement therapies (CEE or CEE+MPA).

Methods
Details about the animals, diets they were fed, and methods used to evaluate plasma lipid and lipoprotein concentrations have been
Clarkson et al Tibolone Does Not Increase Carotid Atherosclerosis 2701

published.\(^\text{19}\) Briefly, 151 surgically postmenopausal cynomolgous monkeys (Macaca fascicularis) were fed a moderately atherogenic diet (0.28 mg cholesterol/cal) and were given either no treatment (control), CEE alone (Premarin, Wyeth-Ayerst) at a dose comparable to a woman’s dose of 0.625 mg/day, or CEE plus MPA added at a dose comparable to a woman’s dose of 2.5 mg/day. There were 2 groups treated with tibolone (Org OD 14, Organon). The low dose (Lo Tib) was intended to approximate a dose for women of \(\approx 1\) mg/day. The high dose (Hi Tib) approximated a dose for women of \(\approx 2.5\) to 3 mg/day. All of the hormone treatments were added to a diet providing the dose desired in 1800 cal of diet (based on the assumptions that the average US woman consumes 1800 cal/day, thus accounting for the difference in metabolic rates of monkeys and women). The treatment period was 2 years (equivalent to 6 years of human treatment). All procedures involving animals were conducted in compliance with state and federal laws, standards of the US Department of Health and Human Services, and guidelines established by the Wake Forest University Institutional Animal Care and Use Committee.

Necropsy Procedures and Carotid Artery Atherosclerosis Evaluations

Monkeys were euthanized using sodium pentobarbital (100 mg/kg administered intravenously). The right common and right internal carotid arteries were pinned flat on cardboard and fixed in 10% neutral buffered formalin. The left common carotid arteries were removed 6 months earlier for a previous experiment. Our intent for that experiment was to evaluate the effect of the treatments on the response of arteries to injury. We used air injury as the model and determined that the degree of injury was not consistent among the subjects in the study and therefore the results were not interpretable. From the right common carotid arteries we took 3 blocks (each 3 mm in length) cut perpendicular to the long axis of the common carotid artery and 2 blocks (each 3 mm in length) from the internal carotid artery.

Each of the blocks was embedded in paraffin and 5-μm sections were made from each block and stained with Verhoeff-van Gieson stain. Each of the sections was projected onto a digitizer plate, and the intimal area (composed of fatty streaks and atherosclerotic plaques) was measured as described previously.\(^\text{20}\) Measurements were made blind to treatment by 1 technician with more than 20 years experience and randomly reevaluated by one of us (T.B.C.). Because of difficulties with tissue processing, data for common carotid artery atherosclerosis for 4 animals were not available. The remaining 147 animals were distributed into the following treatment groups: control, \(n=31\); CEE, \(n=27\); CEE+MPA, \(n=29\); Lo Tib, \(n=30\); and Hi Tib, \(n=30\). Internal carotid artery atherosclerosis data were available for 141 animals distributed as follows: control, \(n=30\); CEE, \(n=25\); CEE+MPA, \(n=28\); Lo Tib, \(n=29\); and Hi Tib, \(n=29\).

Statistical Methods

Analyses were done using BMDP Statistical Software (version 7.0) or Statistical Analysis Software (version 6.08; SAS Institute, Inc.). All variables were first evaluated for their distribution and equality of variances between groups. Log transformations were done for variables that violated the test of equal variances (common carotid and internal carotid intimal area). Average intimal area was calculated for each artery from the mean of 3 sections from the common carotid artery and 2 sections for the internal carotid artery, after verifying that the treatment effects were consistent for the length of the artery. Carotid artery measurements were analyzed by ANCOVA using baseline low-density lipoprotein (LDL)+very low-density lipoprotein cholesterol (VLDLC) concentrations as a covariate, because it was significantly associated with outcome atherosclerosis. The data in this report are means and calculated SEM retransformed into original units. One-way ANOVA and ANCOVA were used to test for differences among groups. \(t\) tests were used for post hoc comparisons (control versus each treatment group) if the ANOVA or ANCOVA probability value was significant. An \(\alpha\) level of <0.05 was used for statistical significance.

Results

Plasma Lipid and Lipoprotein Changes

We have previously published the plasma lipid, lipoprotein, and apolipoprotein concentrations of the animals in this study.\(^\text{19}\) The major changes were in LDL+VLDLC and HDLC and are summarized in Figure 1 as the percentage change from control. CEE and CEE+MPA produced a nearly 20% decrease in LDL+VLDLC reducing HDLC \(\approx 12\%\). Lo Tib had no effect on LDL+VLDLC, whereas with Hi Tib there was a 16% increase. Tibolone produced a dose-dependent 30% or 52% decrease in HDLC.

Common Carotid Artery Atherosclerosis

The mean cross-sectional area of atherosclerosis in the common carotid artery intima is shown in Figure 2A. Both CEE and CEE+MPA treatments resulted in plaque areas that were significantly smaller, by 64% (\(P=0.005\)) and 65% (\(P=0.004\)), respectively, than those of control. The plaque areas of the tibolone-treated groups were not different from those of the control group (Lo Tib, \(P=0.98\); Hi Tib, \(P=0.61\)). All of the animals (100%) in the control, CEE, CEE+MPA, and Lo Tib groups and 97% of the animals in the Hi Tib group had some degree of atherosclerosis in the common carotid artery.

Internal Carotid Artery Atherosclerosis

The mean cross-sectional area of atherosclerosis in the internal carotid artery intima was \(<1\%\) of that in the common carotid (Figure 2A versus 2B). CEE and CEE+MPA treatment resulted in plaque size in these arteries that was significantly smaller, by 92% (\(P=0.001\)) and 86% (\(P=0.004\)), respectively (Figure 2). The tibolone-treated groups were not different from control (Lo Tib, \(P=0.83\); Hi Tib, \(P=0.84\)). The high variability within and across treatment groups for the internal carotid arteries was primarily due to a relatively large number (>50%) of cases that were unaffected with either fatty streaks or plaques. Because of this, we also determined the prevalence of atherosclerosis in the internal carotid artery (ie, percentage of cases affected with any atherosclerosis). Atherosclerosis was present in 40% of the control animals, whereas only 12% in the CEE group were affected (\(P=0.02\)). Fewer cases were affected in the

Figure 1. Plasma LDL+VLDLC and HDLC (percentage difference from control group). Data are means of quarterly evaluations done over the 2-year experimental period. More detailed data on plasma lipid measurements have been reported elsewhere.\(^\text{19}\)
increase LDLC concentrations. It should be noted that the potential of tibolone for inhibiting atherosclerosis in human subjects, because the HDLC decreases were greater than those seen in women, and in women tibolone does not increase LDL concentrations by an average of 52%, there was no exacerbation of either common or internal carotid artery atherosclerosis or, as described elsewhere, coronary artery atherosclerosis. This finding may not fully reflect the exacerbation of either common or internal carotid artery atherosclerosis or, as described elsewhere, coronary artery atherosclerosis. This finding may not fully reflect the potential of tibolone for inhibiting atherosclerosis in human subjects, because the HDLC decreases were greater than those seen in women, and in women tibolone does not increase LDL concentrations. It should be noted that the progression of atherosclerosis in the internal carotid arteries of cynomolgus monkeys is slower than that of other arterial sites such as the coronary arteries. Consequently, the atherosclerotic lesions that were quantified here were quite small. One cannot be certain about the relevance of early arterial changes with respect to atherosclerosis later in life; however, it seems reasonable to speculate that the presence of atherosclerotic plaques early in life would predict the development of larger plaques at a later age. Another point worth noting is that we do not know whether, and to what extent, having removed the left carotid arteries from these monkeys (6 months into the study) changed blood flow in the right carotid arteries or whether there were effects on atherosclerosis progression.

The ≈60% decrease in both common and internal carotid artery atherosclerosis by CEE treatment is consistent with a previous study. In that study, CEE reduced common carotid artery atherosclerosis by ≈50% (P=0.0001) and that of the internal carotid artery by ≈70% (P=0.0003). Thus, both monkey studies provide some pathologic rationale for the conclusion reached by Paganini-Hill, from a review of 7 studies, that postmenopausal estrogen users may have reduced stroke risk. The monkey studies are also consistent with the recent observation of Grodstein et al. who noted a reduced risk from all strokes among women treated with low-dose CEE (0.3 mg CEE/day).

Our group has reported previously that MPA appeared to attenuate the inhibition of coronary artery atherosclerosis by CEE. In the current study we did not find attenuation of the CEE effect by MPA on common and internal carotid artery atherosclerosis (Figure 2). This is consistent with observational studies that considered combined therapy and stroke.

The finding of a lack of exacerbation of carotid artery atherosclerosis associated with the tibolone-induced reductions in HDLC is consistent with our earlier findings in which a decrease in HDLC in monkeys treated with contraceptive steroids did not exacerbate atherosclerosis. In that report we suggested that there were plasma lipid–independent effects of ethinyl estradiol that protected the coronary arteries from the deleterious effects of HDLC reductions. It is possible that by similar mechanisms, the 3-α and 3-β hydroxy estrogen metabolites of tibolone are acting directly on the artery wall to modify potential negative effects associated with the low HDLC. Additionally, the lack of an effect on atherosclerosis of the lower HDLC could be a result of the mechanism by which tibolone lowers HDLC. If the HDLC lowering is secondary to a more rapid clearance, this generally does not exacerbate atherosclerosis, probably because reverse cholesterol transport is not diminished, even though the steady-state HDLC concentrations are reduced. Another possible explanation for the tibolone-induced lowering of HDL not increasing atherosclerosis is that there may be a change in the composition of the HDL that makes it more efficient in promoting efflux of cellular cholesterol. We cannot eliminate this possibility because we did not do compositional or subclass analysis on the HDL in this study, but we did find that in vitro cholesterol efflux using sera from these tibolone-treated animals was not reduced in proportion to HDL lowering.

The fact that tibolone did not increase or decrease atherosclerosis must be considered in light of what appear to be beneficial effects of tibolone on the mammary gland and the...
endometrium as compared with CEE. As a part of this multisystem monkey trial, Cline et al. found that tibolone did not stimulate the endometrium or breast of monkeys treated with either low or high tibolone, whereas CEE treatment increased both endometrial proliferation and mammary gland epithelial area. CEE + MPA treatment partially inhibited the endometrial effects of the CEE but tended to increase the mammary epithelial area.

In summary, there are important similarities and differences between tibolone and CEE. Based on literature reports, the 2 treatments are comparable for relief of menopausal symptoms and prevention of postmenopausal bone loss. Tibolone does not require the coadministration of a progestin, as CEE requires a progestin to protect the endometrium, is mammotrophic, and may increase breast cancer risk. CEE inhibits the progression of coronary, common carotid, and internal carotid artery atherosclerosis, whereas tibolone neither inhibited nor exacerbated atherosclerosis.

Acknowledgments

This study was funded in part by a grant from Organon (Oss, the Netherlands). We thank Dick Meuleman, PhD (Organon), for his scholarly and thoughtful advice at many stages of this study. We also thank Matthew Dwyer, Timothy Vest, Maryanne Post, and Misti Binns for their excellent technical contributions.

References

Comparison of Tibolone and Conjugated Equine Estrogens Effects on Carotid Artery Atherosclerosis of Postmenopausal Monkeys
Thomas B. Clarkson, Mary S. Anthony, Tomi S. Mikkola and Richard W. St Clair

Stroke. 2002;33:2700-2703
doi: 10.1161/01.STR.0000033130.82164.24
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/33/11/2700

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/