Much data from animal research\(^1\) and clinical studies\(^2\)–\(^4\) indicate that estrogen can slow the development of atherosclerosis by inhibiting atherogenesis. Treatment with 17β-estradiol (E\(_2\)) markedly inhibits the initiation and progression of coronary atherosclerotic plaques in several animal models of atherosclerosis.\(^1\) These studies have not only established the atheroprotective effects of E\(_2\) but have also shed light on the mechanisms of atheroprotection. Estrogen lowers major risk factors for developing atheroma plaques, such as lowering the risk for developing hypercholesterolemia. Initiation of the atherogenic response is promoted by hypercholesterolemia because chronic high levels of cholesterol in the bloodstream lead to prolonged retention of low-density lipoproteins in the subendothelial space.\(^4\)\(^,\)\(^5\)

The rennin–angiotensin system (RAS) modulates several components of atherosclerotic process, including inflammation, oxidative stress, and hypertrophy of the vascular wall,\(^6\) and estrogen modulates most of, if not all, the components of the RAS cascade, including the synthesis of angiotensin II (Ang II), the key mediator of the RAS, and the 2 receptor subtypes (AT\(_1\) and AT\(_2\)) that mediate Ang II action. Both animal\(^7\) and clinical studies\(^8\) demonstrate that estrogen inhibits angiotensin-converting enzyme activity, resulting in decreased levels of Ang II in the circulation and in specific tissues, including the aorta. Furthermore, E\(_2\) reduces the membrane density of AT\(_1\) receptors in many Ang II target tissues, including the vasculature,\(^9\) which is the same receptor that is targeted clinically to inhibit the progression of atherosclerosis through the use of AT\(_1\) receptor antagonists.

The NO system is another signaling pathway that is regulated by estrogen and that plays a key role in modulating atherosclerosis. Estrogen upregulates the activity and expression of the endothelial isoform of NO synthase (eNOS). The resulting increase in endothelium-derived NO has many beneficial tissue-specific estrogenic effects, including inhibition of leukocyte adhesion and vascular smooth muscle cell (VSMC) proliferation and vasodilation and blood pressure reduction.\(^10\)\(^,\)\(^11\) E\(_2\) increases eNOS mRNA and protein expression through transcriptional stimulation of eNOS gene expression\(^12\)\(^,\)\(^13\) and eNOS activity through rapid nongenomic mechanisms involving the phosphatidylinositol-3-OH kinase-phosphokinase B-Akt mediated signaling pathway.\(^14\) However, the fact that E\(_2\) is still protective against the development of atherosclerosis in the eNOS\(^{−/−}\) ApoE\(^{−/−}\) knockout mouse suggests that E\(_2\) exerts powerful atheroprotective effects, which are independent of the NO system.\(^15\)

Although a wealth of epidemiological observations and laboratory research indicate that estrogen has cardioprotective effects, the atheroprotective value of estrogen replacement therapy (ERT) have been challenged recently by the negative results of randomized clinical trials in postmenopausal women. A few years ago, the Women’s Health Initiative trial for the primary prevention of cardiovascular disease by estrogen (WHI) was interrupted prematurely because of a small trend toward an increase in cardiovascular risk in healthy women and a slight increase in the incidence of invasive breast cancer.\(^16\) The authors also noted that the current use of postmenopausal ERT resulted in a small increased risk for venous thrombosis. The negative results of this major clinical trial resulted in a 40% reduction in ERT use in this country even among women who were being treated for other postmenopausal symptoms rather than for cardiovascular protection. Recent research has focused on investigating the reasons behind the surprising lack of cardiovascular protection in the ERT trials from a number of different perspectives including: (1) varying the method of ERT administration (transdermal versus oral) and lowering the dose; (2) initiating ERT at the onset of perimenopause rather than starting ERT several years after menopause has been established; and, (3) varying the type of estrogen in the ERT, including the use of naturally occurring estrogens (eg, E\(_2\) and its metabolites), phytoestrogens (plant estrogens), and xenoestrogens (chemically modified estrogens) such as selective estrogen receptor modulators (SERMs) like raloxifene.

Much of the scientific effort of late has been directed toward chemically modifying SERMs so that they possess the beneficial tissue-specific estrogenic effects, but without any adverse estrogenic effects (eg, breast or uterine cancer and increased incidence of venous thrombosis). However, others have made the case that naturally occurring estrogens offer distinct safety advantages over xenoestrogens and deserve exploration for use in ERT. The major circulating estrogen in the human body is E\(_2\), which is rapidly metabolized to 2-methoxyestradiol (2-ME) by methylation. 2-ME has been shown to exert growth-inhibitory effects via an estrogen receptor (ER)-independent mechanism. 2-ME potently inhibits tumor growth and angiogenesis in tumor cell lines devoid of ERs, and an ongoing phase II
clinical trial is currently investigating whether the experimental antitumor effects of 2-ME can be translated to the clinic. Because of its growth-inhibitory properties, scientists hypothesized that 2-ME has similar growth inhibitory effects on VSMCs and thus may possess therapeutic utility for preventing vascular remodeling, atherosclerosis, and injury-induced restenosis. In fact, 2-ME has been shown to inhibit cell migration and proliferation in rat and human VSMCs regardless of whether the stimulus for these processes is serum, platelet-derived growth factor, endothelin-1, or Ang II.18

In this issue of Circulation Research, Barchiesi et al provide the first in vivo evidence that 2-ME protects against injury-induced neointima formation, thus demonstrating the atheroprotective effects of this E2 metabolite. Using flow cytometry studies, the authors demonstrated that 2-ME inhibits its cell division by inducing a concentration-dependent inhibition of the G1/M to G1/G0 transition in the cell cycle. Furthermore, they provide evidence that the potential mechanism for this cell cycle effect involves: (1) inhibition of the expression and activation of cyclin and cyclin-dependent kinases (cdk), which are key proteins responsible for the progression of cell replication and include cyclin-D/ cdk4 as well as retinoblastoma tumor-suppressor protein phosphorylation that is essential for initiation of the cell cycle into the G1 phase and cyclin B/ cdk1, which is essential for G2 to M cell cycle progression (Figure 1); (2) upregulation of the expression of the cdk inhibitor p27 (Figure 1); (3) inhibition of tubulin polymerization, which is a critical step in the mitotic process; and (4) upregulation of the expression of cyclooxygenase-2, which is known to mediate antiproliferative actions in VSMC via prostacyclin synthesis.

Although the authors have made a major contribution to our understanding of the intracellular signaling pathways by which 2-ME acts to inhibit vascular wall thickness, what we still do not know is the first step whereby 2-ME triggers these pathways. To date, no specific receptors for 2-ME have been found, and the authors conclude that the antiproliferative effects of 2-ME in the vascular wall are not mediated by ER activation based on their previous studies showing that the effects of 2-ME on cell proliferation are not blocked by the ER antagonist ICI182780.20,21 Barchiesi et al go on to propose the novel idea that 2-ME is acting as an antiproliferative agent via its interaction with colchicine-binding sites on tubulin.22 Agonist binding to these sites is known to interfere with the dynamics of tubulin polymerization resulting in the dissolution of the mitotic spindle, which is crucial in bringing the new sets of chromosomes toward the opposite poles of the cell.23 Many intracellular signaling pathways require intact microtubule networks to be specific and efficient, perhaps through microtubule-mediated translocation of the signal to the nucleus and through activation of mitogen-activated protein kinase (MAPK).24 There is also evidence that tubulin/G-protein complexes can interact to regulate second-messenger cascades, as is the case for phospholipase C signaling.25

Presently, it is not clear whether inhibition of tubulin polymerization represents the first step and primary mechanism by which 2-ME regulates the expression and activation of the key proteins involved in the proliferation of VSMC (as identified by the authors). It is possible that 2-ME indirectly influences protein transcription and activation through its antioxidant properties since 2-ME contains a phenolic functional group that is known to scavenge reactive oxygen species (ROS).26 The fact that the proliferative effects of several mitogens, including Ang II, are partially mediated by ROS generation27 and that ROS are known to activate key signaling pathways involved in transcription and cell growth (including MAPK and the nuclear factor NFkB),28 suggests that the antioxidant potential of 2-ME could also contribute to the atheroprotective effects described by Barchiesi et al (Figure 2).

The observations from Barchiesi et al raise the possibility that 2-ME replacement therapy holds promise for preventing cardiovascular disease and may provide valuable advantages over treatment with estradiol or specific SERMs. One major benefit to using 2-ME for ERT is the lack of ER-mediated actions. Thus, it is plausible that 2-ME could be used clinically to prevent or treat cardiovascular disease in women without increasing the risk of breast or uterine cancer. Furthermore, 2-ME would have no feminizing effects, and thus could be also used in men. However, before we conclude that 2-ME is the ERT for the next generation regardless of...
one’s sex, much work needs to be done to determine if any other of the undesirable effects of estrogens in the cardiovascular system are retained by 2-ME, such as increasing thrombotic phenomena.26 Although the signaling pathways activated by estradiol and 2-ME are apparently different in VSMC, it will be critical to examine the effects of 2-ME on modulating thrombotic pathways, especially since the mechanisms by which E2 affects homeostasis favoring procoagulation and impairing anticoagulation is unknown.29

The principal question raised by these novel studies of Barchiesi et al is to what extent do the antiproliferative effects of 2-ME reflect the metabolites protective action in preventing atherosclerosis? It is important to bear in mind that atherosclerosis is a progressive multifactorial disease, which is not only characterized by VSMC proliferation, but also by the accumulation of lipids and fibrous elements along with many features of chronic inflammation.30 The fact that 2-ME downregulates VSMC proliferation does not necessarily mean that 2-ME inhibits the atherosclerotic process sufficiently to significantly reduce cardiovascular risk. That being said, it is also of note that clinical treatment of diseases as complex as atherosclerosis often involve a multi-therapeutic approach. Thus, even if 2-ME does not prove to be a first line of defense against atherosclerosis, its potential for synergizing with other therapies, including statins and inhibitors of Ang II action, is indeed significant. Furthermore, the antiproliferative effects of 2-ME also may have clinical relevance in the prevention of injury-induced restenosis and neointima formation. Most importantly though, these studies set the groundwork for achieving a molecular understanding of the cell- and tissue-specific signaling pathways trigged by 2-ME with respect to the cardiovascular system, which ultimately may lead to the development of new therapeutics for the treatment of cardiovascular disease in both men and women.

Sources of Funding
The authors’ work is supported by research funds from the National Institute of Health R01HL57502 and R01AG19291 (to K.S.) and from the American Heart Association Scientist Development Grant and Discovery Fund for Aging Research (to A.P.D.).

Disclosures
None.

References


**KEY WORDS:** hormone replacement therapy ■ estrogen metabolites ■ atherosclerosis ■ VSMC growth
Does 2-Methoxyestradiol Represent the New and Improved Hormone Replacement Therapy for Atherosclerosis?

Ana Paula V. Dantas and Kathryn Sandberg

doi: 10.1161/01.RES.0000236802.00855.cd

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/99/3/234