Estrogen Receptors and Endothelium

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Abstract—Estrogens, and in particular 17β-estradiol (E2), play a pivotal role in sexual development and reproduction and are also implicated in a large number of physiological processes, including the cardiovascular system. Both acetylcholine-induced and flow-dependent vasodilation are preserved or potentiated by estrogen treatment in both animal models and humans. Indeed, E2 increases the endothelial production of nitric oxide and prostacyclin and prevents early atheroma through endothelial-mediated mechanisms. Furthermore, whereas it prevents endothelial activation, E2 potentiates the ability of several subpopulations of the circulating or resident immune cells to produce proinflammatory cytokines. The balance between these 2 actions could determine the final effect in a given pathophysiological process. E2 also promotes endothelial healing, as well as angiogenesis. Estrogen actions are essentially mediated by 2 molecular targets: estrogen receptor-α (ERα) and ERβ. The analysis of mouse models targeted for ERα or ERβ demonstrated a prominent role of ERα in vascular biology. ERα directly modulates transcription of target genes through 2 activation functions (AFs), AF-1 and AF-2. Interestingly, an AF-1-deficient ERα isofrom can be physiologically expressed in the endothelium and appears sufficient to mediate most of the vasculoprotective actions of E2. In contrast, AF-1 is necessary for the E2 actions in reproductive targets. Thus, it appears conceivable to uncouple the vasculoprotective and sexual actions with appropriate selective ER modulators. (Arterioscler Thromb Vasc Biol. 2010;30:1506-1512.)

Key Words: atherosclerosis ■ endothelial function ■ endothelium ■ immune system ■ estrogen ■ hormone ■ menopause

The role of estrogens, particularly the main endogenous one, which is 17β-estradiol (E2), is well described for sexual development and reproduction, but estrogens are also involved in many physiological processes, including the cardiovascular system. The presence of estrogen receptors (ER) in vascular tissues was originally demonstrated by specific binding of estrogens to vascular cells.1 The macrovascular endothelium appears to be a major direct target for estrogens. Indeed, estrogens have been found to be protective of the endothelium in various pathophysiological situations. Furthermore, cultured endothelial cells can produce/synthesize estrogens via the aromatization of androgens.2 We will first summarize the works describing that E2, on the one hand, prevents the activation of endothelium in response to various injuries, whereas on the other hand, it potentiates the production of proinflammatory cytokines by several (circulating or resident) subpopulations of the immune cells. The predominant action of estrogens on the former or on the latter of these 2 populations could represent one key to their final action in many pathophysiological conditions. We will also review the different mouse models targeted for ERα or ERβ that allowed delineating the key role devoted to ERα in the endothelium. Finally, we will analyze the possibility of uncoupling the endothelial/beneficial and the sexual/undesirable actions of estrogens following the partial or selective activation of ERα.

E2 and Endothelial Function

A normal endothelium, both morphologically and functionally, is the major guardian of arterial integrity. Indeed, endothelium not only participates in the regulation of vaso-motor through flow-mediated mechanisms but also confers antithrombotic and antiinflammatory properties to the blood-vessel interface. The production of several endothelium-derived mediators, such as nitric oxide (NO) as well as the mechanisms determining its bioavailability (oxidative stress), prostacyclin and other cyclooxygenase derivatives, and hyperpolarization factors, have stimulated intensive research in the last decades.3–10 Their antispastic and antiaggregating properties appear to play a protective role at various stages of the atheroma pathophysiology.

Chronic estrogen treatment enhances endothelial function in a number of vascular beds. Estrogens participate in the structure adaptation and blood flow control in the uterine vascular system during pregnancy. Endothelium-dependent vasodilation to acetylcholine in the peripheral vasculature is...
preserved or potentiated with chronic estrogen treatment in ovariectomized animals, including rabbits, rats, guinea pigs, and mice. In addition, chronic estrogen treatment enhances endothelium-dependent vasodilation in large peripheral arteries of postmenopausal women.

The cardiovascular risk factors leading to atheroma all favor an “endothelial dysfunction” characterized not only by reduced vasodilative properties but also by a proinflammatory and a prothrombotic state. Indeed, the risk for coronary heart diseases (CHD) in men begins to increase at approximately the same age as flow-mediated vasodilation begins to decline. Women also exhibit this age-related impairment of flow-mediated vasodilation. However, significant reduction of flow-mediated dilation becomes apparent at the age of menopause, more than a decade later than in men, suggesting a protective role of E2. Coronary becomes apparent at the age of menopause, more than a decade earlier than in men, suggesting a protective role of E2.12 Coronary artery disease (CAD) in women begins to increase at approximately the same age as flow-mediated vasodilation begins to decline. Women also exhibit this age-related impairment of flow-mediated vasodilation. However, significant reduction of flow-mediated dilation becomes apparent at the age of menopause, more than a decade later than in men, suggesting a protective role of E2. Coronary artery disease (CAD) in women begins to increase at approximately the same age as flow-mediated vasodilation begins to decline. Women also exhibit this age-related impairment of flow-mediated vasodilation. However, significant reduction of flow-mediated dilation becomes apparent at the age of menopause, more than a decade later than in men, suggesting a protective role of E2.

Figure. Schematic representation of the mouse ERα gene structure and the strategies of Esr1 gene inactivation. A, The Esr1 gene encompasses 8 coding exons and at least 6 noncoding exons. The full-length 66-kDa ERα protein is composed of 6 domains (A to F), comprising a DNA binding domain (DBD), a ligand binding domain (LBD), and 2 transactivation functions (AF-1 and AF-2). B, The expression of the physiologically expressed 46-kDa isoform (not shown) is initiated at AUG2. This isoform lacks the entire A and B domains and hence AF-1. B, The first strategy of ERα gene targeting consisted of inserting a neomycin cassette in the first exon of the ERα gene (referred to as aER-NeoKO). aER-NeoKO expresses at least 2 truncated ERα proteins, because of a natural and nonnatural splicing events, devoid of AF-1 function but with a functional AF-2. The splicing involving the neomycin cassette generates a chimeric 55-kDa isoform. The level of expression of these truncated isoforms is enough to mediate several actions of E2 in the vessel wall. C, To generate ERαAF-1- mice, a knock-in approach consisted of targeting the first exon of ERα gene coding for the A and B domains and therefore AF-1 (deletion corresponding to amino acids 2 to 148). The phenotype of ERαAF-1- mice is reminiscent of that of aER-NeoKO mice, although the leakage and therefore the expression of chimeric 55-kDa isoform is highly variable. D, The second knockout approach (referred to as ERαAF-2- mice) consisted of introducing LoxP sites and then excising the second coding exon of the Esr1 gene (referred to as aER-NeoKO mice). This strain does not allow the expression of any functional ERα, demonstrating that ERα appears to mediate most of the vascular effects of E2.

Molecular Targets: ER

The biological effects of E2 are mediated through binding and activation of intracellular receptors, ERα (NR3A1) and ERβ (NR3A2). They belong to the nuclear receptor subfamily of ligand-inducible transcription factors whose members, based on structural and functional similarities, can be subdivided into 6 distinct regions, termed A to F (Figure A). Ligand-induced transcription of ER involves the action of distinct transactivation functions (AFs), located in the N-terminal A/B (AF-1) and the C-terminal E (AF-2) domains (Figure A). On estrogen binding, ER undergoes a conformational change that facilitates the recruitment of coactivators and the direct (or indirect) binding to cis-acting elements, thereby activating the transcription of target genes. Besides these classic genomic actions, a small pool of ER localized at the plasma membrane can elicit membrane-initiated steroid signaling, leading to the activation of several kinase pathways, such as mitogen-activated protein kinase or phosphatidylinositol 3-kinase, that ultimately also interfere with gene transcription. Several recent reviews have been devoted to the membrane-initiated steroid signaling actions in vascular cells and therefore will not be detailed here.
As detailed later in this review, mouse models targeted for either ERα or ERβ allowed us and others to demonstrate that ERα is absolutely necessary to the beneficial actions of E2 in endothelial NO production, and to influence the pathophysiology of cardiac in the vascular or resident immune cells, that could be resident cells of the vessel wall, in particular endothelial cells, or circulating cells of the immune system.

Studies of the impact of E2 on the cell populations of the immune system revealed a dual response. An acute in vitro E2 treatment of the macrophage cell line RAW or splenic macrophages led to an antiinflammatory effect. In contrast, we observed an increased production of IL-1 (α and β), IL-12, and IL-18 by peritoneal macrophages obtained from chronically E2-treated mice compared with those from ovariectomized mice. Interestingly, a similar proinflammatory effect was observed in microglial cells, the resident macrophages of the brain after an in vivo E2 treatment. Altogether, these striking discrepancies illustrate that the short-term action of E2 does not predict its long-term action, and they underline the importance of the in vivo approach to understand the pathophysiological effects of E2. A similar proinflammatory effect of E2 was also observed in CD4 + T cells as well as in natural killer T lymphocytes. In these studies, the production of proinflammatory cytokines (eg, interferon-γ) was increased, whereas production of IL-4 was decreased, resulting in a strong bias toward a Th1 profile. Altogether, it appears that the long-term exposure of an organism to E2 leads to a novel homeostatic status involving both the endothelium and the immune system. On the one hand, E2 potentiates the ability of several subpopulations of the circulating or resident immune cells to produce proinflammatory cytokines, whereas on the other hand E2 increases the resistance of the endothelium to various injuries and thereby prevents the endothelial activation. Importantly, endothelial apoptosis in response to various stresses is also prevented by E2. We will see that this view of the E2 action could help to explain the discrepancies that characterize the E2 action at the various stages of the atheroma process.

E2 Actions on Endothelial Healing and on Angiogenesis

Angioplasty followed by stent implantation is a commonly used procedure to treat coronary or peripheral artery stenosis. Among the various treatment strategies to promote endothelial regrowth after arterial injury, E2 could be an attractive one. Indeed, E2 increases both migration and proliferation of cultured endothelial cells in vitro, and these direct actions contribute to accelerate reendothelialization in vivo through the retrograde commitment of uninjured endothelium. However, we have recently demonstrated that the stimulation of endothelial ERα by E2 is necessary, but not sufficient, to induce reendothelialization, because a concomitant stimulation of ERα bone marrow derived cell is also required. Indeed, E2 exerts major effects on hematopoietic cells, including mobilization of endothelial progenitors cells as well as actions on inflammatory immune cells and platelets, that could all contribute to endothelial repair. Although it has
been shown that local delivery of E2 decreases neointimal hyperplasia after coronary angioplasty in a porcine model. Strategies aimed at optimizing endothelial healing should take into account this cooperation, which appears to involve several growth factors. For instance, fibroblast growth factor-2, and specifically bone marrow-derived fibroblast growth factor-2, is necessary and sufficient to mediate the accelerative effect of E2 on both reendothelialization and mobilization of endothelial progenitor cells. More recently, the acceleration of the endothelial repair by E2 was also shown to require osteopontin, both for bone marrow–derived cell recruitment and for endothelial cell migration and proliferation.

In addition to endothelial repair, E2 promotes angiogenesis in both in vitro and in vivo models. The mechanisms involved in the proangiogenic effects of estrogens are probably multifactorial. E2 induces 3 of the most important proangiogenic factors: fibroblast growth factor-2 isoforms, vascular endothelial growth factor, and NO. All of the previously mentioned mechanisms could contribute to the neuro- and cardioprotective effects of estrogens observed in models of ischemia/reperfusion injury.

Altogether, these actions of estrogens on inflammation, immunity, and angiogenesis have likely implications in all types of cancer, ie, not only ER-positive but also potentially ER-negative cancers. Indeed, it was recently recognized that estrogens can promote tumor angiogenesis and growth in ER-negative cancer cells. At the same time, estrogens or estrogen plus progesterin were found to decrease the susceptibility to cancer at sites that are not conventional target organs of sex hormones, such as liver and colon, in experimental and human studies respectively. Altogether, these complex actions, including immunosurveillance, could account for the unexpected divergent actions of hormone treatment on breast cancer in the 2 arms of the Women’s Health Initiative (WHI) study (see below: Clinical Implications).

### E2 Action in Early Versus Advanced Atheroma

Epidemiological studies showed that women are protected from CHD before menopause, suggesting a beneficial action of endogenous estrogens (Table). The nurses’ health study suggested that menopausal women taking estrogen or estrogen plus progesterin therapy had a decreased risk of CHD. This suggested that menopaused women taking estrogen or estrogen plus progesterin therapy had a decreased risk of CHD.66 This epidemiological study64,65 (see below: Clinical Implications).

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<tr>
<th>Table. Potential Cellular Targets and Mechanisms of the Estrogen Actions on Arteries</th>
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<td>Prevention of endothelial activation (adhesion molecules such as vascular cell adhesion molecule-1, proinflammatory chemokines)</td>
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<td>Decrease in endothelial permeability</td>
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<tr>
<td>Prevention of endothelial dysfunction (increase in NO and in prostacyclin, decrease in superoxide anion)</td>
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<tr>
<td>Prevention of apoptosis</td>
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<td>Acceleration of endothelial healing, potentially involving an increase in circulating endothelial progenitor cells</td>
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<td><strong>Smooth muscle cells</strong></td>
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<td>Prevention of smooth muscle cell migration and proliferation and therefore neointima hyperplasia</td>
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<td><strong>Liver</strong></td>
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<tr>
<td>Increase in the production of apolipoprotein A and in circulating high-density lipoprotein levels (in humans, not in mouse)</td>
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Potential explanations for the lack of, or even deleterious, action of estrogens in atheromatous arteries

### Endothelium: inhibition of the action of E2

- Medroxyprogesterone acetate antagonizes the beneficial action of estrogens on endothelial dysfunction, favoring vascular spasms.
- 27-Hydroxycholesterol as a competitive antagonist of ER action

### Decreased endothelial ERα expression following E2 deprivation

### Plaque stability/thrombosis

- E2 prevents smooth muscle cell migration and proliferation, which could influence fibromuscular cap formation.
- E2 increases matrix metalloprotease 2 release, which could favor the degradation of the collagen scar.

### Immune cells

- E2 favors the release of proinflammatory cytokines in macrophages.
- E2 favors a strong bias towards a Th1 profile.

### Angiogenesis

- E2 favors angiogenesis and could increase the risk of intraplaque hemorrhage.

### Coagulation

- E2 favors the production of circulating procoagulant factors.

Altogether, these various mechanisms could contribute to plaque instability and rupture in advanced atheroma.
be important at later stages to prevent coronary spasm, as directly demonstrated in hypercholesterolemic ovariectomized monkeys\textsuperscript{15} and in women.\textsuperscript{14}

The antispastic action of E2 could also involve a direct effect of E2 at the level of the vascular smooth muscle. Indeed, in vitro studies showed that E2 inhibits the proliferation and migration of smooth muscle cells.\textsuperscript{59} In addition, medial and myointimal thickening of the carotid and coronary arteries were significantly reduced by E2 treatment in animal models of vascular injury,\textsuperscript{22,55} demonstrating that E2 prevents smooth muscle cell growth in vivo. Conversely, E2 could also elicit deleterious actions at the level of vascular smooth muscle cells, as it enhances their release of matrix metalloproteinase-2,\textsuperscript{7,73} which could also have contributed to early plaque destabilization in the WHI study.

Although the main randomized controlled trial, WHI, did not confirm the preventive action of estrogens against CHD, women who initiated hormone therapy closer to menopause tended to have reduced CHD risk compared with the increase in CHD risk among women more distant from menopause.\textsuperscript{64,74} Interestingly, the importance of age in the estrogen action on endothelial function, a surrogate end point and pathophysiological mechanism, was suggested as early as 2001.\textsuperscript{75} It is noteworthy that Clarkson and Appt demonstrated very early in hypercholesterolemic primates that the efficacy of estrogens on plaque progression was inversely related to the duration of the estrogen deprivation period after ovariectomy.\textsuperscript{67} Furthermore, Rosenfeld et al\textsuperscript{67} reported that E2 inhibits the initiation of fatty streaks throughout the vasculature but does not inhibit the progression of established lesions in ApoE\textsuperscript{−/−} mice. Altogether, these animal studies mimic the discrepancies observed in humans and appear to be relevant models. As summarized above, mouse models already allowed exploration of some molecular and cellular mechanisms of these complex interactions between sex hormones and atheroma. Interestingly, 27-hydroxycholesterol, an abundant cholesterol metabolite that is elevated with hypercholesterolemia and found in atherosclerotic lesions, is a competitive antagonist of ER action in the vasculature, and thus could be a contributing factor in the loss of estrogen protection from vascular disease.\textsuperscript{77}

**Clinical Implications**

According to our current knowledge, the proinflammatory effect of E2 described above cannot account for its preventive effect of fatty streak accumulation in experimental models.\textsuperscript{78} If a similar proinflammatory effect also occurs at the level of atheromatous plaques in postmenopausal women, it could instead favor a destabilization of the most unstable plaques, in conjunction with proangiogenic\textsuperscript{5,59} and procoagulant\textsuperscript{79} effects (Table). These effects could have significantly contributed, in interaction with medroxyprogesterone acetate and its adverse action on endothelial function,\textsuperscript{15} to the increase in the frequency of cardiovascular events in postmenopausal women during the first year of hormone therapy observed in the Heart and Estrogen/Progestin Replacement and WHI studies.\textsuperscript{64,80} Indeed, nonhysterectomized women receiving conjugated equine estrogen combined with medroxyprogesterone acetate had a greater frequency of CHD events than women taking placebo,\textsuperscript{64} although this risk was subsequently found to be restricted to women treated very late after menopause (20 or more years from menopause onset).\textsuperscript{74} However, this was not the case for hysterectomized women receiving conjugated equine estrogen alone, for whom the CHD risk was similar to those taking placebo.\textsuperscript{65} Thus, these 2 arms of the WHI trial underline the deleterious nature of this progestin on arterial risk. It should be noted that medroxyprogesterone acetate, in combination with conjugated equine estrogen, also exerted a deleterious action on the incidence of breast cancer.\textsuperscript{64} Whereas conjugated equine estrogen alone unexpectedly decreased breast cancer.\textsuperscript{65} However, this conclusion was questioned after more detailed analyses of overall hormone use, emphasizing cumulative hormone exposure as related to breast cancer risk in the WHI trial of estrogen plus progestin.\textsuperscript{81} These contrasting findings underline that any effect of hormone therapy on breast cancer remains extremely controversial, and they stress the necessity of further defining the various actions of sex hormones, in particular on the immune system and therefore on immunosurveillance.

Various classes of estrogens and selective ER modulators (SERMs) have been described according to their molecular actions through ER\textalpha and ER\textbeta. Because of the complexity of the mechanisms of action of ER, the in vivo effect of estrogens and SERMs in various cell types and tissues cannot be predicted from in vitro studies. SERMs currently available (tamoxifen, raloxifene) prevent breast cancer but are devoid of effect on menopause symptoms and on cardiovascular risk. Hence, integrated models that allow the screening of present and future SERMs in terms of beneficial and deleterious effects will be valuable tools. Theoretically, it is conceivable to design a SERM (or a combination of molecules) that would retain several of the desired effects of E2 (on endothelium, bone, etc) but that should be devoid of the undesirable effects of E2 (mainly uterus and breast cancer). The optimized action on the immune system and on the angiogenic process is not as easy to delineate. Recent experiments using mice deficient in ER\textalphaAF-1 (ER\textalphaAF-1\textsuperscript{−/−} mice) suggest that SERMs stimulating ER\textalpha with minimal activation of ER\textalpha AF-1 could retain beneficial vascular actions while minimizing the sexual effects. Prevention of both breast cancer and cardiovascular diseases by novel SERMs thus represents the major challenge of the future treatment of menopause.

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Disclosures

None.

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