REVIEW

Cellular mechanisms underlying the cardiovascular actions of oestrogens

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ABSTRACT

Although pre-menopausal women enjoy relative cardiovascular protection, hormone (oestrogen ± progestin)-replacement therapy has not shown cardiovascular benefits in post-menopausal women, suggesting that the effects of oestrogens on the cardiovascular system are much more complex than previously expected. Endothelial cells, smooth muscle cells, cardiac myocytes and fibroblasts, the cellular components of blood vessels and the heart, play important roles in cardiovascular health and disease. During the development and progression of cardiovascular disease, changes occur both in the structure and function of these cells, resulting in a wide range of abnormalities, which affect growth, death and physiological function. These cells contain functional oestrogen receptors and are targets for oestrogen action. This review focuses on recent studies on the effects of oestrogen on cardiovascular cell function. Oestrogens, particularly 17β-oestradiol, exert multiple effects on cardiovascular cells, and these effects may contribute to the gender-associated protection against cardiovascular diseases.

INTRODUCTION

Although CV (cardiovascular) disease is equally prevalent as a cause of death among women and men, it is well recognized that its age of onset is on average delayed in women. CHD (coronary heart disease) is extremely uncommon in pre-menopausal women, even in high-risk populations [1]. The causes for this gender difference remain a matter of debate, but may be related to the beneficial effects of endogenous sex steroid hormones in pre-menopausal women. This hypothesis is based on the following observations: (i) epidemiological data [2] have shown that, in comparison with men, the emergence of CHD as a cause of death is delayed by approx. 5 years in women in whom the risk of ischaemic heart disease is 4–5-fold less than men during the pre-menopausal years; (ii) the risk of CV disease has been found to be significantly increased in pre-menopausal women after surgically induced premature menopause (bilateral oopherectomy) and in women who experience an early natural menopause [3], and hormone (oestrogen ± progestin) therapy appears to provide CV protection in this population of women [4]; and (iii) several observational studies have suggested that

Key words: cardiovascular disease, endothelial cell, gender-associated protection, oestrogen, vascular smooth muscle cell.

Abbreviations: ArKO, aromatase knockout; CEE, conjugated equine oestrogen; CHD, coronary heart disease; COX, cyclooxygenase; CRP, C-reactive protein; CV, cardiovascular; E1, oestrone; E2, oestradiol; E3, oestriol; EC, endothelial cell; BAEC, bovine aortic EC; ER, oestrogen receptor; ERKO, ER-knockout; ET-1, endothelin-1; HDL, high-density lipoprotein; HERS, Heart Oestrogen-Progestin Replacement Study; ICAM-1, intercellular cell-adhesion molecule-1; IL, interleukin; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; MORE, Multiple Outcomes of Raloxifene; MPA, medroxyprogesterone acetate; NF-κB, nuclear factor κB; NO, nitric oxide; NOS, NO synthase; eNOS, endothelial NOS; PDGF, platelet-derived growth factor; SERMS, selective ER modulator; SMC, smooth muscle cell; BASMC, bovine aortic SMC; TNF-α, tumour necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; VSMC, vascular SMC; WHI, Women’s Health Initiative.

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hormone use in post-menopausal women significantly improves a number of surrogate markers of CV risk [5].

In spite of the circumstantial evidence in support of oestrogen and progestogen therapies in the post-menopausal setting, results from the Heart Oestrogen-Progestin Replacement Study (HERS) [6,7] and the Women’s Health Initiative (WHI) [8] did not confirm CV benefits of post-menopausal hormone therapy (oestrogen or oestrogen plus progestin). HERS was the first large-scale randomized clinical trial to test the efficacy and safety of hormone therapy on clinical CV disease outcomes in post-menopausal women. The study population included 2763 women with established coronary artery disease randomized to combined hormone therapy or placebo. There were no significant differences between groups at either 4 or 8 years of follow-up for the primary outcome of non-fatal myocardial infarction or CHD death, or for several secondary CV end points. There was a statistically significant increase in venous thromboembolic events. In addition, there was, in the earlier report, a statistically significant time trend, with more CHD events in the hormone therapy group than in the placebo in year 1, and fewer in years 3 and beyond, although this was not substantiated at 8 years. These results do not support instituting hormone therapy in women with established CHD for the sole purpose of avoiding secondary events. HERS did not address the question of benefit and risk from hormone therapy in primary prevention, nor did it elucidate the mechanism of an apparent bi-phasic effect of early detriment and later benefit, of hormone therapy in women with atherosclerotic disease.

It should be noted that: (i) the patients represented a heterogeneous group and were invariably taking many drugs; and (ii) that the hormone preparation was a combination of CEEs (conjugated equine oestrogens) and MPA (medroxyprogesterone acetate), about which combination of CEEs (conjugated equine oestrogens) and MPA (medroxyprogesterone acetate), about which questions have been raised. Accordingly, this study did not resolve the conceptual question about the role of 17β-E2 (17β-oestradiol) or other physiological hormones in the causation or prevention of CV events.

Similarly, the WHI study did not confirm CV benefits, and also suggested possible increased risks of vascular thrombosis with the same hormonal regimen (CEEs and MPA), as well as an increased risk of breast cancer with prolonged use, as had been well-documented in many previous observational studies. The arm of the study that included oestrogen and progestogen was stopped due to an increase in the incidence of breast cancer and a perceived lack of overall benefit after an average follow-up of 5.2 years, and the arm that included oestrogen only was stopped after 7 years due to no improvement in the overall global index and, in addition, to increasing the risk of uterine cancer and rates of uterine bleeding, biopsy and hysterectomy. In the latter case, it is noted that no increase in the incidence of breast cancer was seen; however, it must be recognized that the study was not powered to test this end point so this negative result must be interpreted with caution. As a result, hormone therapy is now not recommended for primary prevention of CHD in post-menopausal women [8].

In light of current evidence, the effects of sex hormones on the CV system are likely to be more complex than previously expected. Oestrogens undoubtedly have a role in CV physiology. Exactly what this is, how they interact with the effects of other hormones – such as androgens and progestogens – in different physiological and clinical settings and their possible therapeutic implications, if any, for the prevention or treatment of vascular disease, however, remains to be elucidated. This review is focused on recent studies of the actions of oestrogen on CV cells. It will not consider the actions of progesterone or other progestogens, or of androgens.

**OESTROGENS, ERs (OESTROGEN RECEPTORS) AND OESTROGEN-ASSOCIATED PROTEINS**

The naturally occurring oestrogens are E1 (oestrone), 17β-E2 and E3 (oestril), C18 steroids derived from cholesterol. Cholesterol is taken up by steroidogenic cells, stored and moved to the sites of steroid synthesis. Different steroids are formed by reduction in the number of carbon atoms from 27 to 18. Aromatization, catalysed by the P450 aromatase mono-oxygenase enzyme complex, is the last step in oestrogen formation in which E1 and E2 are formed from their obligatory precursors androstenedione and testosterone respectively.

The primary sources of oestrogens in pre-menopausal women are the theca and granulosa cells of the ovaries and the luteinized derivatives of these cells. In pre-menopausal women, 17β-E2, the most bioactive oestrogen produced by the ovaries, is the chief circulating oestrogen. Serum E2 concentrations are low in pre-adolescent girls and increase at menarche. In women, they range from approx. 100 pg/ml in the follicular phase to approx. 600 pg/ml at the time of ovulation. They may rise to nearly 20000 pg/ml during pregnancy. As with other hormones, they are secreted in a pulsatile manner; however, relatively little is understood about the relevance of variations in pulse frequency and amplitude, although there are suggestions that they may be related to pathological phenomena. After menopause, serum E2 concentrations decline to values similar to, or lower than, those in men of similar age (5–20 pg/ml). Oestrogens are mainly metabolized by sulphation or glucuronidation, and the conjugates are excreted into the bile or urine. Oestrogens are also metabolized by hydroxylation and subsequent methylation to form catechol and methoxylated oestrogens. In this setting,
oestrogen production continues as a result of peripheral aromatization, in which E1 may become biologically more important than E2.

CV cells are exposed to oestrogens not only from endogenous sources, such as hormonal secretion from the ovaries and aromatization of androgenic precursors in a variety of tissues, but also from exogenous ones. Post-menopausal women may receive treatment with a variety of preparations, such as CEEs, E2 and E1, through oral, transdermal, subcutaneous or inhaled routes of administration, with or without a progestogen or, in some cases, an androgen. In addition, other compounds in clinical use, such as tamoxifen, raloxifene and tibolone, can activate vascular ERs. So-called 'phyto-oestrogens', a diverse group of compounds found in various plant-derived foods and beverages, can also have oestrogenic effects [9]. CV cells in both men and women are also exposed to oestrogens as a result of local conversion of androgens by the enzyme P450 aromatase [10].

There are two known ERs, ERα and ERβ, both of which are members of the superfamily of steroid hormone receptors. These molecules have considerable homology and, like all steroid hormone receptors, act as transcription factors that alter gene expression when they are activated by oestrogen binding. ERs may be activated by growth factors in the absence of oestrogens when local concentrations of growth factors are high or when serum oestrogen concentrations are low (as in men and post-menopausal women), and are also activated by different intracellular pathways (oestrogen-independent activation) in vascular and non-vascular cells [11,12].

Both ERα and ERβ have been identified in CV tissues, with the levels of expression of each ER subtype varying in different CV cells in normal women and men [13,14]. Studies using ERKO (ER-knockout) mouse models show that both ERα and ERβ can mediate potentially protective effects of oestrogens on the CV system [15,16], although the exact function of each ER subtype remains unclear.

Complexes of oestrogens and ERs associate, and act in concert, with other proteins, known as 'coactivators', to facilitate gene expression [17,18]. Coactivator proteins work in at least two ways: (i) they recruit proteins of the general transcriptional apparatus, the multiprotein complex, that transcribes DNA into RNA; and (ii) they also have enzymatic activity that can facilitate the transcription of RNA by the general transcriptional apparatus. In addition, there are proteins, known as 'corepressors', that bind to steroid hormone receptors and silence transcription [18]. The first ER-specific corepressor was cloned in 1998 [19]; however, the molecular mechanisms by which corepressors inhibit gene expression are not well understood at this time.

The presence of various types or amounts of ER-associated proteins may contribute to differences between the actions of oestrogens in CV and non-CV cells. Control of gene expression by complexes of oestrogens and ERs thus involves a series of specific molecular interactions among oestrogens, ERs, ER-associated proteins and control regions for the different oestrogen target genes present in each cell.

Besides the classical activation of target genes after binding nuclear receptors, E2 also has rapid non-genomic effects, and these have been attributed to cell-membrane-initiated signalling [20]. At the cell surface, a small population of ERs bind E2 and activate G-proteins. Multiple signalling pathways are then rapidly stimulated by oestrogen in target cells that express endogenous ERα and ERβ, and these pathways have been linked to discrete cellular actions of the steroid. In this respect, a 10-min infusion of intracoronary E2 (1 nM) was found to attenuate ET-1 (endothelin-1)-induced coronary vasoconstriction [21]. It is proposed therefore that the integration of cell-surface and nuclear signalling impacts overall cell biology.

EFFECTS OF OESTROGEN ON CV CELLS

Oestrogens alter serum lipid concentrations, coagulation and fibrinolytic systems, antioxidant systems and the production of other CV active molecules, such as NO (nitric oxide) and prostaglandins, all of which might mediate CV protection or damage. However, as described below, considerable evidence now exists showing that CV cells are themselves targets for oestrogens.

Vascular ECs (endothelial cells)

Possible effects of oestrogens on ECs have been widely explored in experimental animal as well as cell culture studies, and they are listed in Table 1.

Using an ovariectomized rat model of local ischaemia, pre-treatment with E2 was shown to increase GLUT1 (glucose transporter 1) expression and EC survival in cerebral blood vessels, which probably contributed to the significant reduction in the ischaemic damage following middle cerebral artery occlusion [22]. Physiological concentrations of E2 attenuated ET-1-induced coronary vasoconstriction in pigs, an effect possibly mediated via the ET-1-receptor and related to the anti-anginal properties of oestrogens [21]. It has been reported that male rabbits developed more monocyte adhesion and subendothelial migration in aortic tissue than do female rabbits in response to hypercholesterolaemia; ovariectomized rabbits given physiological levels of E2 supplementation demonstrated fewer adherent and subendothelial monocytes than do ovariectomized rabbits given placebo; and VCAM-1 (vascular cell adhesion molecule-1) protein expression was increased in aortas from hypercholesterolaemic ovariectomized animals supplemented with placebo, whereas E2 attenuated this increase.
Table 1  Effects of oestrogens on vascular ECs

<table>
<thead>
<tr>
<th>Activities</th>
<th>Experimental models</th>
<th>Oestrogen effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth or repair</td>
<td>Mouse cerebral blood vessels (in vivo study)</td>
<td>↑</td>
<td>[22]</td>
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<td></td>
<td>Monkey coronary arteries (in vivo study)</td>
<td>↑</td>
<td>[24]</td>
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<td></td>
<td>HUVECs and MAECs (in vitro culture)</td>
<td>↑</td>
<td>[29]</td>
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<td></td>
<td>BAECS (in vitro culture)</td>
<td>↑</td>
<td>[30]</td>
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<tr>
<td></td>
<td>HUVECs (in vitro culture)</td>
<td>↑ (LC), ↓ (HC)</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>HUVECs (in vitro culture)</td>
<td>↓</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>HUVECs (in vitro culture)</td>
<td>→</td>
<td>[33, 53]</td>
</tr>
<tr>
<td></td>
<td>BAECS (in vitro culture)</td>
<td>→ or ↓</td>
<td>[34]</td>
</tr>
<tr>
<td>Injury or apoptosis</td>
<td>Rat arteries (in vivo study)</td>
<td>↓</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>Mouse aortas (in vivo study)</td>
<td>↓</td>
<td>[26]</td>
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<tr>
<td></td>
<td>HAECS and BAECS (in vitro culture)</td>
<td>↓</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td>HUVECs (in vitro culture)</td>
<td>↓</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>BAECS (in vitro culture)</td>
<td>↓</td>
<td>[37]</td>
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<tr>
<td></td>
<td>BPAECs (in vitro culture)</td>
<td>→ or ↑</td>
<td>[38]</td>
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<td></td>
<td>BCAECs (in vitro culture)</td>
<td>↑</td>
<td>[39]</td>
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<tr>
<td>Expression of adhesion molecules</td>
<td>Mouse aortas (in vivo study)</td>
<td>↓</td>
<td>[23]</td>
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<td></td>
<td>HUVECs and HSVECs (in vitro culture)</td>
<td>↓</td>
<td>[28, 40, 41, 44]</td>
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<tr>
<td></td>
<td>HUVECs (in vitro culture)</td>
<td>↑</td>
<td>[42, 43]</td>
</tr>
<tr>
<td>NO release or NOS expression</td>
<td>Pig coronary arteries (in vivo study)</td>
<td>↑</td>
<td>[21]</td>
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<td></td>
<td>ERKO mouse aortas (in vivo study)</td>
<td>↑</td>
<td>[27]</td>
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<td></td>
<td>HAECS (in vitro culture)</td>
<td>↑</td>
<td>[44]</td>
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<td></td>
<td>HUVECS and BAECS (in vitro culture)</td>
<td>↑</td>
<td>[47]</td>
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<tr>
<td></td>
<td>BAECS (in vitro culture)</td>
<td>↑ or →</td>
<td>[48]</td>
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<td></td>
<td>Canine coronary arteries (in vivo study)</td>
<td>↑</td>
<td>[51]</td>
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<tr>
<td>Prostacyclin</td>
<td>HUVECs (in vitro culture)</td>
<td>↑</td>
<td>[52]</td>
</tr>
<tr>
<td></td>
<td>HUVECs (in vitro culture)</td>
<td>→</td>
<td>[53]</td>
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</table>

In VCAM-1 [23]. Using quantitative coronary angiography to determine vasomotor responses of atherosclerotic coronary arteries in overiectomized cynomolagus monkeys, oestrogen ‘replacement’ was shown to modulate vasomotion of atherosclerotic coronary arteries. At the same time, the extent of atherosclerotic diet-induced plaques was slightly less in oestrogen-treated arteries than in controls [24]. Using pentosidine as a biomarker of glycoxidative damage, it was found that arteries from ovariectomized rats receiving E2 exhibited a 50 % reduction in damage compared with placebo and control vessels [25]. Chronic treatment with E2 was found to lead to a significant decrease in blood glucose and triacylglycerol (triglyceride) levels, reduce the lesion area in all vascular segments studied and prevent cartilaginous metaplasia in steptozotocin-treated ApoE (apolipoprotein E) knockout mice [26]. In studies of ERKO mouse models, atheroprotective effects of oestrogen therapy, such as modulation of the release of endothelium-derived NO [27] and inhibition of vascular injury [15], have been reported, and these actions are possibly mediated by either classical ERs (α or β) or other unidentified pathways. Furthermore, production of oestrogens in vascular endothelium by P450 aromatase, which converts testosterone into E2, was found to attenuate expression of VCAM-1 [28]. This local oestrogen production may also contribute to protection of arteries from atherosclerosis [28].

EC growth and death are important in vascular health and disease. In cell culture studies, oestrogen may promote growth of human ECs [29–31], although this finding is not universal [32–34]. E2 has been reported to inhibit EC apoptosis induced by the inflammatory factor TNF-α (tumour necrosis factor-α) [35, 36] or hypoxia [37]. The endogenous oestrogen metabolite, 2-methoxyoestrodiol, induced EC apoptosis [38, 39], whereas E2 and its other metabolites, oestrial and 2-methoxyestriol, did not [38]. The cellular and molecular mechanisms underlying effects of oestrogens on EC apoptosis are still largely unclear and may involve an ER-dependent and/or ER-independent process, with possible roles for ICE (IL (interleukin)-1β-converting enzyme (caspase)), c-myc gene, pp125 FAK (local adhesion kinase), MAPKs (mitogen-activated protein kinases)
Cardiovascular actions of oestrogens

Table 2. Effects of oestrogens on VSMCs

<table>
<thead>
<tr>
<th>Activities</th>
<th>Experimental models</th>
<th>Oestrogen effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSMC growth/proliferation</td>
<td>Rat carotid arteries (in vivo study)</td>
<td>↓</td>
<td>[55,57,58]</td>
</tr>
<tr>
<td></td>
<td>Monkey iliac arteries (in vivo study)</td>
<td>↓</td>
<td>[61]</td>
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<td></td>
<td>Rabbit aortic SMCs (in vitro culture)</td>
<td>↓</td>
<td>[63]</td>
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<td>HSVSMCs (in vitro culture)</td>
<td>↓</td>
<td>[64]</td>
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<td></td>
<td>HUVSMCs (in vitro culture)</td>
<td>↓</td>
<td>[65]</td>
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<tr>
<td></td>
<td>HIMASMCs (in vitro culture)</td>
<td>↓</td>
<td>[66]</td>
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<td></td>
<td>HASMCs (in vitro culture)</td>
<td>↓</td>
<td>[67–69]</td>
</tr>
<tr>
<td></td>
<td>HUVSMCs (in vitro culture) (LC) ↓ (HC)</td>
<td>↑</td>
<td>[31]</td>
</tr>
<tr>
<td>Collagen synthesis</td>
<td>BASMCs (in vitro culture)</td>
<td>↓</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>RASMCs (in vitro culture)</td>
<td>↓</td>
<td>[76]</td>
</tr>
<tr>
<td>Prostacyclin</td>
<td>RASMCs (in vitro culture)</td>
<td>↑</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>SMCs in human uterine artery</td>
<td>→</td>
<td>[78]</td>
</tr>
</tbody>
</table>

[ERK1/2 (extracellular-signal-regulated kinase), p38 and JNK (c-Jun N-terminal kinase), Fas, Bcl-2, β-galactosidase and NOS (NO synthase)].

A key event in the development of atherosclerosis is the attachment of monocytes to the vascular wall, followed by migration into the peri-vascular space, events controlled by selective expression of cell-surface adhesion molecules, some of which appear to be modulated by oestrogens. In endothelial tissue derived from human umbilical vein, oestrogens reduce expression of VCAM-1 in response to IL-1 [40], and in some instances inhibit [41], whereas in others enhance [42,43], TNF-α-induced expression of adhesion molecule VCAM-1, ICAM-1 (intercellular cell-adhesion molecule-1) and E-selectin. Estrogen, as well as the selective ER modulator LY117018, but not tamoxifen, inhibits LPS (lipopolysaccharide)-induced expression of VCAM-1 in human saphenous vein ECs, and inhibits further the cell adhesiveness towards monocytoyid cells [44]. It is unclear at which point of the signalling cascade oestrogens act, but these steroids have been shown to influence activation of the nuclear transcription factor NF-κB (nuclear factor κB), which is involved in the regulation of adhesion molecule expression in ECs [45].

The labile gas NO has been implicated in the cytoprotective actions of E2. NO is synthesized from L-arginine via a particulate Ca2+/calmodulin-sensitive NO synthase (eNOS (endothelial NOS)), which is expressed constitutively in ECs and is activated by vasoactive agonists known to elevate intracellular Ca2+ concentration. The gene encoding eNOS (NOS3) possesses ER-binding elements, suggesting a receptor-mediated action of oestrogen on eNOS expression. Treatment of cultured ECs with E2, but not testosterone, up-regulates eNOS mRNA levels [46], an effect inhibited by pretreatment with the anti-oestrogen tamoxifen [47]. However, an elegant study has established that treatment (> 24 h) of cultured BAECs (bovine aortic ECs) with ethinyloestradiol, a synthetic oestrogen with properties that overlap and extend beyond those of E2, has no effect on either the activity or expression of eNOS, whereas O2 production and mRNA levels for SOD (superoxide dismutase) were increased [48]. The relative contributions of genomic increases in eNOS expression to vascular function and rapid ‘non-genomic’ activation of eNOS, which may also be regulated by ERs [49,50], but may be ER-independent under some circumstances [51], remain to be fully elucidated.

NO is not the only endothelium-derived vasoactive factor reported to be modulated by oestrogens. Synthesis of the vasodilator prostacyclin and vasoconstrictor thromboxone (TXA2), products of the COX (cyclooxygenase) pathway, may also be modulated by oestrogens. However, data on effects of oestrogens on prostanoid synthesis in cultured ECs are conflicting, with one report [52] showing an increase in prostacyclin production in response to E2, and another [53] showing no detectable changes in either basal or agonist-induced prostacyclin release. Interestingly, the rapid potentiation of acetylcholine-induced vasodilation in skin resistance arteries by E2 has been shown to be mediated via COX-2, one of the two isoforms of COX, a key enzyme in prostaglandin synthesis [54].

VSMCs [vascular SMCs (smooth muscle cells)]

VSMCs are widely affected by oestrogens, and the possible effects of the hormone on these cells are summarized in Table 2.

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Abnormal migration and proliferation of VSMCs play a key role in the development of atherosclerotic plaque. Direct inhibition of atherosclerotic intimal thickening by oestrogens has been explored in experimental animal studies. Using balloon injury of the common carotid artery of the rat as an experimental model of localized and highly controllable vascular damage in which the response to injury can be studied in vivo, it has been shown that balloon inflation denudes endothelium and induces a highly reproducible intimal migration/proliferation of SMCs over the entire length of the affected vessel [55]. In addition, the early response proto-oncogene c-myc plays an important role in regulating neointima formation after balloon denudation [56]. Sexual dimorphism in this animal model has been examined further [57–59] and it has been shown that: (i) neointima formation after balloon injury of the carotid artery is significantly greater in intact male Sprague–Dawley rats than in age-matched intact females; (ii) gonadectomy of male rats does not alter the neointimal response; (iii) gonadectomy of female rats is associated with a more robust neointimal proliferative response to injury, comparable with that seen in the male; and (iv) E2 replacement markedly attenuates neointima formation in gonadectomized rats of both sexes. In these studies, steady-state c-myc mRNA levels were significantly greater in uninjured carotid arteries of intact male rats than in intact females and, in contrast, the c-myc response in females was delayed and greatly attenuated. As with the action of oestrogens, selective ER modulators also inhibited the neointimal formation in balloon-denudated carotid arteries of ovariectomized rats, possibly via inhibition of smooth muscle proliferation and enhancement of re-endothelialization [60].

Most animal studies suggest that oestrogenic compounds inhibit the neointimal response in acutely injured blood vessels; however, in a non-human primate model with pre-existing atherosclerosis [61,62], oestrogen treatment was shown not to inhibit neointimal formation. In these studies, oestrogens only effected a transient decrease in arterial cell proliferation rate immediately after balloon injury, but did not alter either neointimal area or arterial remodelling. Oestrogen treatment administered concurrently with a high-fat diet slowed the progression of experimentally induced atherosclerosis in this model [62], but it did not modulate the acute injury response in the atherosclerotic artery.

Oestrogens can regulate a number of VSMC functions, including contractility, proliferation, matrix formation and composition. A rapid effect of oestrogens on vascular smooth muscle contractility has been explored both in vivo and in vitro in isolated organ and arterial segment preparations: these studies have shown that acute E2 administration, possibly via non-genomic mechanisms, results in direct vasodilation or improvement of vasodilatory response of blood vessels, including coronary, aortic, cerebral, brachial and peripheral arteries. VSMCs in culture appear mainly as a synthetic phenotype and, in recent years, the effects of oestrogens on VSMC migration, proliferation and matrix formation have been examined in cultured VSMC.

Consistent with findings from in vivo studies, most VSMC culture studies have shown that oestrogen treatment inhibits VSMC proliferation in response to biochemical or mechanical stimulation. Addition of E2 into the culture medium inhibits the proliferation of rabbit aortic SMC cultures stimulated with hyperlipidaemic serum [63]. The inhibitory effect of oestrogens was also observed in cultured human VSMCs from different vessels, including saphenous vein [64], umbilical vein [31,65], mammary artery [66] and aorta [67,68]. E2, at physiological and high physiological concentrations, inhibits DNA synthesis and/or cell proliferation in response to various stimuli, including serum, PDGF (platelet-derived growth factor), IGF (insulin-like growth factor), ET-1 or mechanical stress [69]. The effect may be mediated through various subcellular pathways, including ERs, MAPKs, pre-oncogenes (c-fos and c-myc), early growth response genes (Sp-1 and Egr-1) and creatine kinase. Similarly, the inhibitory effect on VSMC proliferation was also elicited by progesterone [65,67] or phyto-oestrogens [70,71]. The actions of phyto-oestrogen compounds may also be mediated by ERs, and possibly through the MAPK pathway. The cellular and subcellular effects of oestrogens on VSMCs are complex, with oestrogens producing varying effects under different conditions. For example, E2 at low physiological concentrations may stimulate VSMC proliferation [31]; E2 may lose its inhibitory effects on PDGF-induced VSMC proliferation under high glucose conditions [66]; phyto-oestrogen compounds may appear to enhance effects on VSMC growth [72]; and pregnancy as well as E2 supplementation may enhance PDGF-induced growth in uterine artery SMCs, possibly through activation of PKC (protein kinase C) [73]. We have recently found that aortic SMCs from mice with a genetic deficiency of endogenous oestrogens [ArKO (aromatase knockout) model] had altered cell proliferation and death patterns, with delayed proliferation in serum- or growth-factor-enriched cultures and increased apoptotic death induced by TNF-α stimulation [74]. Thus many aspects of the cellular and molecular mechanisms underlying the effects of oestrogen on VSMCs remain largely unclear.

In vitro, treatment of cultured BASMCs (bovine aortic SMCs) with E2 (10⁻⁸ M) decreases proline hydroxylation and alters the ratio of type I to type III procollagen fractions produced, without a significant effect on prolyl hydroxylase activity or proline incorporation into protein [75]. Phyto-oestrogens have also been found, in a previous study, to affect production of collagens in rat aortic VSMCs via ER mediation [76]. These observations indicate that oestrogens may affect...
remodelling of the vessel wall in response to injury. The effect of oestrogens on prostaglandin synthesis in cultured VSMCs is not consistent, with some studies [77] showing E2 (10⁻⁸–10⁻⁶ M) stimulation of prostacyclin production by stimulation of both COX and prostacyclin synthetase activities, and others [78] showing that both synthetic and natural oestrogens had no significant effect on stimulated prostacyclin production.

Cardiac cells

Although studies concerning direct effects of oestrogens on cardiac cells are still relatively few compared with those on vascular cells, oestrogens have been found to possess multiple effects on cardiac myocytes. In particular, changes in oestrogen levels affect the balance between myocyte life and death and cell function. Expression of HSPs (heat shock proteins), an important family of endogenous and protective proteins found in all tissues, was reported to be much higher in cardiac myocytes from female rats than in cells from males, and ovariectomy significantly reduced this protein expression, which was subsequently restored by oestrogen replacement [79]. Cardiac myocytes appear to show higher expression of β₁-adrenoceptor and are more sensitive to ischaemic injury under conditions of endogenous oestrogens deficiency (ovariectomized animals), and oestrogen supplementation suppresses this protein expression and reduces cardiac injury caused by sympathetic hyperactivity during ischaemia [80]. Myocardial ischaemia/reperfusion injury has also been shown to be more severe in ERKO than wild-type mice [81].

The occurrence of programmed cell death (apoptosis) in the myocardium has been demonstrated in several different models of heart failure, including ischaemic heart disease, as well as non-ischaemic heart failure, such as myocarditis or dilated cardiomyopathy [82]. The inhibition of apoptosis in cardiac myocytes may conserve the number of contractile myocytes and thus delay or prevent left ventricular dysfunction and development of heart failure. E2 at physiological concentrations inhibits staurosporine-induced apoptosis in ventricular myocytes in culture, possibly through pathways associated with NF-κB transcription factors and caspase proteins [83]. Oestrogen has been found to induce the expression of cellular adhesion molecules and cytokines. Activation of NF-κB can be induced by elevated levels of several factors such as TNF-α, IL-1 and phorbol esters. TNF-α is an important inflammatory factor that is found to accumulate in the heart following myocardial infarction and can induce both myocyte hypertrophy and apoptosis in culture. It is known that binding of TNF-α to its cognate receptor directly provokes caspase and NF-κB activation, resulting in opposite effects on cell survival. The finding of an inhibitory effect of oestrogens on NF-κB activation in cardiac myocytes increases interest in examining the possible myocardial protection by oestrogens during the inflammatory process. To date, however, studies in this field are generally lacking.

Ventricular myocytes from rats subjected to bilateral ovariectomy appeared to have an elevated resting intracellular Ca²⁺ level and delayed intracellular Ca²⁺ clearing, a decrease in contractile response, including less peak shortening amplitude and prolonged time to peak shortening and relengthening, and reduction of Akt activation. E2 replacement reversed all of these abnormalities, with the exception of the time to peak shortening and, in addition, enhanced peak shortening and maximal velocity of shortening/relengthening, effects mediated by ERs [85].

Oestrogens are found to affect electrophysiological activity in cardiac myocytes. Clinical studies have reported that an increase in the number and duration of episodes of paroxysmal supraventricular tachycardia in pre-menopausal women was associated with cyclical variation in plasma ovarian hormones, with a significant positive correlation with plasma progesterone and inverse correlation with plasma E2 levels [86]. The mechanisms of these effects are still unknown. E2 (3–10 µM), but not progesterone, shortened the action potential duration without changes in the resting membrane potential in single guinea-pig atrial myocytes, possibly by inhibition of the voltage-dependent Ca²⁺ current, thus providing anti-arrhythmic effects [87].

Cardiac fibroblasts comprise 60 % of the total heart cells and contribute to pathological structural changes in the heart by undergoing proliferation, depositing extracellular matrix proteins and replacing myocytes with fibrotic scar tissue. Oestrogens, mediated by ERs, were found to induce expression of the immediate early gene c-Fos in neonatal rat cardiac fibroblasts and stimulate the cell growth [88]. Interestingly, in this study, E1 and its metabolite 2-hydroxyestrone were the most potent stimulators, whereas E2 appeared relatively weak and its metabolite, 2-methoxyoestradiol, had no such action. Another study also showed that E1, but not E2, stimulated cardiac fibroblast growth, and growth was attenuated by the ACE (angiotensin-converting enzyme) inhibitor moexiprilat [89]. Previous studies found that E2 and its metabolites (2-hydroxyestradiol and 2-methoxyestradiol), but not 17α-E2, E1 or E3, inhibit serum-induced proliferation and collagen synthesis in rat cardiac fibroblasts in culture, and that the E2 metabolites were more potent than E2 itself [90]. The inhibitory effect appears to be mediated by ER-independent mechanisms [91]. These findings suggest that a decrease in E2 and its metabolites and an increase in E1 and 2-hydroxyestrone,
conditions found in the post-menopausal state, may contribute to the progressive fibrosis found in women after menopause.

**POTENTIALLY ADVERSE METABOLIC EFFECTS OF E2**

**Effect of oestrogens on inflammatory markers**
E2 has been reported to influence levels of inflammatory biomarkers. The PEPI (Postmenopausal Estrogen Progestin Intervention) study [92] showed that women assigned to CEE alone or CEE plus a progestin for 1 year had 121 and 150% increase in CRP (C-reactive protein) levels respectively. However, post-menopausal hormone therapy decreases many other markers of inflammation, including IL-6, ICAM-1, VCAM-1, E-selectin and s-thrombomodulin [93]. The discrepancy between increased plasma levels of CRP and reduced plasma levels of all other markers of inflammation suggests that the increased CRP levels after oral hormone therapy may be related to metabolic hepatic activation and not to an acute-phase response [94]. Of note, in patients with a longer time since menopause, post-menopausal hormone therapy may increase inflammation and worsen endothelial function [93], thus the timing of introduction of hormonal therapy may be very important.

**Effect of oestrogens on clotting factors**
Studies have shown that supplementation with E2 and CEE in post-menopausal women may affect blood coagulation and fibrinolysis [95,96]. Most studies, including two recent reports [97,98], demonstrate that hormone therapy increases procoagulant factors, such as factor VII, factor VIII, vWF (von Willebrand factor), factor IX and D-dimer, and decreases anticoagulant factors, such as antithrombin, protein C, protein S and PAI-1 (plasminogen-activator inhibitor). Overall, oestrogen-induced changes in the haemostatic system appear to favour thrombosis.

**Effect of oestrogens on triacylglycerols**
Oestrogens have been shown to increase VLDL [very low-density lipoprotein]-cholesterol and triacylglycerol levels, despite other favourable lipid effects, namely, increases in HDL (high-density lipoprotein)-cholesterol and a decrease in LDL-cholesterol [98]. Triacylglycerol levels during daily hormone therapy with conventional doses of conjugated oestrogens and MPA have been reported to increase more in overweight and obese post-menopausal women in association with increased oestrogen levels [99]. Of note is the finding that some progestins (e.g. norgestimate) minimize the increase in triacylglycerols that occurs with exogenous oestrogen, whereas others (e.g. MPA) do not [100]. By contrast, transdermal E2 has been shown to decrease triacylglycerol levels by approx. 11% in both normotriglyceridaemic and hypertriglyceridaemic post-menopausal women [101].

**CONJUGATED OESTROGENS COMPARED WITH E2**

Differences have been reported in the effects of E2 compared with CEEs. For example, it has been suggested that E2 may in fact induce greater CV benefit. In a porcine model, Jayachandran et al. [102] reported that, although both 17β-E2 and CEE reversed increases in platelet aggregation caused by ovariectomy, only 17β-E2 increased platelet RNA and release of platelet-derived NO. Differential effects have also been reported for the combination of E2 + dydrogesterone compared with CEE + MPA on lipids, apolipoproteins and lipoprotein (a) [103]. The influence of these differences on clinical endpoints, however, is unclear.

**SERMS (SELECTIVE ER MODULATORS)**

SERMs are non-hormonal pharmacological agents that bind to the ER, resulting in oestrogen agonist effects on bone and the CV system and oestrogen antagonist effects on endometrial and breast tissue. Tamoxifen, raloxifene and tibolone are the SERMs used currently. It has been reported in a 4-year trial study that raloxifene treatment (60 mg/day or 120 mg/day) had a neutral effect overall on the incidence of CV events in women participating in the MORE (Multiple Outcomes of Raloxifene) trial \( (n = 7705) \), but in those at increased risk of CV events \( (n = 1035) \) raloxifene treatment was associated with a lower incidence of CV events \[104,105\]. Further year-by-year analysis of CV events consistently showed that the risk of CV events was not increased in any single year of the MORE trial in women taking raloxifene, either in the low- or high-CV risk subsets \[106\]. Further studies are required to determine if SERMs are cardioprotective in post-menopausal women at increased CV risk.

**OESTROGENS AND OESTROGEN THERAPY IN MALES**

Several studies have shown that oestrogen has CV effects in males. Studies in the male ArKO mouse have shown that oestrogen deficiency is associated with impaired endothelium-dependent vasorelaxation [107]. Physiological levels of E2 help maintain normal plasma levels of HDL-cholesterol in men [108]. Oestrogens in men may promote both bone and vascular health: a man lacking functional ERα has been reported to have a number of abnormalities in bone and mineral
metabolism [109,109a], as well as impairment of flow-mediated endothelial vasodilatation and premature coronary arterial calcification [110,111]. In young men, inhibition of aromatase with anastrozole has been shown to result in impaired flow-mediated vasodilation in the brachial artery [112]. Finally, previous work has demonstrated that genetic variation in ERα can substantially modify the risk for myocardial infarction in men. In studies of subjects from the Framingham Heart Study Offspring Study, Shearman et al. [113] have shown that a common ERα variant is associated with a 3-fold increase in the risk of myocardial infarction in men. These studies, taken together, strongly suggest a role for oestrogens in male CV physiology.

SUMMARY AND FUTURE DIRECTIONS

Oestrogens have been found to exert multiple effects on CV cells and their function, and these effects may contribute to gender-associated protection against atherosclerosis and heart failure. Physiological levels of E2: (i) enhance growth of ECs and protect them from inflammatory-factor-induced adhesion molecule expression and apoptosis; (ii) inhibit migration, proliferation and collagen production in vascular smooth muscle under various conditions of chemical and mechanical stimulation; (iii) reverse survival and contractile dysfunction in cardiac myocytes under ischaemic stress; and (iv) inhibit abnormal proliferation in cardiac fibroblasts. However, oestrogens could also induce potentially adverse effects on the CV system through increases in inflammatory and procoagulant factors and triacylglycerols.

Further studies are needed to determine the situations in which the effects of oestrogens on CV cells influence physiology and pathophysiology, the molecular mechanisms underlying these actions, the relative contribution of genomic versus non-genomic actions, the actions of local oestrogens generated via vascular aromatase, and the role of oestrogens in males. The results of such studies may provide clarification of the clinical questions regarding the role of oestrogens in both disease causation and treatment.

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