Towards a molecular understanding of the atheroprotective effects of estrogens: a review of estrogen effects on endothelial activation

Tommaso Simoncini*§, Andrea Riccardo Genazzani*, Raffaele De Caterina**§

**Department of Reproductive Medicine and Child Development, University of Pisa, Pisa, §Scuola Superiore di Studi Universitari e di Perfezionamento "S. Anna", **CNR Institute of Clinical Physiology, Pisa, and Chair of Cardiology, "G. D'Annunzio" University of Chieti, Chieti, Italy*

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Early phases of atherosclerosis are characterized by an increased adhesion of leukocytes to vascular endothelium, leading to a recruitment of white blood cells into the intima. Leukocyte adhesion is mediated by the expression of specific adhesion molecules on endothelial cells, dependent on a dysfunctional status of vascular endothelium (endothelial activation), potentially caused by the exposure to diverse atherogenic stimuli.

Female sex steroid hormones regulate vascular function acting directly on vascular cells, and producing a net anti-inflammatory effect. Among the various mechanisms mediating the anti-atherogenic effects of estrogens, the inhibition of endothelial activation process and of leukocyte adhesion molecule expression are particularly important, for the strategic pathophysiological role played by these processes during atherogenesis.

This brief review discusses recent discoveries on the molecular mechanisms of estrogens on endothelial activation, as well as pathophysiological and clinical implications of these effects.

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Address:

Prof. Raffaele De Caterina

Sezione di Scienze Cardiovascolari
Universit degli Studi G. D Annunzio
c/o Ospedale San Camillo de Lellis
Via Forlanini, 50
66100 Chieti
E-mail:
rdecater@ifc.pi.cnr.it

Several large epidemiological studies have suggested that hormone replacement therapy in postmenopausal women is associated with a decreased risk of cardiovascular events[1-3] and with a decrease in overall mortality, particularly in women at higher cardiovascular risk[4]. This decrease in mortality has not been demonstrated in a recent large prospective intervention trial with hormone replacement therapy in postmenopausal women[5], but the relatively short follow-up of this study, sufficient to show an increased burden of likely thrombotic events in the short term, together with the secondary prevention nature of the study probably precluded an evaluation of the long-term atheroprotective effects of these substances. These are more likely to emerge with longer duration of hormone replacement therapy administration[6]. In epidemiological studies, some of the atheroprotective effects have been attributed to modifications of the lipid profile[2,7]. It has however been concluded that at least half of the protection is lipid-independent, and likely occurring through a direct action at the vascular level[8].

Atherosclerosis is a disease with important inflammatory aspects[9], and the endothelium plays a central role in its pathogenesis. Inflammatory stimuli can activate functional changes on endothelial cells, and trigger monocyte adhesion to the vessel wall and the intimal migration of these cells[10]. These molecular events proceed because of a coordinated activation of several genes encoding for leukocyte adhesion molecules – that include vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and E-selectin –, leukocyte chemotactic factors – such as monocyte chemoattractant protein-1 –, and growth factors – such as macrophage-colony stimulating factor – all acting together in directing circulating monocytes from the bloodstream toward the vascular wall[10] (Fig. 1). This complex process depends on the interplay of various transcription factors (nuclear factor-kB, activator protein-1 and the GATA family being the most important ones) that promote the expression of the genes linked to endothelial activation[10] (Fig. 1).

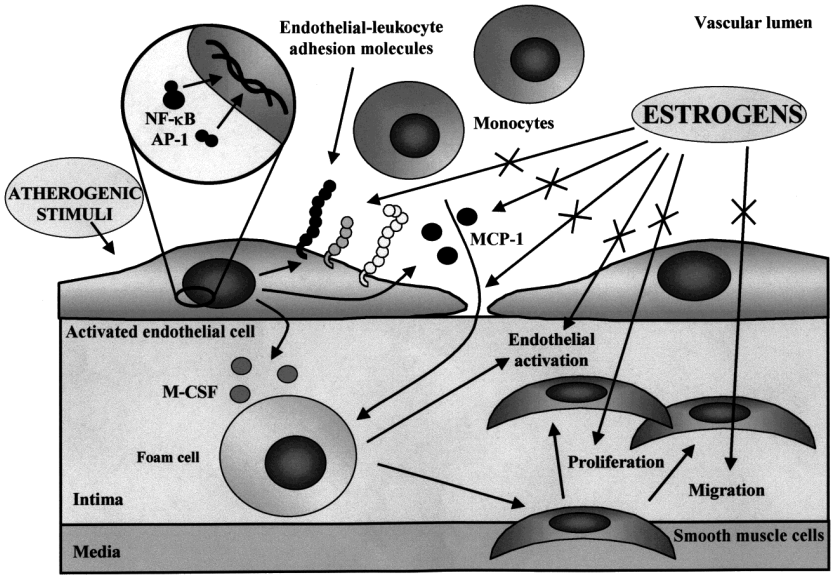

Figure 1. *Endothelial activation and the molecular events taking place during early atherogenesis. Inflammatory stimuli, such as bacterial lipopolysaccharide or cytokines, can induce functional changes in endothelial cells (endothelial activation), and trigger monocyte adhesion to the vessel wall and their intimal accumulation. These molecular events depend upon a coordinated activation of several genes encoding for leukocyte adhesion molecules, such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and E-selectin, leukocyte chemotactic factors like monocyte chemoattractant protein-1 (MCP-1) and growth factors such as macrophage-colony stimulating factor (M-CSF), all acting together in directing circulating monocytes from the bloodstream toward the vessel wall. This complex process depends on the interplay of various transcription factors (nuclear factor-κB - NF-κB, activator protein-1 - AP-1, and the GATA family being the most prominent ones) that promote the expression of the genes linked to endothelial activation. Once in the arterial intima, monocytes differentiate to lipid laden macrophages (foam cells), and continue to locally release inflammatory mediators, which further augment endothelial activation, as well as growth factors and chemoattractants, that induce the migration and proliferation of media-derived smooth muscle cells. Estrogens regulate several of the steps implicated in this process, possibly inhibiting transcription factors involved in endothelial activation.*

17b-estradiol, the major natural estrogen, has been shown to protect endothelial integrity and function in experimental animal models of atherosclerosis[11], and this action has been studied also *in vitro*, where 17b-estradiol[12] and other estrogenic compounds[13] decrease cytokine-induced expression of adhesion molecules, as well as increase the release of endothelium-derived anti-atherogenic molecules such as nitric oxide[14]. All these pieces of evidence together indicate that 17b-estradiol plays a critical anti-atherogenic action by regulating endothelial cell function.

Several reports have been published in the last years on the effects of estrogen on endothelial function, and many of these have focused on the effects of estrogens on endothelial leukocyte adhesion molecules. Clinical studies have shown that a short estrogen treatment in both males[15] and females[16] as well as longer-term hormone replacement therapy in healthy[17-20] or atherosclerotic women[21] reduce circulating levels of soluble endothelial leukocyte adhesion molecules. Soluble endothelial leukocyte adhesion molecules enter the bloodstream after cleavage of their extracellular domain (shedding) from the endothelial surface. Their level is thus thought to represent a marker of the status of vascular cell activation. To this regard, several recent studies have assessed that soluble leukocyte adhesion molecules are markers of the extent or severity of atherosclerosis[22,23], and possibly a useful tool for the prediction of future cardiovascular events[24]. Changes in serum levels of soluble adhesion molecules during estrogen treatments go along with the well-known improvement of endothelium-dependent vascular relaxation associated with these treatments[25,26], and provide an additional way to monitor the endothelial status in physiological and pathological conditions.

Reductions in the peripheral levels of adhesion molecules are associated with (and probably depend on) reduced endothelial production. In fact, recent studies show that, at the cellular level, natural estrogens exert anti-inflammatory effects reducing the expression of endothelial leukocyte adhesion molecules induced by inflammatory cytokines[12]. Mechanisms for this effect are yet under investigation, but a specific conformational modulation of the estrogen receptor(s) is probably a critical factor in determining the final effect. In fact, not only estrogen action can be blocked by estrogen receptor antagonists, but different selective estrogen receptor modulators (i.e. molecules with mixed estrogen agonistic/antagonistic properties due to the different conformational changes induced on the estrogen receptor upon interaction with the hormone binding domain) have been shown to have differential effects on the expression of adhesion molecules[13].

The inhibition of adhesion molecule expression at the endothelial level is one consequence of a general repression in the transcription of endothelial genes which are induced during endothelial activation, including

monocyte chemoattractant protein-1[27] (Fig. 1). Several of the genes induced during endothelial activation are controlled by transcriptional enhancement by inflammatory transcription factors, such as nuclear factor-kB and activator protein-1 (Fig. 1). This effect is due to binding of the activated factors to specific response elements located on the promoter region of these genes. Since the genes encoding for leukocyte adhesion molecules have no known estrogen response elements[28], it is possible that estrogen inhibitory action on adhesion molecule expression may be mediated by functional interferences with the activation of these transcription factors, and indeed preliminary data obtained in our lab confirm this hypothesis[29]. Another possibility is that estrogen action may be mediated by increased production of nitric oxide, dependent on both transcriptional induction of endothelial nitric oxide synthase[30] as well as on acute activation of the enzyme[14,31]. In fact, nitric oxide is a well-known anti-inflammatory molecule, and has been shown to inhibit nuclear factor-kB activation and leukocyte adhesion molecule expression[32]. Preliminary findings (Simoncini T. et al., unpublished data) also support a role for this mechanism.

In summary, recent research indicates that actions exerted directly on the vessel wall are critical for estrogen cardiovascular beneficial effects. Anti-inflammatory effects on endothelial cells emerge as primary actors in this process. The understanding of the molecular mechanisms through which these effects occur may provide new tools to optimize cardiovascular disease prevention in postmenopausal women.

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