State of the Art Lecture

Hormone Replacement Therapy and Inflammation
Interactions in Cardiovascular Disease

Andrew P. Miller, Yiu-Fai Chen, Dongqi Xing, Wenguang Feng, Suzanne Oparil

***Abstract*—**Inflammation plays a central role in the pathogenesis of many forms of vascular disease, including atherosclerosis. Atherogenesis begins with endothelial damage, and the damaged endothelium expresses adhesion molecules, chemokines, and proinflammatory cytokines that direct atherosclerotic plaque formation and spill into the circulation as biomarkers of atherosclerotic disease risk. Menopausal hormone therapy, including a variety of estrogen preparations with or without a progestin, has negative modulatory effects on most of these soluble inflammatory markers, including E-selectin, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, monocyte chemoattractant protein-1, and tumor necrosis factor-α, inconsistent effects on interleukin-6, and stimulatory effects on transforming growth factor-β, a vasoprotective cytokine. In contrast, C-reactive protein, a circulating proinflammatory cytokine produced in both liver and atherosclerotic arteries, increases in response to oral conjugated estrogens but not to transdermal estrogen. Although C-reactive protein is clearly linked to increased cardiovascular disease risk in women, the hormone-induced rise in this biomarker is not associated with increased risk and may be related to a first-pass effect of C-reactive protein production in the liver after oral estrogen absorption. Many important questions about the effects of ovarian hormones on vascular inflammation and the pathogenesis of vascular disease cannot be answered in human subjects. Insights from fundamental mechanistic studies in animal models are needed to delineate the cellular/molecular events that determine whether these hormones protect or injure blood vessels. **(*Hypertension*. 2003;42[part 2]:657-663.)**

Key Words: atherosclerosis ■ leukocytes ■ vessels ■ women ■ risk factors

Cardiovascular disease is the leading cause of death in women.[1,2] There is a strong link between menopause and an increased incidence of cardiovascular disease, and observational studies suggest that postmenopausal hormone therapy, including various estrogen preparations with or without a progestin (most commonly a synthetic progestin), reduce cardiovascular disease risk by about half.[2,3] These studies, although provocative, have the limitations of noncomparability of the two treatment groups: Women who take menopausal hormone therapy are on average better educated, have higher incomes and better access to health care, and are healthier even before starting therapy.[4] A meta-analysis that adjusted for socioeconomic status as well as other risk factors showed no cardiovascular disease risk reduction in women taking menopausal hormones.[5] Controversy about the risks and benefits of menopausal hormone treatment[6] has stimulated the design and performance of randomized controlled trials of this therapy. These trials have reported no net benefit and some early (first 1 to 2 years of treatment) harm from menopausal hormone therapy with respect to cardiovascular disease prevention.[7,8] Limitations of published studies include inclusion of a predominance of elderly participants after many hormone-free years, during which estrogen receptors and other components of the hormone response pathway may have disappeared; use of a restricted range of hormone preparations (usually conjugated equine estrogen with or without medroxyprogesterone acetate, MPA), which may have unwanted adverse effects, and the unavailability of biomarkers for susceptibility to adverse effects of menopausal hormones.

Among the mechanisms that may account for the effects of menopausal hormones on cardiovascular disease in women is inflammation. Clinical studies relating menopausal hormone use to vascular inflammation have relied on measurement of circulating biomarkers of inflammation. This review will discuss the role of inflammation in the pathogenesis of vascular disease and its modulation by ovarian hormones, with particular emphasis on circulating biomarkers of inflammation as nontraditional cardiovascular risk factors. The urgent need for fundamental mechanistic studies to delineate putative proinflammatory and anti-inflammatory effects of ovarian hormones on the vasculature will be emphasized.

Received May 12, 2003; first decision June 9, 2003; revision accepted June 26, 2003.

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Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000085560.02979.0C**

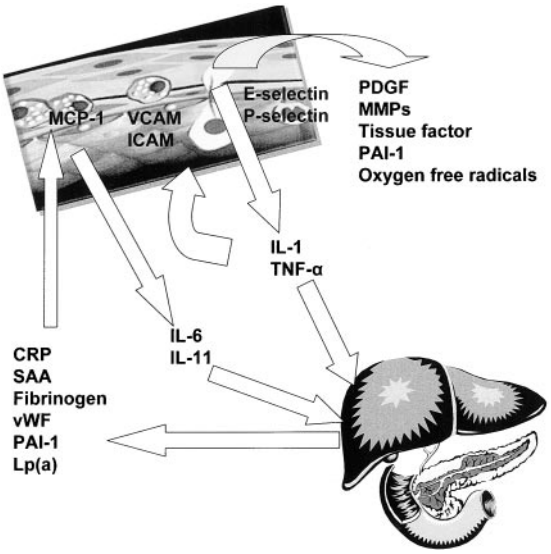

Figure 1. Vascular injury leads to adhesion molecule expression and leukocyte infiltration. Cytokines released from the injured artery initiate hepatic synthesis of several proteins (acute phase reactants), including CRP. Cytokines and some acute phase reactants have effects on the injured artery and contribute to the inflammatory response. Modified with permission of the authors from Koh KK[13] and Libby P, www.lipidsonline.org.[14] CRP indicates C-reactive protein; ICAM, intercellular adhesion molecule-1; IL, interleukin; Lp(a), lipoprotein(a); MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor-1; PDGF, platelet-derived growth factor; SAA, serum amyloid A; TNF-α, tumor necrosis factor-α; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor.

Inflammation and Vascular Disease

Inflammation plays a central role in the pathogenesis of many forms of vascular disease, including atherosclerosis.[9–14] Atherogenesis begins with endothelial damage. The damaged endothelium expresses adhesion molecules, including P-selectin and E-selectin, that tether circulating inflammatory cells (monocyte/macrophages, neutrophils and T-lymphocytes) and initiate their rolling across the damaged endothelial surface (Figure 1). A functional role for selectins in the initiation of atherogenesis has been shown in animal models in which P-selectin expression precedes inflammatory cell infiltration into vessel walls and elimination of P-selectin with neutralizing antibodies or homologous deletion of the gene attenuates both leukocyte rolling and attachment to endothelium, as well as the development of atheromatous and neointimal lesions in damaged vessels. Rolling leukocytes attach to endothelium by binding to adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). VCAM-1 expression has been shown to precede inflammatory cell infiltration into atheromatous lesions in animal models, and genetically altered animals that do not express VCAM-1 have greatly attenuated atheroma formation, confirming the functional role of this adhesion molecule.

The attached leukocytes migrate into the subendothelial space under the influence of monocyte chemoattractant protein-1 (MCP-1) and other chemokines (Figure 1). Mediators of the tethering, adhesion, and infiltration of leukocytes in injured arteries are expressed in endothelial and smooth muscle cells activated by inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and interferon-γ. Leukocytes trapped in the subendothelial space become activated, express proinflammatory cytokines and scavenger receptors for modified lipoproteins and transform their phenotype to become lipid-laden macrophages or foam cells, the core of the nascent atheroma. Release of proinflammatory cytokines from early lesions creates a paracrine-positive feedback loop, further damaging the endothelium, recruiting more leukocytes from the circulation and the adjacent tissues and amplifying the vascular inflammatory response (Figure 1). Proinflammatory cytokines, principally IL-6, are released into the circulation and stimulate the liver to synthesize and secrete the acute-phase reactant C-reactive protein (CRP), as well as procoagulant/anti fibrinolytic factors such as fibrinogen, lipoprotein(a) [Lp(a)], and plasminogen activator inhibitor-1 (PAI-1). Thus, there is a second endocrine-positive feedback loop by which injured vessels may promote generalized vascular inflammatory damage.

CRP is proinflammatory, stimulates expression of VCAM-1, ICAM-1, and MCP-1 by endothelial cells,[15] activates complement,[16] and facilitates LDL cholesterol uptake by macrophages.[17] CRP is synthesized in extrahepatic sites, including smooth muscle cells and macrophages in atherosclerotic lesions, and its colocalization with complement in these lesions adds support to its putative pro-inflammatory role in atherogenesis.

Clinical Foundations for Measuring Soluble Inflammatory Markers

Inflammation plays a pivotal role in atherogenesis, and a variety of soluble forms of inflammatory markers have been evaluated as predictors of cardiovascular risk. Measurement of these has been embedded in the design of large observational studies, as well as smaller interventional trials of cardiovascular disease therapies. In general, these trials have validated the usefulness of soluble inflammatory markers as nontraditional cardiovascular risk factors (Figure 2).

P-Selectin

As a mediator of the earliest event of vascular inflammation, P-selectin was chosen as a potential identifier of persons in the early stages of atherogenesis and, hence, at increased risk of developing clinical cardiovascular disease. A substudy of the Women's Health Study compared baseline plasma P-selectin levels from apparently healthy women who subsequently had a cardiovascular event to those from matched control subjects.[18] Participants with plasma P-selectin levels in the highest quartile had a relative risk of cardiovascular disease 2.2 times higher than those in the lowest quartile ($P=0.02$). This predictive effect was independent of traditional risk factors.

Soluble ICAM-1

In 14 916 healthy men enrolled in the Physicians' Health Study, baseline levels of soluble ICAM-1 (sICAM-1) were higher among those who had a myocardial infarction than among those who did not; the relative risk for men in the highest quartile was 1.8 times that of the lowest quartile

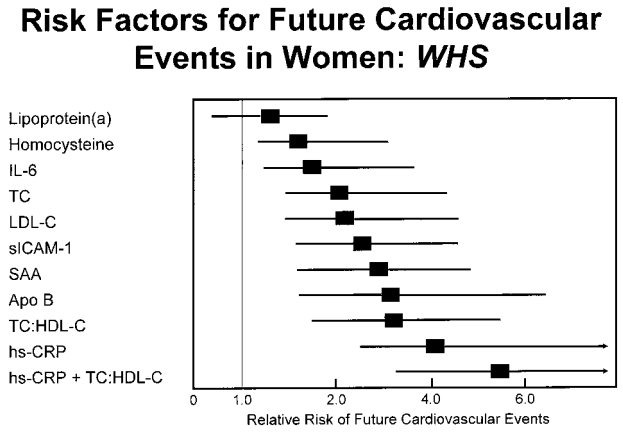

Figure 2. Direct comparison of relative risk of future cardiovascular events associated with levels of lipid components and inflammatory risk factors in the Women's' Health Study. Relative risks and 95% confidence intervals are shown for women in top vs bottom quartile for each factor. TC indicates total cholesterol; LDL-C, low-density lipoprotein cholesterol; sICAM-1, soluble intercellular adhesion molecule-1; Apo B, apolipoprotein B-100; HDL-C, high-density lipoprotein cholesterol; other abbreviations as in Figure 1. Modified from Blake and Ridker, copyright ©2002, Massachusetts Medical Society. Published with permission of Blackwell Publishing. All rights reserved.[12]

($P=0.03$).[19] The Atherosclerosis Risk in Communities (ARIC) study showed that the relative risk for coronary artery disease among women and men in the highest quartile of baseline sICAM-1 was 5.5 times that of the lowest quartile.[20] Similarly, in the Women's Health Study, the relative risk of a future cardiovascular event for women in the highest quartile of sICAM-1 at baseline was 2.6 times that of women in the lowest quartile[21] (Figure 2).

Soluble VCAM-1

As shown in both the ARIC study and the Physicians Health Study, soluble VCAM-1 (sVCAM-1) levels do not appear to predict future cardiovascular events in healthy persons.[20,22] However, in persons with established cardiovascular disease, sVCAM-1 is predictive of risk, with a >2-fold increase in death in the highest quartile compared with the lowest in a prospective cohort of 1246 patients with coronary artery disease.[23] In contrast to ICAM-1, which is expressed by many cell types, including circulating leukocytes and fibroblasts, VCAM-1 expression is localized to the atherosclerotic plaque surface. This restricted pattern of expression may explain its unreliability as a biomarker of cardiovascular risk in healthy persons.

Tumor Necrosis Factor-α

TNF-α predicts cardiovascular events in both apparently healthy individuals[24] and persons with established cardiovascular disease, as shown in the Cholesterol And Recurrent Events (CARE) trial.[25]

Interleukin-6

IL-6 has predictive value in both healthy populations and persons with cardiovascular disease. In the Physicians' Health Study, men with IL-6 levels in the highest quartile were at a 2.3-fold-increased risk of future cardiovascular events compared with those in the lowest quartile.[26] This marker has also been validated in women: Participants in the Women's Health Study with IL-6 levels in the highest quartile suffered a relative risk of future cardiovascular events of 2.7 compared with those in the lowest quartile[21] (Figure 2). Furthermore, in the Fragmin and Fast Revascularization during Instability in Coronary artery disease II trial (FRISC II), elevated IL-6 was a robust predictor of increased mortality rates in participants with acute coronary syndromes and identified those most likely to benefit from an early invasive strategy.[27]

C-Reactive Protein

CRP is the best-characterized biomarker of cardiovascular risk identified to date. Although the other biomarkers are biologically important, their clinical value has not been as well established as that of CRP, in part because of lack of standardization of assay conditions and the instability and short half lives of the proteins.[28]

Baseline levels of CRP are robust and independent predictors of risk for future cardiovascular events and death in apparently healthy individuals as well as those with known cardiovascular disease.[11] A prospective nested, case-control study from the Women's Health Study tested 12 markers of risk for their predictive strength[21] (Figure 2). Relative risk of future cardiovascular events among women in the highest quartile of CRP levels compared with the lowest was 4.4, the highest risk assessment for any of the markers tested, including lipid levels (Figure 2). The only plasma markers that independently predicted future risk were CRP (relative risk, 1.5) and total cholesterol to high-density lipoprotein ratio (relative risk, 1.4).

CRP levels may also guide therapeutic interventions. Both aspirin and statin therapy appear to provide more benefit to those with elevated CRP, as shown in the Physician's Health Study and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS).[29,30] Interestingly, statin therapy has been shown to lower CRP in persons with elevated pretreatment levels, suggesting that a component of the vasoprotection conferred by these agents may be related to an anti-inflammatory effect.[31] CRP levels are elevated in postmenopausal women taking oral hormone therapy, and whether CRP is a source of the harm attributed to hormone treatment is an important open question, as is the utility of CRP as a biomarker of cardiovascular disease risk in women using menopausal hormones.

Hormone Replacement Therapy and Inflammatory Markers

Clinical trial data demonstrate that hormone replacement therapy has significant impact on soluble levels of a variety of inflammatory markers.

Soluble E-Selectin

Seven randomized, controlled trials including 942 participants have demonstrated an overall 15% reduction in soluble E-selectin (sE-selectin) levels with menopausal hormone therapy (Figure 3A). Reductions in sE-selectin were seen with oral conjugated estrogen with or without MPA or progesterone in the Postmenopausal Estrogen/Progestin In-

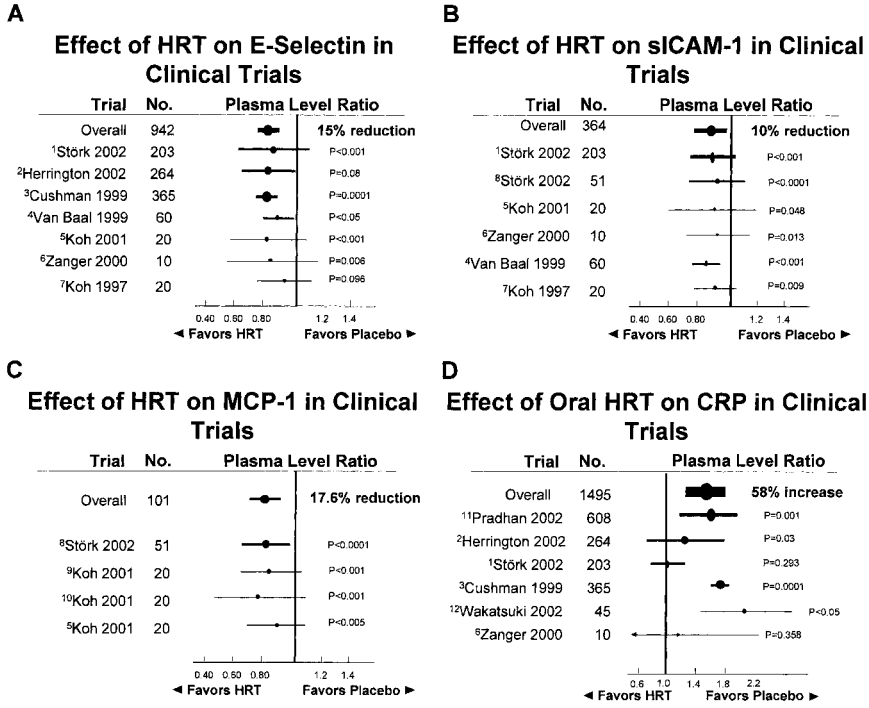

Figure 3. Effects of hormone therapy on plasma levels of various inflammatory markers (A, E-selectin; B, sICAM-1; C, MCP-1; D, CRP). Graph shows median change and 95% confidence interval weighted for study population. Abbreviations as in Figures 1 and 2. (1) Störk et al[33]; (2) Herrington et al[34]; (3) Cushman et al[32]; (4) van Baal et al[35]; (5) Koh et al[36]; (6) Zanger et al[37]; (7) Koh et al[38]; (8) Störk et al[39]; (9) Koh et al[40]; (10) Koh et al[41]; (11) Pradhan et al[42]; (12) Wakatsuki et al.[43]

terventions (PEPI) trial,[32] with 17β-estradiol plus progestin in a substudy of the Postmenopausal Hormone Replacement against Atherosclerosis (PHOREA) trial[33] and with conjugated estrogen with or without MPA in the Estrogen Replacement and Atherosclerosis (ERA) trial.[34] Interestingly, participants in ERA with a common estrogen receptor polymorphism (ER-α IVS1–401 C/C genotype) demonstrated nearly a 2-fold-greater reduction in E-selectin than those with the C/T or T/T genotypes. Four smaller studies confirm the inhibitory effect of a variety of menopausal hormone regimens on sE-selectin levels (Figure 3A).[35–38]

sVCAM-1 and sICAM-1

Six randomized, controlled trials with a pooled population of 364 women demonstrated an overall 10% reduction in these adhesion molecules with hormone therapy. These reductions were statistically significant in all of the trials, which tested a variety of menopausal hormone preparations (Figure 3B).[33,35–39]

MCP-1

Limited (4 trials, 101 pooled participants) trial experience has demonstrated robust (mean, 17.6%) and significant decreases in circulating MCP-1 levels with hormone treatment (Figure 3C).[36,39–41]

C-Reactive Protein

Most studies have shown that oral menopausal hormone (estrogen with or without progestin) treatment is associated with increased (mean 58%) circulating CRP levels (Figure 3D). The largest of these, a nested, case-control study from the Women's Health Initiative observational study, and data from the ERA and PEPI trials, showed highly significant 63%, 31%, and 85% increases in CRP with a variety of oral hormone regimens.[32,34,42] However, increased CRP levels in these studies were not accompanied by elevations in IL-6, E-selectin, fibrinogen, or other acute phase reactants.[32,42] In fact, decreases in circulating levels of adhesion molecules (E-selectin, ICAM-1, VCAM-1) have been reported with oral menopausal hormone therapy in both healthy postmenopausal women and postmenopausal women with coronary artery disease (Figures 3A and 3B). Furthermore, small studies have confirmed the inconsistent effect of menopausal hormones on IL-6[37,43] and have found significant reductions in TNF-α[40] and increases in the vasoprotective cytokine TGF-β in postmenopausal women treated with hormones.[44] These findings strongly suggest that the effects of menopausal hormones on CRP do not represent a generalized proinflammatory effect mediated through upstream cytokines such as IL-6 but rather are related to a secondary mechanism.[42] The finding that transdermal estradiol, unlike oral conjugated estrogen, does not elevate circulating CRP levels suggests that this secondary mechanism may be a first pass effect of CRP production in the liver after oral estrogen absorption, which is avoided by transdermal delivery.[45,46]

Importantly, the clinical significance of hormone-related increases in CRP is open to question. In the Women's Health Initiative observational study, CRP was an independent predictor of cardiovascular risk for both users and nonusers of hormone therapy.[42] However, use or nonuse of hormone therapy had less importance as a predictor of cardiovascular risk than CRP levels, and hormone therapy assignment did not significantly change stratified odds ratios based on CRP levels. Thus, although hormone therapy was associated with an increase in CRP, this change could not be equated with increased cardiovascular risk.

Mechanistic Studies of Hormones and Vascular Inflammation

Clinical studies raise many important questions about the effects of ovarian hormones on vascular inflammation and the

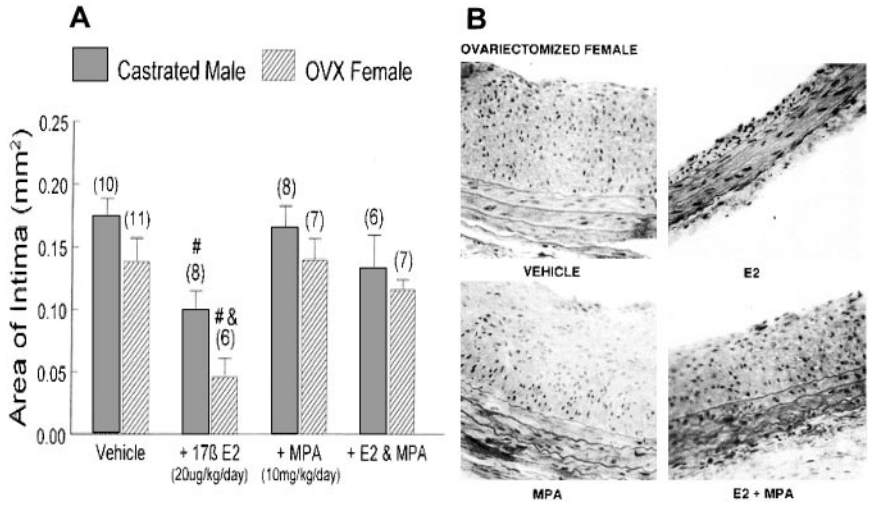

Figure 4. A, Effects of estrogen (E2) and medroxyprogesterone acetate (MPA) on neointima formation in balloon-injured carotid artery of gonadectomized male and female rats at 14 days after injury. Cross-sectional areas of neointimal are presented as mean±SEM. #$P<0.05$ compared with their respective vehicle control groups; &$P<0.05$ compared with their respective castrated male groups. B, Representative light micrographs of carotid arteries from ovariectomized rats 14 days after balloon injury. Balloon-injured right carotid arteries from vehicle control (top left), E2-treated (top right), MPA-treated (bottom left), and E2+MPA-treated (bottom right) rats. Magnification ×400. Modified from Levine et al, reprinted with permission.[48]

pathogenesis of vascular disease that cannot easily be answered in human subjects. Insights from fundamental mechanistic studies in animal models are needed to delineate the cellular/molecular events that determine whether these hormones protect or injure blood vessels. Balloon injury of the rat carotid artery has been used as an experimental model of localized and highly controllable vascular damage in which the injury response can be studied in vivo.[47–49] In this model, balloon inflation denudes endothelium and induces highly reproducible neointima formation over the entire length of the affected vessel. Our laboratory has used this model to show that ovarian hormones modulate the neointimal response to endoluminal vascular injury.

Initially, we demonstrated a sexual dimorphism in the vascular injury response that was estrogen-dependent.[47–49] Neointima formation after carotid injury was greater in male rats than in female rats; orchidectomy with or without testosterone replacement did not alter the injury response in males, but ovariectomy greatly enhanced neointima formation in female rats, and testosterone administration had no additional effect. Systemic administration of 17β-estradiol in a dose that resulted in physiological levels of circulating hormone markedly attenuated neointima formation in gonadectomized rats of both sexes (Figure 4), providing strong evidence that the sexual dimorphism of neointima formation after endoluminal vascular injury is mediated by estrogen. Addition of the synthetic progestin MPA to the estrogen blunted its inhibitory effects, restoring neointima formation to vehicle control levels, whereas MPA alone had no independent effect. These effects of combined hormone administration mirror the findings of clinical trials, in which preparations of estrogen plus MPA fail to prevent cardiovascular events.[7,8]

Subsequent studies demonstrated that estrogen inhibits neointima formation in the balloon injury model by estrogen receptor (ER)-dependent modulation of molecular/cellular events that occur early (first 72 hours) after injury.[50,51] This ER-dependent effect involves negative modulation of synthesis and release of chemotactic/adhesion molecules and proinflammatory cytokines in damaged

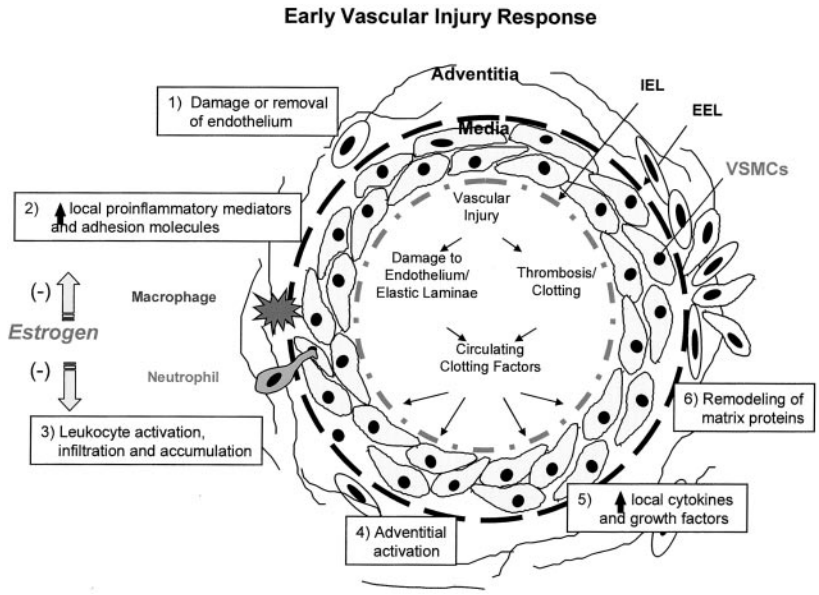

Figure 5. Simplified schematic illustration of cellular responses to endoluminal vascular injury.

smooth muscle cells within the injured vessel[52–54] (Figure 5). The pattern of expression of these chemokines/cytokines/adhesion molecules may resemble that observed in the early phase of atherogenesis.

Activated smooth muscle cells synthesize and release a variety of proinflammatory cytokines and chemotactic/adhesion molecules, leading to activation of adventitial cells within the first 24 hours after injury.[50,51,55] The signaling pathway responsible probably involves both diffusion of chemoattractant/mitogenic factors from damaged SMCs through the media and external elastic lamina to the adventitia and delivery of these factors to the adventitia via the circulation (ie, the vasa vasorum).

Although our previous studies focused on adventitial fibroblasts as target cells for activation and migration into media and neointima after endoluminal vascular injury,[53] we have recently observed extensive inflammatory cell infiltration (by hematoxylin and eosin staining) of the adventitia and perivascular tissues of balloon-injured carotid arteries of ovariectomized rats within 24 hours of the insult. These cells were not present in the adventitia of carotid arteries that had been dissected and exposed but not subjected to balloon injury. Immunohistochemical analysis is being used to localize specific inflammatory cell types in injured vessels and provide a semiquantitative assessment of their density. Preliminary studies using flow cytometry have revealed extensive infiltration of neutrophils, monocyte/macrophages and T-lymphocytes into arteries of ovariectomized rats at 24 hours after injury; estrogen treatment greatly reduced neutrophil and monocyte/macrophage numbers, whereas addition of MPA blocked the estrogen effect.[56] We hypothesize that the previously demonstrated opposing effects of estrogen and MPA on neointima formation after acute vascular injury may be mediated, at least in part, through modulation of this inflammatory response. Further study is needed to assess the relevance of these observations to the vascular effects of menopausal hormone therapy in women.

Perspectives

As surrogate markers of vascular inflammation, plasma levels of a variety of adhesion molecules, cytokines, and acute phase reactants have been studied and validated as predictors of future cardiovascular events in both women and men. In prospective studies, administration of ovarian hormones to postmenopausal women has been shown to negatively modulate most of these soluble markers, with significant decreases in E-selectin, sVCAM1, sICAM-1, and TNF-α. In contrast, oral hormone therapy increases CRP. However, the biological importance of this effect is disputable, with evidence that the mechanism is through a first-pass effect in the liver and that the hormone-induced rise does not confer additional cardiovascular risk. Studies in animal models of acute vascular injury have demonstrated a clear vasoprotective effect of estrogen and have given preliminary evidence that this vasoprotection is, at least in part, a consequence of an anti-inflammatory action. Further insights from fundamental mechanistic studies in animal models are needed to delineate the cellular/molecular effects of ovarian hormones on vascular inflammation and on the pathogenesis of vascular disease, both topics that are difficult to address in human subjects.

Acknowledgments

This work was supported in part by National Heart, Lung, and Blood Institute grants HL-64614, HL-07457, HL-44195, HL-50147, and HL-44195.

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