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Estradiol and the vascular wall (additive 1998-2000)

Several additional observations confirm or discover novel mechanisms for the protective effects exerted by estradiol in cardiovascular diseases, and atherosclerosis in particular. Most cardioprotective effects of estrogens are receptor-dependent. Two recent studies suggest, however, that some of the cardioprotective effects of estrogen are non-receptor mediated and occur at the plasma membrane level (see below, paragraph protection against oxidative modification and vasorelaxation).

Protection against oxidative stress

It is known that estradiol (E2) protects the vascular wall against LDL oxidative metabolism. In addition, hydroxy-estradiol, a major metabolite of 17β-estradiol that is a strong antioxidant but a weak ER ligand, protects membrane phospholipids of vascular smooth muscle cell (SMC) against free radical-induced peroxidation (Dubey et al., 1999). This direct anti-oxydant effect occurs at physiological concentrations, by a mechanism independent of the estrogen receptor. Anti-oxydant estrogens could also protect membrane associated signal transduction mechanisms controlling cell migration and proliferation (Dubey et al., 1999).

Vasorelaxation

Until now, the major positive effects of estrogen on vascular tissue were known to involve intracellular estrogen receptors. Recently, a novel mechanism of action was elucidated involving the direct interaction of estradiol with a voltage-gated channel subunit (Maxi-K channel, hSlo) present on vascular smooth muscle cells; estradiol-induced acute activation of this channel accelerates the efflux of potassium from the cell, leading to vasodilation (Valverde et al., 1999).

Several mechanisms could be responsible for the changes in nitric oxide (NO) synthesis in the vascular wall, observed during vascular pathology (hypertension, atherosclerosis) :

• reduced NO synthesis due to impaired constitutive NO synthase III (endothelial, eNOS) activity ; several studies have provided evidence that 17β-estradiol directly increase endothelium-dependent vasorelaxation, via interaction with the constitutively expressed nitric oxide synthase present in the endothelium.

• excessive NO synthase II (iNOS) production induced by proinflammatory stimuli has also been associated with atherosclerosis. 17β-estradiol decreases the content and activity of nitric oxide synthase II (iNOS) in smooth muscle cells from rat aorta (Zancan et al., 1999).

Inhibition of proliferation of smooth muscle cells and fibroblasts

SMC proliferation and migration are critical for SMC accumulation in the intima. *In vivo* observations further indicate that the protective effects of E2 are not restricted to E2 present in normal females or used in HRT, but also increase with E2 increase seen in pregnancy (Zhang et al., 1999). Other recent results using a model of steroid deprivation (ovarian ablation) indicate that estradiol decreases the accumulation of bFGF *in vivo* (sheep aorta) and inhibits the mitogenic effect of bFGF on human aortic SMC *in vitro* (Selzman et al., 1998). Inhibition of IGF-R1 could also been involved in E2-inhibition of SMC proliferation and transplant arteriosclerosis (Lou et al., 1998).

Recent *in vivo* results, obtained in a rat model of endothelial denudation of carotid artery, show that ERβ mRNA dramatically increases in SMC in the media and neointima as a consequence of injury ; they also provide evidence that genistein, a weak estrogen on the reproductive tract, is a potent vasculoprotective agent in regulating the proliferation of SMC in the vascular wall (Mäkelä et al., 1999).

In addition to vascular SMC, estradiol decreases growth and migration of adventitial fibroblasts in response to vascular surgery *in vivo* ; estradiol directly modulates SMC expression of factor(s) controlling migration of adventitial fibroblasts. In addition to estrogen, the dietary phytoestrogens biochanin and daidzein, and progesterone inhibit serum-induced cardiac fibroblasts proliferation *in vitro*, a process which could be involved in cardiac remodeling (Dubey et al., 1998).

Stimulation of endothelial cell proliferation

The cytoplasmic targets of direct E2 action on endothelial cell proliferation are still unknown. Estradiol induces mitogen-activated protein (MAP) kinase

activity in HUVEC, and this activation could result, at least in part, from an autocrine stimulation of bFGF (Kim-Schulze et al., 1998).

Other effects of estradiol

Estradiol inhibits monocyte adhesion by inhibiting expression of VCAM-1 *in vivo* (Nathan et al., 1999), in addition to analogous results in previously described *in vitro* experiments. It also reduces lipid accumulation in monocyte-derived macrophages, and this effect appears specific for the female sex (McCrohon et al., 1999).

Effects of progesterone on endothelial cell function

Micronized progesterone, when added to estrogen replacement therapy, does not attenuate the favorable effect of estradiol on endothelium-dependent vasodilation in women with mild hypercholesterolemia (Gerhard et al., 1998).

Functional nuclear PR are reported in endothelial cells ; physiological levels of progesterone inhibit proliferation of endothelial cells *in vivo* and *in vitro* (Vazquez et al., 1999). *In vivo* analysis of aorta re-endothelialization in wild-type and PR-knock-out mice shows that the rate of re-endothelialization is significantly decreased in wild-type mice in the presence of progesterone, whereas there is no difference between control and PR-knock-out mice.

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