Editorial

Interference of Progestins With Endothelial Actions of Estrogens
A Matter of Glucocorticoid Action or Deprivation?

J.F. Arnal, P. Gourdy, T. Simoncini

Estrogens play a pivotal role in sexual development and reproduction and are also implicated in a number of physiological processes in various tissues including the cardiovascular system. Epidemiological evidence suggests that endogenous estrogens protect women against coronary heart disease (CHD) before the age of menopause, and decreased CHD risk among postmenopausal women has been for a long time the main expected benefit of hormone therapy (HT).1 However, from the publication of the Women Health Initiative (WHI) results,2 medical practices of HT have been dramatically altered.

Vascular Effects of HT on the Coronary Heart Disease in Randomized Trials and on Experimental Models

Unexpectedly, large randomized controlled trials failed to demonstrate a beneficial effect of HT (conjugated equine estrogens combined [CEE] with medroxyprogesterone acetate [MPA]) for both secondary (Heart and Estrogen/Progestin Replacement Study [HERS])3 and primary CHD prevention (Women’s Health Initiative study [WHI]),2 and even revealed a detrimental effect during the year after the initiation of the HT. When women enrolled in the WHI study were divided according to the delay between onset of menopause and initiation of HT, the coronary risk tended to be lowered compared to placebo when HT is initiated during the first 10 years after menopause (hazard ratio for CHD=0.88).4 However, this risk tended to increase when HT was started 10 to 19 years postmenopause (hazard ratio for CHD=1.23), and the increase was significant when HT was started after more than 20 years (hazard ratio for CHD=1.66).

See accompanying article on page 586

In striking contrast, and in line with epidemiological and cohort studies suggesting a protective effect of estradiol (E2), a large amount of data from experimental models of atherosclerosis (from mouse to monkey) demonstrated that endogenous as well as exogenous E2 prevents the development of fatty streaks in comparison with castrated animals given a placebo. Several reviews summarized our current knowledge of the cellular or molecular mechanisms of E2 action, mainly in mouse models.5–7 Furthermore, in primate models, Clarkson et al8 provided convincing evidence for the primary prevention of coronary artery atherosclerosis when estrogens are administered soon after surgical castration. Noteworthy, the efficacy of estrogens on plaque progression was inversely related to the duration of the estrogen deprivation period after ovariectomy, revealing a total loss of the beneficial effects when the E2 treatment was delayed for a period corresponding to 6 postmenopausal years in women.8

Interference of Progestins With Estrogen Actions In Vivo

More than 3 decades ago, unopposed estrogens were recognized to increase the risk of endometrial cancer, a deleterious effect prevented by the association to progestin. However, in these last years, several studies evidenced that this interaction could not be limited to the endometrium.

First, the comparison of 2 arms of the WHI trial demonstrated the deleterious role of the progestogen MPA on CHD. Whereas nonhysterectomized women receiving CEE combined with MPA had increased frequency of CHD events than women taking placebo;2 it was not the case of hysterectomized women receiving CEE alone.9 Unfortunately, this randomized trial did not provide information concerning natural progesterone.10 Interestingly, more than 10 years ago, Miyagawa et al11 compared MPA with progesterone as the progestin in HT from the standpoint of coronary artery vasospasm. In ovarioctomized rhesus monkeys, they demonstrated that coronary vasospasm was prevented in monkeys chronically given E2 alone or E2 plus progesterone, whereas those given E2 plus MPA were not protected from vasospasm, a finding making great sense after the publication of the 2 arms of WHI.2,9

Second, compared to the oral route, transdermal E2 administration allows to avoid the hepatic first-pass and consequently to limit certain deleterious effects. The ESTrogen and THromboEmbolism Risk (ESTHER) study showed indeed that oral estrogens increased VTE risk, whereas transdermal estrogens had little or no impact on the development of thrombosis.12 Noteworthy, the ESTHER cohort Study also indicated that the combination of transdermal estrogens and 19-norpregnane progestins was associated with an increased venous thromboembolic risk, whereas transdermal estrogen alone or combined with either progesterone or pregnant derivatives appeared safe with respect to thrombotic risk.13

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Thus, although these latter clinical findings have to be confirmed by randomized clinical trials, they suggest that both the route of estrogen administration and the type of progestogen may be critical determinants of the benefit-to-risk profile of HT.13

**Cultured Cell Models to Study the Actions of E2 In Vitro**

In the present issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Zerr-Fouineau et al14 examined whether progestins affect the stimulatory effect of E2 for 24 hours on the NO production in a model of cultured endothelial cells. They report that E2 increased eNOS expression as well as NO production, and that E2 potentiated the inhibitory effect of endothelial cells on platelet aggregation. Both MPA and progesterone inhibited these beneficial effects of E2, whereas mifepristone, a glucocorticoid and progesterone receptor antagonist, prevented the inhibitory action of both progestins. Other progestins as levonorgestrel and nomegestrol acetate elicited more complex actions, inhibiting the E2 action on eNOS expression, but not on platelet aggregation. Some of these findings are in contrast with previous publications, where no antagonistic effects of progesterone or nomegestrol acetate on eNOS expression were found,15 but altogether they reinforce the emerging concept that progesterone and progestins are not a single class of hormonal compounds. This may well depend on the “flexible” nature of progesterone receptors, which are driven to recruit different signaling intermediates based on the specific hormone engaging the ligand binding pocket, but could also depend on the level of glucocorticoid activity in the model. This results in different biological responses, including nitric oxide synthesis in endothelial cells.16

**What Is the Relevance of This In Vitro Study for In Vivo Situations?**

At first glance, in line with the clinical studies summarized above, this study supports, that progestin can alter the beneficial actions of E2, as the endothelial NO production in the Zerr-Fouineau’s work. However, it should be reminded that cell culture medium devoid of steroid hormones are classically used to study the action of steroids in general, and of estrogen or progestin in particular. Consequently, these medium are deficient not only in sexual hormones, but also in other physiological steroid hormones including glucocorticoids. Importantly, in their Figure 2,14 a physiological concentration of hydrocortisone (1 nmol/L, an activity corresponding to the circulating levels of the free glucocorticoid hormone in mammals) fully abrogated the stimulatory action of E2 on endothelial NOS expression. It should be kept in mind that glucocorticoids and progestins bind to receptors that share many structural and functional similarities, including virtually identical DNA recognition specificity.17 It is thus possible that the antagonistic actions of these 2 progestins on the E2 effects would have been attenuated or even abolished if the culture medium had been supplemented with hydrocortisone, and thereby undetectable in vivo. Unfortunately, neither in vitro nor in vivo experiments to address this question were performed in the present work.14 However, the interrelationship between progestin and glucocorticoid signaling was previously explored in LPS-activated endothelial cells on factor nuclear factor kB and endothelial leukocyte adhesion molecules expression.16 When administered together with physiological amounts of glucocorticoids, MPA (which also binds glucocorticoid receptor) markedly interfered with the hydrocortisone-dependent stabilization of the transcription factor nuclear factor kB and with the expression of adhesion molecules, acting as a partial glucocorticoid receptor antagonist. In contrast, the addition of progesterone did not alter the antiinflammatory action of hydrocortisone.16

To conclude, this work highlights once again the importance of the modelization. The culture conditions to study the action of steroids reveal the antagonist effect of glucocorticoids on the induction of eNOS expression by estrogens in vitro. Accordingly, eNOS expression was not found to be influenced by E2 in rat18 or in mouse,19 potentially as a consequence of the circulating glucocorticoids in vivo. Similar in vitro models were used to study the action of E2 on endothelial NO production through an acute (10 to 30 minutes) stimulation of endothelial NO synthase activity (reviewed in15,20,21). Thus, future studies should address the question whether glucocorticoid hormones interfere with short-term membrane-initiated actions of estrogens in these experimental settings? In addition, it appears that established physiological actions are often less obvious in the context of disease. Plus, they act in ways that are much more complex than we previously thought. This underlines the requirement to tightly connect the in vitro and the in vivo models and constantly remind the concept of homeostasis defined by Claude Bernard.

**Disclosures**

None.

**References**


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