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Effects of progestogens on thrombosis and atherosclerosis

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In contrast with past practice, current hormone replacement usually includes a combination of oestrogens and progestogens. In this article, we review the effect of progestins on haemostasis and in the development of atherosclerosis. Second-generation progestogens produce minor haemostatic changes, and in lipid metabolism they decrease the synthesis of triglycerides and very low density lipoproteins (VLDL) and stimulate hepatic lipoprotein lipase. In combination, progestogens modify the effect of oestrogens on hepatic metabolism, endothelium and platelets. Several new progestins (known as third-generation) have less effect on lipid profiles. In vessel walls, animal studies have shown that progestogens are able dose-dependently to inhibit the beneficial effect of oestrogen without significant changes in lipid concentrations. The endothelium-dependent vasoconstrictor effect of progestogens on the arterial wall has been also evaluated. Large epidemiological studies show a two-fold increase in risk of venous thromboembolism with the use of third-generation progestins. Regarding the risk of myocardial infarction, no definite evidence is yet available with the use of third-generation progestins. The clinical consequence is therefore that second-generation progestins are the first choice in prescription for first-time users.

Key words: atherosclerosis/haemostasis/progestogens

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Introduction

Premenopausal women have a lower incidence of cardiovascular events compared with age-matched men (Levy and Boas, 1936; Glendy *et al.*, 1937; Robinson *et al.*, 1959; Parrish *et al.*, 1967; McGill and Stern, 1979). However, after menopause the incidence of coronary artery disease increases in women. Oestrogen deficiency may be responsible for this phenomenon (Barrett-Connor and Bush, 1991), although the mechanisms of this cardioprotective (or atheroprotective) effect of oestrogen have not been completely defined. A direct effect of oestrogen on the arterial wall has been suggested from animal and human experimental data. In recent years, a variety of direct and indirect oestrogen effects on the metabolism of the vascular wall have been demonstrated.

Less is known about the effect of progesterone in the process of atherosclerosis. Progesterone has contradictory effects on serum lipids (Tikkanen, 1990), and little is known about the influence of progesterone on the beneficial action of oestrogen in the arterial vessel wall.

The representatives of first-generation progestins are derivatives of nortestosterone, and include norethisterone and lynestrenol. A second generation of progestogens, levonorgestrel, which is also a nortestosterone derivative, was introduced at the end of the 1960s. The third-generation progestins, derivatives of levonorgestrel, were developed to reduce androgenic metabolic side effects, which may cause atherosclerosis and arterial diseases, with a similar or even higher contraceptive efficacy. Representatives of this group are desogestrel and gestodene. A third derivative, norgestimate, is difficult to classify because it is partially metabolized to levonorgestrel and partially to other intermediates (Helmerhorst *et al.*, 1997) (Table I). Another classification of progestins relates specifically to their androgenic properties. Cyproterone acetate, chlormadinone acetate and dienogest are anti-androgenic progestins, levonorgestrel and

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gestodene have mainly androgenic properties, and medroxyprogesterone acetate is relatively neutral (Table II).

Table I. Classification of progestins according to time of appearance

First generation	Second generation	Third generation
Norethisterone	Levonorgestrol	Desogestrel
Lynestrenol		Gestodene
		Norgestimate

Table II. Classification of progestins according to androgenic properties

Anti-androgenic	Androgenic	Neutral
Cyproterone acetate	Levonorgestrol	Medroxyprogesteron e acetate
Chlormadinone acetate	Gestodene	
Dienogest		

Most of the clinical studies are based on women taking unopposed oral oestrogen. In contrast with past practice, current hormone replacement in women with an intact uterus usually includes a progestogen. Few studies have examined the risk of cardiovascular disease in women taking oestrogen plus progestogen. A case-control study from the United Kingdom (Thompson *et al.*, 1989) found a non-significantly increased relative risk (1.2) for heart disease or stroke associated with oestrogen plus progestogen. On the other hand, a cohort study from Sweden (Ingemar *et al.*, 1990), where one-third of women also received a progestogen, found a significantly reduced relative risk (0.5) in women treated with oestrogen and progestin. Two clinical trials of hormone replacement therapy have focused on long-term heart disease end-points. In the first study (Nachtigall *et al.*, 1979), women were assigned to oestrogen and progestogen or placebo. After 10 years, 1.2% of the 84 hormone-treated women and 3.7% of the placebo-treated women had a heart attack (relative risk of 0.33, not statistically significant). The other study was the Nurses' Health Study (Grodstein *et al.*, 1996), that evaluated cardiovascular disease and postmenopausal hormone therapy after 16 years follow-up in 59 337 women. Women who took oestrogen with progestin, when compared with women who did not take hormone therapy, had a relative risk ratio of 0.39, which was equivalent to that with oestrogen therapy alone. Thus, in this clinical trial the addition of progestin did not attenuate the cardioprotective effects of postmenopausal oestrogen therapy.

However, observational studies may be misleading because women who take postmenopausal hormones tend to have a better coronary heart disease (CHD) risk profile (Matthews *et al.*, 1996) and to obtain more preventive care than non-users (Barret-Connor, 1991). The HERS (Heart and Estrogen/Progestin Replacement Study) trial is the first randomized, double-blind, placebo-controlled trial of daily use of oestrogen plus progestin (medroxyprogesterone acetate) on the combined rate of death from non-fatal myocardial infarction and CHD death among postmenopausal women with established coronary artery disease and advanced age (average of 66.7 years). After a follow-up of 4.1 years, treatment with this combination did not reduce the overall rate of CHD events. The lack of an overall effect occurred despite a 11% net reduction of low-density lipoprotein concentrations and a 10% increase of high-density lipoprotein concentrations in the hormone group compared with the placebo. Therefore, the authors do not recommend starting this treatment for the purpose of secondary prevention of CHD, although it could be appropriate for women already receiving hormone treatment to continue (Hulley *et al.*, 1998).

Another important issue to address is whether preparations containing third-generation progestogens reduce the risk of myocardial infarction (Thorogood, 1997). The WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception found the completely unexpected result that the risk of a thrombosis (venous thromboembolism, stroke and myocardial infarction) was related to the type of progestogen used in the oral contraceptives, with the third-generation progestogens desogestrel and gestodene being associated with a relative risk more than twice as large as that associated with use of combined preparations containing levonorgestrel (Meirik *et al.*, 1995).

Interim data from the Transnational Study (Lewis *et al.*, 1996) show that there is no increase in risk associated with third-generation preparations, and the ratio of risk for third as opposed to second generation was 0.36. This estimation of the odds ratio for third-generation preparations is based on just six cases, and should be interpreted with extreme caution. A further analysis has been carried out on data from a computerized database of primary care records in the UK (the GPRD database). The study included 600 000 women-years at risk among women aged under 45 years with no known risk factors for myocardial infarction and who had received at least one oral contraceptive prescription. Eleven women with the diagnosis of myocardial infarction or sudden death were identified, and a nested case control study was carried out to estimate relative risks of myocardial infarction by type of progestogen in the preparations. This analysis also suggests less risk with third-generation pills, but with very small numbers of cases and wide confidence intervals (Jick *et al.*, 1996).

Effect on haemostasis

The numerous investigations on the influence of combinations of oestrogens and progestogens on various haemostatic serum parameters have provided no convincing explanation for the increased risk of venous and arterial thromboses during treatment with oral contraceptives. However, a general

pattern in the haemostatic changes is that coagulation changes are unfavourable both on account of reductions in anticoagulant proteins and increases in procoagulant proteins, while fibrinolytic changes seem to show a favourable pattern of increase in potency. The magnitude of the changes induced by oral contraception has generally shown a decrease when the dosage of the oestrogenic component was reduced from 100 to 50 μg and lower. At the lower oestrogen dosages, the progestogenic effects may become more prominent. Reports have suggested that third-generation oral contraceptives might prove a greater risk of developing venous thrombosis than the second generation.

Some reviews (Winkler, 1993; Kuhl, 1996b) indicate that the haemostatic changes occur mostly due to oestrogenic effects. Particularly, norethisterone (first generation) might have contributed to the dose of oestrogen since some of the metabolic products have oestrogenic activity (Meade *et al.*, 1977). Other progestogens alone have no effects, or only minor ones, and exert mainly a modulating action in combination with the oestrogenic component by their anti-oestrogenic potency and possible androgenic activity.

Four epidemiological studies (Koster *et al.*, 1993; Jick *et al.*, 1995; WHO, 1995; Spitzer *et al.*, 1996) showed a two-fold increase risk of venous thromboembolism with the use of oral contraceptives containing third-generation progestins (gestodene and desogestrel), relative to second-generation products (levonorgestrel). Biases cannot devaluate the conclusion that the increased risk of thromboembolism, especially in first-time and younger users of third-generation oral contraceptives, is highly likely (Helmerhorst *et al.*, 1997).

Progestogen-only formulations have no or only minor effects on haemostasis, depending on type and dose (Basdevant *et al.*, 1991). However, the progestogen component may modulate the action of ethinyloestradiol. The pronounced effect of orally taken oestrogens on hepatic metabolism may be counteracted by progestogens, particularly by compounds with androgenic properties. On the other hand, the progestogen component may modify the effect of ethinyloestradiol on platelets, endothelium or macrophages, resulting in different alterations of coagulatory and fibrinolytic activity in the vessels.

A comparison between formulations containing ethinyloestradiol 30 μg and desogestrel 150 μg or levonorgestrel 150 μg revealed that factors II and VII were elevated only during treatment with the ethinyloestradiol/desogestrel combination, which also caused a more pronounced elevation of plasminogen. On the other hand, only the use of the ethinyloestradiol/levonorgestrel combination increased factor V and platelet aggregation and shortened activated partial thromboplastin time, while the ethinyloestradiol/desogestrel formulation had no effect (Prasad *et al.*, 1989).

Comparisons of the influence of combinations of ethinyloestradiol with gestodene or levonorgestrel on haemostatic parameters indicate a more pronounced effect of the gestodene-containing preparation on fibrinogen, factors VII, X and XII and protein C, which increased, and on antithrombin III, which decreased (Cohen *et al.*, 1988; Omsjo *et al.*, 1989; Ball *et al.*, 1990; Refn *et al.*, 1990). No effect was observed on factors V and VIII and on blood clot lysis time, while whole blood clotting time was shortened to the same degree, indicating an increased sensitivity to platelet activation (Cohen *et al.*, 1988).

In another study, treatment with a triphasic ethinyloestradiol/levonorgestrel combination did not influence antithrombin III, while ethinyloestradiol 30 μg plus desogestrel 150 μg caused a transitory 15% reduction after 6 months (Bonnar, 1987). On the other hand, the levonorgestrel-containing formulation caused a more pronounced rise in fibrinolytic activity (Bonnar, 1987).

The results indicate that oestrogen-dominant formulations like the combinations of ethinyloestradiol with third-generation progestogens cause a stronger reduction in antithrombin III and exert a marked stimulatory effect on hepatic synthesis of plasminogen, fibrinogen, factors II, VII, X, XII and protein, while progestogens with androgenic properties (namely first and second generation) may counteract this. In contrast, combinations of ethinyloestradiol with levonorgestrel seem to enhance not only platelet aggregation and the intrinsic system for coagulation, but also fibrinolytic activity.

In recent reports, the prothrombin fragment 1+2 (F1+2) and thrombin–antithrombin complex (TAT) have been evaluated. A median increase of 45% in F1+2 has been recorded in women taking gestodene (Winkler *et al.*, 1996).

Most changes in haemostasis factors summarized above concern factors synthesized in the liver. Very limited experimental information is available on the response elements in haemostasis genes, and until now only in the promoter of factor XII has a functional oestrogen response element been definitely identified and characterized (Farsetti *et al.*, 1995). The plasma concentration of a molecule is the result of two processes: secretion into the blood stream and clearance. Oestrogens and progestogens may influence haemostasis variables by changing their hepatic clearance. Orally administered oestrogens are known to influence various aspects of hepatic metabolism because of the first-pass effect, whereas with transdermally administered oestrogens this influence on the liver is lessened (Kluft and Lansink, 1997).

Effect on fibrinolysis

Recent investigations have revealed that combinations of ethinyloestradiol with newer progestogens stimulate the activity of the extrinsic pathway of coagulation, which is generally balanced by an increase in fibrinolysis. The increase in the turnover of fibrin is reflected by a rise in serum markers of thrombin generation and fibrin degradation (Winkler *et al.*, 1989). For example, there was a strong increase in fibrinopeptide A concentrations during the first treatment cycles with combinations of ethinyloestradiol and gestodene or desogestrel

(Inauen *et al.*, 1987; Melis *et al.*, 1991). Similarly, the concentrations of fibrin degradation products or D-dimer were markedly elevated (Gram *et al.*, 1990; Bonnar, 1991; Thomson *et al.*, 1991; Winkler *et al.*, 1991; Petersen *et al.*, 1993).

Moreover, the interaction of oestrogens and progestogens with the endothelium may modulate the production of tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1). During treatment with combinations of ethinyloestradiol with desogestrel, gestodene or dienogest, a reduction in the levels of both t-PA and PAI-1, resulting in an increase in t-PA activity, is observed (Bonnar, 1991; Thomson *et al.*, 1991; Winkler *et al.*, 1991). Several experiments demonstrate the potential of the t-PA gene to respond to sex steroids. In human endometrial cells, progesterone increases t-PA mRNA concentrations (Miyachi *et al.*, 1995).

Increase in fibrinolysis potency is frequently suggested to provide a counterforce for coagulation changes and activation upon oral contraceptives use. There exist potentially counteracting (balancing) effects in coagulation and fibrinolysis activation, but they are not part of a regulated process and individual mismatches may appear (Kluft and Lansink, 1997).

Effect on lipid metabolism

Even though high-density lipoprotein cholesterol (HDL) is decreased, and triglycerides and low-density lipoproteins (LDL) are increased, during treatment with preparations containing an 'androgenic' progestogen, the strong direct effect of ethinyloestradiol on the arterial wall protects from the formation of atherosclerotic plaques, probably by preventing oxidation of LDL (Wahl *et al.*, 1983). The increased risk of arterial diseases during oral contraception, particularly in smokers, is due to the pronounced effect of ethinyloestradiol on haemostasis and the vasoconstrictor effect of the progestogen which may facilitate vasospasm and clotting at the site of an intimal lesion (Sarrel, 1989; Hillard *et al.*, 1992).

Unopposed progestogen treatment

It is known that progestogens with androgenic properties may decrease the synthesis of triglycerides and very low-density lipoproteins (VLDL), and stimulate hepatic lipoprotein lipase which is involved in the degradation of HDL_2. Therefore, treatment of postmenopausal women with levonorgestrel 125 μg/day resulted in a stimulation of hepatic lipase by 50% and a reduction of the serum concentration of HDL_2 by 20%. Moreover, the concentrations of total triglycerides, total phospholipids, VLDL-triglycerides, LDL-triglycerides, LDL-phospholipids, HDL, HDL_2, HDL-phospholipids and apolipoprotein A1 were decreased by 10 to 30% (Kuusi *et al.*, 1985; Kauppinen-Mäkelin *et al.*, 1992).

The newer nortestosterone derivative desogestrel, which has only a weak androgenic activity, stimulated hepatic lipoprotein lipase by only 15% and reduced HDL_2 concentrations by 12%. Desogestrel had a less suppressing effect on triglycerides and VLDL-triglyceride concentrations and did not affect the other lipid parameters (e.g. HDL and LDL cholesterol) (Kuusi *et al.*, 1985; Tikkanen *et al.*, 1986; Kauppinen-Mäkelin *et al.*, 1992). LDL and HDL_3 were influenced neither by levonorgestrel nor by desogestrel (Kauppinen-Mäkelin *et al.*, 1992). Neither gestogen influenced peripheral lipoprotein lipase or lecithin–cholesterol acyltransferase (LCAT) (Kuusi *et al.*, 1985).

Combination with oestrogens

Progestogens, particularly those with androgenic properties, may counteract many of the effects of oestrogens on lipid metabolism. Because of their weak 'androgenicity', the antagonistic effects of newer progestogens on ethinyloestradiol-induced changes in lipid composition are less pronounced.

Levonorgestrel may markedly reduce the ethinyloestradiol-induced synthesis of triglycerides and VLDL, and of apolipoprotein A1 and A2, but not apolipoprotein B, and accelerate the clearance of VLDL remnants. When a combination of ethinyloestradiol 30 μg and levonorgestrel 150 μg is used, however, the effect of the oestrogen outweighs that of levonorgestrel on hepatic lipoprotein lipase, resulting in a 30% increase. In spite of this there was a significant reduction in the concentrations of HDL and HDL_2, which had been assumed to increase the risk of atherosclerosis during long-term treatment, although there is no alteration in total cholesterol, triglycerides and phospholipids, LDL and HDL_3 (Kauppinen-Mäkelin *et al.*, 1992).

The newer progestogens with weak androgenic properties may counteract the actions of ethinyloestradiol on lipid metabolism to a much lesser extent than levonorgestrel. During a 12-month trial with a combination of ethinyloestradiol 30 μg and desogestrel 150 μg or gestodene 75 μg, it became obvious that some parameters changed with the duration of treatment. There was a transitory decrease in LDL and LDL-phospholipids with both formulations after the first cycle. After 12 months, the concentrations of total cholesterol and phospholipids were not altered, while those of total triglycerides, VLDL and apolipoprotein B were increased. The concentrations of all components of HDL and HDL_3, including apolipoprotein A2, were significantly elevated, while those of HDL_2 and apolipoprotein A1 were not altered (Kuhl *et al.*, 1990).

The alterations in the composition and concentrations of the lipoproteins appear to be beneficial rather than deleterious with the preparations using newer progestogens. Although the rise in HDL_2 is partly due to an inhibition of hepatic lipoprotein lipase, the ethinyloestradiol-induced stimulation of HDL_3 synthesis appears to play a role. Moreover, the unaltered concentrations of total cholesterol and LDL indicate no increased risk of atherosclerosis.

In addition, accumulating evidence suggests that ethinyloestradiol may prevent oxidation of LDL and remnants, which is

the first event in the chain of plaque formation in the arterial intima, by effectively capturing free oxygen radicals (Huber *et al.*, 1990; Maziere *et al.*, 1991; Rifici and Khachadurian, 1992; Subbiah *et al.*, 1993; Sach *et al.*, 1994). LDL composition and atherogenic potential after contraceptive treatment (oestrogen and progestin), alone or in combination, has been studied in cynomolgus monkeys fed with an atherogenic diet (Manning *et al.*, 1997). The triphasic oral contraception (combination of ethinyloestradiol with levonorgestrel) altered the composition of LDL toward a less atherogenic particle that are more atherosclerotic, contained less cholesterol and less apolipoprotein E, and was less reactive with arterial proteoglycans compared with L-norgestrel alone. The inclusion of ethinyloestradiol in the triphasic oral contraceptive treatment was sufficient to negate the potentially atherogenic effects of L-norgestrel on LDL composition. In contrast, in another study (Suzuki *et al.*, 1997) it was found that minimally oxidized LDL significantly increased adhesion of human monocytic THP-1 cells to human aortic endothelial cells as compared with native LDL at the same concentration. Although 17β-oestradiol inhibited minimally oxidized LDL-induced THP-1 cell adhesion in a dose-dependent manner, progesterone had no significant effects. These findings also suggest a beneficial effect of hormone replacement therapy on atherosclerosis.

On the other hand, high triglyceride concentrations are known as a risk factor for cardiovascular disease. It is necessary, however, to distinguish between high triglyceride concentrations caused by impaired catabolism and elimination of VLDL and remnants which represent a high atherosclerotic risk, and a rise in triglycerides induced by the stimulatory effect of oestrogens on hepatic triglyceride synthesis. As ethinyloestradiol also enhances the B and E receptor-mediated uptake of remnants and LDL in the liver, the turnover of these lipoproteins is increased. This results in a shortening of the residence time in the circulation and consequently in a reduction of LDL oxidation which may occur in the arterial intima. Therefore, ethinyloestradiol-induced rises in triglycerides probably do not represent an increased cardiovascular risk (Kuhl, 1996a).

It is known that androgens and progestogens may decrease the serum concentration of lipoprotein (a) [Lp(a)]. Treatment with desogestrel-containing oral contraceptives has been demonstrated transitorily to reduce Lp(a) concentrations (Kuhl *et al.*, 1993). However, the physiological significance of suppressing Lp(a) by sex steroids remains unknown (Godsland *et al.*, 1987).

Effect on vessel wall

There is now strong epidemiological evidence that oestrogen replacement therapy has a protective effect in postmenopausal women (Stampfer and Colditz, 1991; Sullivan and Fowlkes, 1996). The cardiovascular protective action of oestrogen is mediated indirectly by an effect on lipoprotein metabolism and by a direct effect on the vessel wall itself. A variety of oestrogen effects on cells of the arterial wall have been described that could form the basis of oestrogen protection at this level. These include oestrogen effects on endothelial cells resulting in decreased endothelin production (Akishita *et al.*, 1996), decreased cytokine-induced endothelial cell adhesion molecule expression (Caulin-Glaser *et al.*, 1996) and increases in nitric oxide secretion (Arnal *et al.*, 1996) and prostaglandin I_2 production (Mikkola *et al.*, 1996). In smooth muscle cells, oestrogens have been reported to decrease the rate of cell proliferation (Rhee *et al.*, 1977; Jacobson *et al.*, 1992; Chen *et al.*, 1996), migration (Kolodgie *et al.*, 1996) and calcium flux (Han *et al.*, 1995). Although macrophages have been less extensively studied, there is some evidence to suggest that oestrogens can increase phagocytosis (Chao *et al.*, 1996) and enhance cholesterol ester hydrolysis (Tomita *et al.*, 1996) as well as inhibit the synthesis of the chemotactic cytokine, monocyte chemoattractant protein-1 (MCP-1) (Frazier-Jessen *et al.*, 1995). The extent to which one or a combination of these mechanisms can explain the protection from atherosclerosis by oestrogens is unclear.

Effect on cell proliferation

Proliferation of smooth muscle cells is an essential event in the process of atherosclerotic lesion formation (Ross, 1986; Fuster *et al.*, 1992). Contradictory results have been reported in experimental animals regarding the ability of progestogens to affect muscle cell proliferation. The experimental animal model of choice and the parameters evaluated may have a significant influence in these dissenting results.

The effect of progesterone in experimental atherosclerosis in hypercholesterolaemic rabbits has been reported (Hanke *et al.*, 1996a,b). An inhibitory effect of oestrogen of intimal thickening was found, in comparison with the control group, whereas progesterone alone did not show a significant effect on intimal plaque size. In combination with progesterone (high dose), oestrogen was not able to reduce intimal atherosclerosis. However, the beneficial effect of oestrogen was not affected by progesterone, when this was reduced respectively to one-third or to one-ninth of the highest dosage. Interestingly, these differences in atherosclerotic plaque development were observed without significant changes in plasma cholesterol concentrations by the administered hormones. Thus, progesterone was able dose-dependently to inhibit completely the beneficial effect of oestrogen in experimental atherosclerosis, probably by affecting arterial sex hormone receptors. Oestrogen and progesterone receptors have been identified in arterial endothelial cells and smooth muscle cells in the baboon (Lin *et al.*, 1986) and in human coronary arteries (Ingegno *et al.*, 1988). In ovariectomized baboons, 17β-oestradiol was apparently able to affect the intracellular distribution of cardiovascular oestrogen receptors and increase the cytoplasmic concentration of proges-

terone receptors, suggesting that these oestrogen receptors in the baboon are physiologically functional (Lin *et al.*, 1986).

The ability of oestrogens and progestogens to inhibit the growth of human umbilical vein smooth muscle cells in culture has been examined (Morey *et al.*, 1997). Mitogen-stimulated mitogen activated protein (MAP)-kinase and MAP-kinase activities were inhibited significantly by either oestrogens or progestogens. The steroids also inhibited mitogen-stimulated c-fos and c-myc, downstream targets for MAP-kinase action. Critical signalling and molecular events through which mitogens stimulated smooth muscle cell proliferation could be significantly inhibited by oestrogens or progestogens, providing a potential cellular mechanism for their vascular protective actions.

Progesterone receptors were detected in human and rat aortic smooth muscle cells, important constituents of atherosclerotic plaques. Progesterone at physiological concentrations inhibited DNA synthesis and proliferation in these cells in a dose-dependent manner, and pretreatment with the progesterone receptor antagonist RU486 blocked inhibition. Cyclin A and E mRNA levels decreased after progesterone treatment, but those of cyclin B and D1 did not change. This cell cycle-dependent inhibition of arterial smooth muscle cell proliferation by progesterone could represent a mechanism for the hormone's protective effect against atherosclerosis (Lee *et al.*, 1997).

Sex hormone-binding globulin (SHBG) is a glycoprotein in human plasma that binds with high affinity to androgens and has a lower affinity for oestradiol (Raudaskoski *et al.*, 1998). The main biological function of SHBG is the plasma transport of sex steroids, but descriptions of a specific membrane receptor (SHBG-R) in human tissue (Fortunati *et al.*, 1991) and cultured cells (Fortunati *et al.*, 1993) have suggested other potential functions at a cellular level. Indeed, SHBG-R initiates a cascade that signals through adenylyl cyclase and cAMP (Rosner *et al.*, 1998). It is well known that SHBG is increased by androgenic sex steroids (Bang *et al.*, 1992; Nakhla *et al.*, 1997), including androgenic progestins, and this may have an impact on their availability to target organs. In a prostate cancer cell line, dihydrotestosterone (DHT) increases growth in the presence of SHBG. The DHT–SHBG-mediated growth is enhanced by inhibiting protein dephosphorylation with okadaic acid (Rosner *et al.*, 1998). This growth-stimulatory effect of androgenic steroids, though not proven, could also be responsible for smooth muscle cell growth and proliferation in the process of atherosclerosis.

On the other hand, the combination of 17β-oestradiol and progesterone inhibited 2.5% of cardiac fibroblast-induced proliferation (DNA synthesis and cell number) and collagen synthesis ([^{3}H]proline incorporation) in a concentration-dependent manner and to a similar extent in male and female cardiac fibroblasts (Dubey *et al.*, 1998). Moreover, hormone replacement therapy using 17β-oestradiol and progesterone may protect postmenopausal women against cardiovascular disease by inhibiting cardiac fibroblast growth and cardiac remodelling.

Effect on endothelium vasoreactivity

Progestogens have been shown to exert a direct constrictor effect on the arterial wall (Sarrel, 1989; Hillard *et al.*, 1992) and to enhance the distensibility and capacitance of veins (Goodrich and Wood, 1964; Fawer *et al.*, 1978). In the arteries, progestogens may antagonize the vasodilator action of oestrogens and in this way enhance the risk of vasospasm at the site of an endothelial lesion. By using ultrasound and Doppler colour flow mapping, it has been demonstrated in the aorta of ovariectomized rabbits that there is a significant dose-dependent increase in blood flow after treatment with 17β-oestradiol. The administration of progesterone did not attenuate the beneficial effect of oestrogens on arterial tone (Hegele-Hartung *et al.*, 1997). In the veins of disposed women (e.g. women with varicose veins), progestogens may intensify the vasodilatation caused by ethinyloestradiol, leading to a slowing of blood flow and ultimately to stasis.

A recent study has shown that rats subjected to balloon injury of the carotid artery present a different hormone response depending on the treatment administered and gender. Neither oestradiol nor progestin (medroxyprogesterone acetate) altered the neointimal response in males, whereas in females oestradiol reduced and progestin enhanced the neointimal response. The combined medroxyprogesterone acetate + oestradiol treatment enhanced the neointimal response in intact females, presumably by blocking the production and thus the vasoprotective effects of endogenous oestrogen (Oparil *et al.*, 1997). This may be related to progestin–oestrogen interactions at the receptor level (Kraus *et al.*, 1994) and/or to opposing effects on growth factors/mitogens in damaged vascular tissue (Levine *et al.*, 1996). Whatever the cellular mechanism(s) involved, these results are consistent with previous observations that addition of progestin to oestrogen treatment reduces the vasoprotective effects of oestrogen.

The endothelium is thought to play an important role in the genesis of atherosclerosis, and changes in endothelial function have been reported within an hour of oestrogen administration (Gilligan *et al.*, 1994). In non-human primates, oestrogen has been shown to improve endothelium-mediated vasodilatation in ovariectomized normocholesterolaemic and hypercholesterolaemic animals. Oestrogen has been found to increase basal arterial diameter and decrease basal vascular resistance. In experimental models, concurrent progesterone treatment may significantly modify the beneficial effects of oestrogens on vascular reactivity (Williams *et al.*, 1994). In isolated rabbit aortic rings, progesterone was found to antagonize short-term endothelium-dependent vasodilator responses to oestrogens (Miller and Vanhoute, 1991). In ovariectomized rats, unopposed oestrogen replacement

preserved endothelial reactivity, whereas combined oestrogen and progesterone treatment led to vascular responses similar to those seen in endothelium-denuded aortic rings (Rudd and Loscalzo, 1995). In monkeys with diet-induced atherosclerosis, the addition of medroxyprogesterone diminished the beneficial effect of oestrogen on endothelium-dependent coronary vasoreactivity (Williams *et al*., 1994).

Ca^{2+} homeostasis is central to both endothelial and vascular smooth muscle cell function. Endothelial release of NO and prostacyclin is Ca^{2+}-dependent (Adams *et al*., 1989), and increased intracellular Ca^{2+} prompts vascular smooth muscle contraction (Khalil *et al*., 1987). The immediate effects of progesterone on Ca^{2+} homeostasis in vascular as well as non-vascular tissue support the existence of non-genomic influences. Acute administration of progesterone alone caused relaxation in endothelium-denuded rabbit coronary artery rings (Jiang *et al*., 1992) preconstricted with BayK8644, a voltage-gated Ca^{2+}-channel antagonist. Clearly, further studies are needed on genomic and non-genomic effects of progesterone and its derivatives on Ca^{2+} homeostasis in vascular smooth muscle and endothelial cells (White *et al*., 1995).

Cyclical oestradiol and norethisterone hormone replacement therapy administered for 2.9 ± 0.5 years did not improve endothelial function, measured as brachial artery flow-mediated vasodilatation (Sorensen *et al*., 1998). Again, the addition of a progestin in a hormone replacement regimen may counteract the beneficial effects of oestrogen on cardiovascular disease.

Conclusions

The available clinical experience, the results of experiments with primates and rabbits, and the findings on radical scavenger properties of oestrogens led to the conclusion that long-term treatment with oral contraceptives containing progestogens does not cause atherosclerosis. In postmenopausal healthy women, there seems to be a protective effect derived from hormone replacement therapy. In postmenopausal women with pre-existing disease, results are uncertain. Extended follow-up of the Heart and Estrogen/Progestin Replacement Study (HERS) and additional randomized trials, including the ongoing Women's Health Initiative, are needed to clarify the cardiovascular effects of postmenopausal hormone replacement therapy.

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