Understanding the oestrogen action in experimental and clinical atherosclerosis


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INTRODUCTION

Oestrogens play a pivotal role in sexual development and reproduction and are also implicated in a number of physiological processes in various tissues including cardiovascular (CV) system. Numerous epidemiological studies suggest that oestrogens protect women against CV diseases before the age of menopause. After menopause, the CV risk of women becomes progressively closer to that of men, reinforcing the hypothesis of an atheroprotective effect of oestrogens. However, the two controlled prospective and randomized studies published so far did not demonstrate a beneficial effect of hormone replacement therapy (HRT), neither in secondary prevention (Heart and Estrogen/Progestin Replacement Study, HERS) [1] nor in primary prevention (Women’s Health Initiative study, WHI) [2]. This is in contrast to the large amount of data from experimental models of atherosclerosis, where oestradiol (E2) treatment prevents the development of fatty streaks in comparison with castrated animals given a placebo [3].

The American Heart Association [4] recently defined five main priorities in the area of menopause treatment and CV risk including (i) the determination of the

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ABSTRACT

Whereas hormone replacement/menopause therapy (HRT) in postmenopausal women increases the coronary artery risk, epidemiological studies (protection in premenopausal women) suggest and experimental studies (prevention of the development of fatty streaks in animals) demonstrate a major atheroprotective action of oestradiol (E2). The understanding of the deleterious and beneficial effects of oestrogens is thus required. The immuno-inflammatory system plays a key role in the development of fatty streak deposit as well as in the rupture of the atherosclerotic plaque. Whereas E2 favours an anti-inflammatory effect in vitro (cultured cells), it rather elicits in vivo a proinflammation at the level of several subpopulations of the immuno-inflammatory system, which could contribute to plaque destabilization. Endothelium is another important target for E2, as it potentiates endothelial NO and prostacyclin production, thus promoting the beneficial effects as vasorelaxation and inhibition of platelet aggregation. Prostacyclin, but not NO, appears to be involved in the atheroprotective effect of E2. E2 also accelerates endothelial regrowth, thus favouring vascular healing. Finally, most of these effects of E2 are mediated by oestrogen receptor α, and are independent of oestrogen receptor β. In summary, a better understanding of the mechanisms of oestrogen action not only on the normal and atheromatous arteries, but also on innate and adaptive immune responses is required and should help to optimize the prevention of cardiovascular disease after menopause. These mouse models should help to screen existing and future selective oestrogen receptor modulators.
mechanisms of the CV events during the first year of the HRT, and (ii) the understanding of the beneficial effects of endogenous oestrogens.

After a brief summary of our current knowledge of atherosclerosis, we will detail the atheroprotective action of E2 in experimental models, and the cell populations that are the target of E2 and that could mediate the protective effect. Unexpectedly, we found that E2 elicited a proinflammatory action at the level of several cell populations of the inflammatory-immune system. Whereas this effect cannot account for the prevention of the fatty streak deposit in experimental models, it could contribute to plaque destabilization in postmenopausal women. Endothelium, another major target for E2, and endothelial messengers, such as NO and prostacyclin, are both increased by E2. Although the atheroprotective effect of E2 appears independent of NO production, induction of COX-2 and prostacyclin production plays an important role in the prevention of fatty streak. We finally detail the respective roles of oestrogen receptor alpha (ERα) and ERβ in the vascular effects of E2. We conclude by an attempt of a comprehensive view of experimental and clinical studies.

THE ATEROMATOUS PROCESS: NUMEROUS CELLULAR ACTORS FOR SEVERAL SCENARIOS

The first step of the atheromatous process is the penetration of atherogenic lipoproteins, in particular low density lipoproteins (LDL) through the endothelial monolayer [5]. LDL oxidation occurs in the sub-endothelial space and probably represents a necessary modification to the subsequent steps, because oxidized LDL induces in turn an activation of the endothelium, consisting in particular of an increased expression of adhesion molecules, such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1. These molecules are required to slow down circulating monocytes, to stop them and to allow their subsequent migration into the intima. In the sub-endothelial space, the activation of the monocytes induces their differentiation into macrophages, and this probably contributes to increase the level of LDL oxidation. These modified LDLs can be recognized by scavenger receptors expressed by macrophages. Thus, macrophages tempt to clean the intima, thereby preventing the accumulation of oxidized LDL. As oxidized LDL accumulates intracellularly, macrophages progressively turn into foam cells which constitute the major component of fatty streaks.

Blood flow shear stress represents a crucial protective factor, where abnormal shear stress promotes endothelial activation and dysfunction which represent so far the best and well-recognized explanation for a focal location of atheroma [6,7]. Classical risk factors (high blood pressure, hypercholesterolaemia, smoking, diabetes) appear to favour and/or even aggravate endothelial dysfunction. They can also favour the production of the chemokines and cytokines by the different cellular actors (endothelium, monocytes-macrophages, smooth muscle cells but also lymphocytes). Moreover, abnormal in vivo local shear stress appears to favour endothelial cell apoptosis and may be a major determinant of plaque erosion and thrombosis [8]. Protective factors are less recognized, although high density lipoproteins (HDL) appear of major importance.

Expansion of the fatty streak tends to be limited and circumvented by a scarring reaction of the smooth muscle cells migrating into the intima, secreting collagen and other extracellular matrix proteins. The balance between the inflammation level and the strength of fibro-muscular cap determines the stability of the atheromatous plaque [9]. Plaque rupture exposes thrombogenic materials leading to the formation of a thrombus, which threatens the viability of the tissue downstream the occluded artery. Unfortunately, plaque rupture is not satisfactorily modelled in mouse, and this probably represents the most important limitation of the current experimental approach.

Many groups have been working to describe the cellular and molecular mechanisms leading to the aggravation or to the protection from atheroma [9–12]. This was allowed by the generation of the two major models of hypercholesterolaemic mice: mice deficient in apolipoprotein E (apo E-KO) and mice deficient in LDL-receptor (LDLR-KO). apo E-KO mice are hypercholesterolaemic (3–4 g cholesterol/litre) under a chow diet and have very low levels of HDL cholesterol [13]. Accordingly, they spontaneously and rapidly (within a few weeks) develop fatty streaks at the root of the aorta. LDLR-KO must be given a Western diet (fat plus cholesterol) to develop fatty streaks, because their lipoprotein profile under chow diet is less severe than the apo E-KO mice.

So far, the cellular and molecular dissection of the pathophysiology of atheroma was explored by breeding hypercholesterolaemic mice and mice deficient in another specific gene. For instance, hypercholesterolaemic mice also deficient either in monocyte-macrophage (through a deficit of macrophage colony-stimulating
factor) [14], or in mature B and T lymphocytes (RAG-1 gene deficient) [15] develop respectively 10- and twofold less fatty streaks than control hypercholesterolaemic mice. It has been proposed that T-cell-mediated and antibody-mediated responses directed against plaque-associated antigens could influence the progression of arterial disease in a variety of animal models [16]. CD4+ T cells can be divided into functional subsets according to the type of cytokines they produce. T helper (Th1) cells mainly produce interferon (IFN)-γ, activate macrophages, and are implicated in the elimination of intracellular pathogens and tumours. This Th subset is also implicated in the development of autoimmune diseases. On the other hand, Th2 cells produce IL-4, IL-5, IL-10 and IL-13 which are associated with allergic diseases, and down-regulate Th1-mediated responses. Both subsets promote humoral immune responses with Th1 cells inducing the production of the Th1 cytokine IFN-γ appears to play a central role in the atherogenic process through its capacity to activate macrophages, to inhibit smooth muscle cell proliferation and collagen synthesis, thereby promoting plaque destabilization.

Mice deficient in various cytokines in general demonstrated an aggravating role of pro-inflammatory cytokines (such as IFN-γ, IL-1α, IL-12, IL-18) and a protective role of anti-inflammatory cytokines (mainly IL-10) in the development of the atherosclerotic process (refs in [10]). Platelets have also been recognized to participate to the constitution of fatty streak lesions at a very early stage, in particular at the level of the carotid bifurcation, a lesion-prone site, by interacting with the activated endothelium before any macrophage infiltration [17]. This process involves the platelet GPIbα (glycoprotein 1b, alpha polypeptide), and the adhesive proteins P-selectin and/or von Willebrand factor which mediate the attachment of platelets to activated endothelial cells. Blocking these interactions completely (~100%) prevented fatty streak formation at the level of the carotid bifurcation in apo E-KO mice, but only partially (~30%) at the level of the aortic sinus [17]. While this points out the platelets as a target for anti-atherosclerotic therapies, this also suggests that the modulation of the adhesive properties of the endothelium may be of pathophysiological relevance, especially at the level of the carotid bifurcation.

**E2 IS ATHEROPROTECTIVE IN EXPERIMENTAL MODELS DESPITE PROINFLAMMATORY ACTIONS**

**E2 prevents fatty streak in all animal species**

Studies in primates, mainly conducted by Clarkson and Appt [18] have provided convincing evidence for the primary prevention of coronary artery atherosclerosis when oestrogens are administered soon after the development of oestrogen deficiency. Equally convincing are the data from studies in cynomolgus monkeys indicating the total loss of the beneficial effects of oestrogens if the treatment is delayed for a period equal to six postmenopausal years for women [18]. Moreover, in the monkey model, an attempt has been made to identify the most effective hormone treatment regimen in preventing the progression of coronary artery atherosclerosis. By far, the most successful approach is that of using oestrogen containing oral contraceptive during the perimenopausal transition, followed directly by HRT postmenopausally. However, monkey model does not allow the understanding of the cellular or molecular mechanisms of E2 action.

Several groups, including us, have been working to describe the vascular effects of E2 and to elucidate the cellular and molecular mechanisms [3,19,20]. Ovariectomy of apo E-KO or LDLR-KO mice is followed by an increase in fatty streak lesion area and E2 prevents in both models the fatty streak deposit. However, serum E2 concentrations of the order of those encountered during gestation are necessary for maximal protection [21,22]. Although E2 treatment induces a decrease in serum cholesterol concentrations, the decrease involves both LDL and HDL fractions and is too minor to explain the hormone atheroprotective effect [3,22]. This effect seems rather to be the consequence of a direct effect of E2 on the cells of the arterial wall [19]. A similar conclusion was previously obtained by other groups [23,24] using hypercholesterolaemic rabbits. In addition, they showed the crucial role of intact endothelium, as the antiatherogenic effect of E2 was abolished, or even reversed, after balloon catheter injury [24].

**Involvement of the inflammatory-immune system**

As mentioned above, cell populations of the inflammatory-immune system (monocytes-macrophages, lymphocytes, etc.) play crucial roles in the pathophysiology of atherosclerosis [10–12]. Indeed, we demonstrated that E2 prevents the deposit of fatty streaks in immunocompetent apo E-KO mice, whereas it has no effect in mice...
deficient in both apo E and RAG-2 gene expression, lacking mature B and T lymphocytes [25]. One hypothesis resulting from these observations was that lymphocytes, or at least a subpopulation of them, were the mediators of the atheroprotective effect. After crossing apo E-KO mice with mice deficient in either T-cell receptor (TCRαβ, CD4, CD8 or TCRγδ T lymphocytes, we reported that TCRαβ T lymphocytes play a major role in fatty streak development [26]. However, the protective effect of E2 persisted in all these strains, showing that none of these lymphocyte subpopulations specifically mediated the atheroprotective effect of E2 [27]. This led us to investigate the effect of E2 on the cytokine production of different cell populations of the inflammatory-immune system, as it was shown to play a crucial role in the pathophysiology of atheroma [10].

Pro- or anti-inflammatory effect of E2?

Cell populations of the inflammatory-immune system are heterogenous and it is not easy to isolate them, especially in mouse models

At variance with macrophages in fatty streaks, peritoneal macrophages represent a cell population that can be obtained in considerable amounts, and thus the chronic effect of E2 on the inflammatory-immune system can be easily studied in these cells. We have first to mention that a chronic in vivo treatment of mice by E2 led to a proinflammatory response in peritoneal macrophages (B. Calippe, P. Gourdy, unpublished data), whereas an acute (only few hours of E2) in vitro treatment of the macrophage cell line RAW 264.7 by E2 led to an anti-inflammatory effect [28]. In vitro experiments have also suggested a selective inhibitory effect of E2 on the production of some inflammatory cytokines, such as TNF-α, IL-6, but not IL-12 or IL-10 by lipopolysaccharide (LPS)-activated splenic macrophages [29]. Similar differences were also reported by comparing the effect of E2 on microglial cells, the resident macrophages of the brain [30–32]. Whereas E2 was found to prevent LPS-induced microglial reactivity both in vitro and in vivo, based on the changes in inducible NO synthase, prostaglandin E2, and metalloproteinase-9 levels [30,31]; another study has clearly shown that E2, through ERα-signalling, was required for optimal transcriptional activation of TNF-α and IL-12 genes in the brain-resident microglia upon endotoxin challenge [32]. Although all these parameters were not simultaneously assessed in these two studies, the anti-inflammatory effect of E2 could depend on the target genes. Altogether, these striking discrepancies illustrate the importance of the in vivo approach to understand the pathophysiological effects of E2.

We have thus evaluated the influence of a chronic stimulation of E2 on the production of pro- and anti-inflammatory cytokines in lymphocytes and macrophages. We have demonstrated that the profile of cytokine secretion in CD4+ [33] as well as in natural killer (NK) T lymphocytes [34] is altered by E2. In these studies, an increase in IFN-γ production and a decrease in anti-inflammatory cytokine production were observed, resulting in a strong bias towards a Th1 profile. This effect of E2 was observed in vivo upon induction of T-cell responses but not in T cells stimulated in vitro, suggesting that E2 may not directly upregulate cytokine production in T cells as previously suggested. Likewise, we recently found that starting hormonal treatment at the time of immunization had almost no effect on the establishment of T-cell responses [35]. By contrast, E2 treatment limited to the 3-week period before immunization was necessary and sufficient to promote antigen (Ag)-specific Th1 cell expansion and the production of type-1-dependent IgG2a and IgG2b isotypes [35]. Furthermore, we found that the E2-mediated increase in Ag-specific Th1 cell development was abolished in IL-12R-deficient mice. These observations suggest that E2 acts on the antigen presenting cell (APC) compartment rather than T cells, although the latter cell type cannot be excluded as a potential target. In favour of a role for E2 on APC, it has been shown that oestrogens were needed for the optimal development of mouse dendritic cells (DC) from bone marrow precursors in vitro [36]. The requirement for oestrogens during DC differentiation suggests a mechanism by which E2 levels in peripheral tissues may modulate both number and functional properties of DC in vivo, thereby influencing immune responses. Indeed splenic DC numbers were increased in E2-treated mice, and this increase seems to affect preferentially CD8α+ DCs that have been shown to secrete higher amounts of IL-12 [35]. Similarly, we observed an increased production of IL-1 (α and β), IL-12 and IL-18 by macrophages obtained from chronically E2-treated mice compared with those from ovariectomized mice (B. Calippe, P. Gourdy, in preparation).

According to our current knowledge, the proinflammatory effect of E2 cannot account for its preventive effect of fatty streak accumulation [10–12]. In contrast, it could contribute to the destabilization of atheromatous plaques, and thus represent a good candidate to explain the increase in CV events during the year following the onset of HRT [1,2].
Understanding the protective and deleterious effects of E2 in atheroma

In summary, E2 exerts an atheroprotective effect in all experimental models and most likely in women before menopause. Although E2 decreases serum cholesterol, this influence on lipid metabolism is negligible. Endothelium is involved in the regulation of coagulation, leucocyte adhesion in inflammation, transvascular flux of cells, vascular smooth muscle growth, etc. and also represents a major target for E2. Endothelial messengers, such as NO (ref in [37]) and prostacyclin are increased by E2. Indeed, E2 can increase NO bioactivity acutely through a stimulation of endothelial NO synthase activity [37] and chronically through a decreased breakdown of NO, as a consequence of a decreased production of reactive oxygen species [38,39]. Although the atheroprotective effect of E2 appears as independent of NO production [40], induction of COX-2 and prostacyclin production was recently proposed to play an important role in the prevention of fatty streak at the level of thoraco-abdominal aorta [41]. Thus, the endothelial effect of E2 could represent one key mechanism of the atheroprotective effect of E2.

On the other hand, E2 also promotes the production of IFN-γ, a prototypic pro-inflammatory cytokine, by enhancing antigen-specific Th1 cell development. This proinflammatory effect could have been prominent in advanced atheromatous plaques in postmenopausal women, favouring a destabilization of the most unstable plaques through IFN-γ-dependent activation of macrophages. This previously unrecognized effect could significantly contribute for the increase in the frequency of CV events in postmenopausal women during the first year of the HRT, as observed in the HERS and WHI studies. It is of importance to note that the women enrolled in these studies were postmenopausal since several years (on average, more than 10 years after the onset of menopause). A subgroup analysis in the WHI study [42], suggests the absence of coronary risk when HRT is initiated during the first 10 years after menopause, whereas this risk increases 10 years and mainly 20 years after menopause. Although these differences do not reach the statistical significance, the trend is clear. Nevertheless, it is in perfect concordance with the conclusions drawn from the above-mentioned studies on primates [18].

Thus, it is likely that endogenous oestrogens protect against the coronary risk, whereas exogenous oestrogens are completely lost after 10 years of untreated menopause. In addition, hysterectomized patients treated with ‘oestrogens alone’ does not present any increase in coronary risk, irrespective of their age [43], underlining a potential deleterious role of the progestin (medroxyprogesterone acetate) used in HERS and WHI [44]. Indeed, this non-natural progestin used in these clinical trials possesses undesirable, deleterious effects [45].

Oestrogen receptor α mediates most of the vascular effects of E2

E2 effects can be mediated by ERα and ERβ, two members of the nuclear receptor superfamily, that are encoded by two distinct genes [46]. A collaborative effort with the Krust/Chambon group led to the clear-cut demonstration of a prominent role of ERα in vascular physiology in vivo. Full length ERα (66 kD) is composed of six ‘domains’ (named from A to F). The two transactivation function domains, AF1 and AF2, are found within domains B and E, respectively [47,48]. Both the ERα and ERβ genes have been subjected to targeted mutagenesis [46]. The first gene disruption mice model of ERα was generated by Lubahn et al. [49], through the insertion of the neomycin resistance gene in exon 1 (thus named ER-αNeoKO). These mice were subsequently shown to present a transcriptional leakage due to a non-natural alternative splicing of the ERα mRNA resulting in the expression of a truncated 55 kD isoform [50–52]. However, such an ERα isoform, lacking a major part of the B domain and thus the AF-1 transactivating function, was sufficient to mediate the E2 effect on the endothelial NO production [51]. Interestingly, an ERα 46 kD isoform, lacking the N-terminal portion (domains A/B), can be physiologically expressed through an alternative splicing [53,54] in the uterus [51,55] and in cultured endothelial cells [56]. This isoform is also expressed in the ER-αNeoKO mice [51].

In contrast, the generation and studies of mice that fully and unambiguously lack ERα [57] showed that ERα is necessary in the response of E2 on NO production [51]. ERβ-deficient mice had, however, a normal NO production [58]. Altogether, these data allow us to conclude that an ERα lacking the AF-1 function is sufficient to mediate some of the vascular effects of oestrogen.

However, the prevention of fatty streak appears to require the full length ERα (66 kD) [59]. Indeed, in contrast to the E2 protection elicited in apo E-KO mice, E2 treatment of ovariecotomized ERα-Neo/apo E double KO female mice caused a nonsignificant (P = 0.12)
reduction in lesion size and no reduction in total plasma cholesterol [59]. It should be however mentioned that E2 treatment significantly reduced the complexity of plaques in ERα-Neo/apo E double KO female mice, although not to the same degree as in apo E-KO female mice. Although this could have been due to the existence of ERβ-dependent atheroprotective effects of E2, the expression of the truncated 55 kD ER-α isofrom could also have been responsible of this E2 effect.

As mentioned previously, the loss of the integrity of the endothelial monolayer represent another important aspect at early steps of atherosclerosis, but also after the destruction provoked by endoluminal angioplasty (often followed by stent implantation) [60]. In this context, the acceleration of vascular healing, where re-endothelialization plays a key role, is considered as a major protective event against short- as well as long-term complications of endovascular therapy.

Endovascular desendothelialization in mice is a complex and delicate manipulation as a consequence of the very small size of carotid artery. Carmeliet et al. [61] proposed to destroy the endothelium using perivascular electric injury approach, and an adaptation of this model was proposed by our group [62]. Endovascular and electric perivascular injury are identical for their efficiency to destroy the endothelium, which will be temporarily replaced by a monolayer of platelets, but they differ in at least two major points. First, whereas endovascular injury preserves most of the medial smooth muscle cells as well as cells in the adventitia [63], electric injury destroys the cells of the three layers of the injured area, and in particular the smooth muscle cells which do not recolonize the media even after several weeks. Secondly, the main cell population in the perivascular electric injury model susceptible to interact with regenerating endothelium is immuno-inflammatory cells (mainly macrophages).

The accelerative effect of E2 on re-endothelialization is mediated by ERα [62] and endothelial NO synthase appears absolutely required for this effect [64]. Interestingly, the ERα isoforms (55 and 46 kD) expressed in the ER-αNeoKO mice [49] is sufficient to mediate the E2 effect on the postinjury medial hyperplasia [65,66]. Thus, in analogy with vascular NO production [51], the AF-1 transactivating function may be dispensable to mediate the beneficial effect of E2 on artery healing. Finally, E2 increases the number of circulating endothelial progenitor cells [64,67], and this effect could be a key actor of the acceleration of re-endothelialization by E2.

**TOWARDS A COMPREHENSIVE VIEW OF EXPERIMENTAL AND CLINICAL STUDIES**

Thus, experimental studies demonstrate and epidemiological studies suggest that oestrogens prevent atherosclerosis, whereas randomized clinical trials did not confirm a cardioprotective effect. Although observational studies may have overestimated the coronary benefit conferred by HRT, there are other plausible explanations for the apparent discrepancy between previous results and the less favourable findings from WHI. It is likely that age or time after onset of the menopause may importantly influence the CV benefit-risk ratio associated with HRT, and that the method of administration, dose, and formulation of exogenous hormones may also be relevant [20,68].

Compared with oral route, transdermal E2 administration allow to avoid the hepatic first-pass and consequently to limit certain deleterious effects. The ESTHER case–control study [69] confirmed that oral oestrogens favour a significant increase in the thrombo-embolic risk, and suggests that the transdermal route is not associated with an increase in the incidence of thrombo-embolic events. However, no large, controlled study allowed to definitively demonstrate this difference. Whatever may be the informative value of C-reactive protein levels in terms of CV risk, it is of interest to mention that oral oestradiol increases these levels, whereas the transdermal route does not [70].

Although increasing amount of evidence indicates that older women and those with subclinical or overt coronary heart disease should not take HRT, oestrogen remains the most effective treatment currently available for vasomotor symptoms (vasomotor flushes). The HRT effects on the development of coronary disease in newly postmenopausal women remain unclear. An apparently surprising absence of effect of HRT on the quality of life was reported in the WHI study [71]. However, it is worth mentioning that women with hot flushes were discouraged from participating in the WHI trial for ethical reasons. The investigators did not consider their participation to be ethical as it would deprive women with these symptoms from receiving the most active treatment (i.e. oestrogens) for years as mentioned in the editorial accompanying the WHI study [72].

Clearly, the effects of HRT on quality of life and cognitive function in recently postmenopausal women deserve further studies. These unresolved clinical issues provide the rationale for the design of the Kronos Early
Estrogen Prevention Study, a 5-year randomized trial that will evaluate the effectiveness of low-dose oral oestrogen and transdermal oestradiol in preventing progression of atherosclerosis in recently postmenopausal women [73].

Finally, HRT decreases the risk of bone fracture in menopausal women, as clearly established in WHI study with a reduction of about 40% of all fractures. This effect is noteworthy as the population included was at low risk of osteoporosis (in particular as a high percentage of overweight women were included). Thus, <15% of the women had a densitometric osteoporosis, at variance with other large trials conducted to evaluate osteoporosis or effects of bisphosphonates that included women with a higher risk of fracture.

UNRESOLVED QUESTIONS, PRESENT AND FUTURE TREATMENTS

Breast cancer represents the most obvious risk feared by women taking HRT. Indeed, cohort or intervention trials (HERS and WHI 2002) consistently showed a 1.3-fold increase in breast cancer by HRT. Unexpectedly, WHI 2004 ‘oestrogens alone’ in hysterectomized women showed an almost significant trend (P = 0.05) towards protection against breast cancer (RR: 0.77 [43]). Even if this study raised the question of a potential role of the consequences of associated ovariectomy or the nature of the oestrogens used (potential protective role of conjugated equine oestrogens), most of the recent studies underline again the deleterious role of progestin. Indeed, a cohort study [74] and more recently the E3N INSERM study [75] showed no increased risk when HRT included natural progesterone, strikingly contrasting with an increased risk of breast cancer when oestrogen are associated with synthetic progestin having androgenic (19-nortestosterone) or anti-inflammatory (medroxyprogesterone acetate) properties.

Although ERs are classically defined as ligand-activated transcription factors [48], it has become clear that ‘extragenomic membrane short-term’ responses play an important role in cultured endothelial cells [37], but also in osteoblasts (as for instance the activation of PI3kinases-AKT pathways as well as MAP kinase pathways) [76]. An important challenge for the next years will be to characterize the respective roles of these ‘membrane’ effects and the ‘classical’ effects [20,37,77,78].

These new acquisitions constitute a basis for new pharmacological developments allowing the prevention of deleterious effects and preserving the beneficial ones [68,79]. The effects of selective oestrogen receptor modulators (SERMs) on the different actors of the atheroma plaque formation have now to be analysed on the basis of their specific regulation of the ERα but also of the ERβ, the specific activation of which elicit some anti-inflammatory actions in vivo. Various classes of oestrogens and SERMs have been described according their molecular actions through ER α [80–82]. Due to the complexity of the mechanisms of action of oestrogens and SERMs, their effect on various cell types and tissues cannot be predicted from their structure. Hence, integrated models that allow the screening of present and future SERMs in terms of the beneficial and deleterious effects will be valuable, important tools. Theoretically, it is conceivable to design an SERM (or a combination of molecules) which would retain most (if not all) of the desired effects of E2 (on the central nervous system to prevent vasomotor flushes, on bone, on endothelium, etc.), but which should be devoid of the undesirable effects of E2 (mainly breast cancer, thromboembolism and probably pro-inflammatory effect). SERMs currently available (tamoxifen, raloxifene) prevent breast cancer, but are devoid of effect on menopause symptoms and on CV risk. Prevention of both breast cancer and CV diseases by novel SERMs thus represents the major challenges of the future treatment of menopause.

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