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The risk for cardiovascular disease in women: from estrogens to selective estrogen receptor modulators

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1. ABSTRACT

Cardiovascular disease, a generic denomination including coronary heart disease (CHD), stroke, and venous thromboembolic disease (VTED), has shown sensitivity to estrogens. The relative protection of women as compared with men has nourished a debate about a possible protective role for estrogens, but the prejudicial effects detected in clinical trials has created confusion on the risk/benefit ratio induced by hormone administration. The hypothesis that agonists distinct to estrogens might improve the effects associated with estrogens is at the base of the increasing interest on the role of selective estrogen receptor modulators (SERMs). There is a lack of definitive clearcut clinical data on the effects of SERMs in CVD, although the available information suggests a neutral balance on CHD and stroke and an increase in risk similar to estrogens for VTED. Research on pathogenetic mechanisms concentrates in atherosclerosis as the main determinant of the arterial forms of the disease and in hypercoagulability as the counterpart for venous disease. The different experimental models used up to the present moment suggest that, compared with estrogens, SERMs play a less active protection against atherogenesis but do not increase vulnerability of unstable plaques. There is not a clear notion on the mechanisms promoted by SERMs to increase risk for VTED.

2. INTRODUCTION

The detection of estrogen receptors in tissues and organs distinct from the genital tract or the breast has favoured the concept of estrogens as systemic agents. In agreement with this notion estrogens have been confirmed to exert functional actions in organs as diverse as the brain, the skeleton, the liver, or the vasculature. Among these extragenital effects, the sensitivity of the cardiovascular tree to estrogens has gained momentum as a result of the significance of cardiovascular pathology.

Cardiovascular disease (CVD) defines the leading cause of mortality and morbidity in both men and women. The epidemiological weight of the disease is unequally divided into its three main forms, coronary heart disease (CHD), cerebrovascular disease, which includes stroke and transient ischemic attacks, and venous thromboembolic disease (VTED). In the USA, a 53% of deaths from CVD has been assigned to CHD and a 18% to stroke (1). The figures are very similar in Europe (2), and the World Health Organisation estimates that CVD will be the first cause of death in developing countries by 2010 (3). VTED has a lower prevalence (approximately 1/1000 persons-year) but it raises exponentially with age from <5 cases per 100,000 persons <15 years old to ≅500 cases per 100,000 (0.5%) at age 80 years (4).

CVD offers the profile of a disease with a strong gender influence although the picture changes depending on the form that is considered. Specific gender patterns have been detected for the prevalence and the behavior of CHD, where women show a lower frequency, a later presentation of the clinical events, and specific clinical patterns (5). Differences are somewhat less for stroke, although in some countries of the Mediterranean areas, such as Spain, stroke is still the first leading cause of death and disease in women (6). Incidence is similar for men and women in the case of VTED (7,8).

The differences in risk between men and women have called the attention on a possible influence of sexual hormones. Particularly, a possible role of estrogens has been suggested by epidemiological, clinical, and experimental studies (9). The Framingham Heart Study (10), a longitudinal cohort designed to identify common contributing factors to CVD, included women to clarify why they exhibit this relative protection. Together with a series of differences of the disease between men and women, the Framingham Study revealed that background sex steroid availability might be affecting the specific profile of CVD in women. For example, menopausal women up to age 55 had a higher risk of CVD than premenopausal women of the same age (11).

Given this role of estrogens as assumed, it is interesting that some differences seem to exist between the effects attributed to endogenous or exogenous estrogens. This contrast is evident in the case of CHD, where the possible protection mediated by endogenous estrogens has not been observed in postmenopausal women treated with this hormone, as confirmed by recent randomized clinical trials (12-14). The lack of benefit associated with exogenous estrogens has created an important debate because it is contrary to a substantial body of experimental work and observational clinical studies that have consistently supported a protective role for estrogens. A similar contrasting effect between endogenous and exogenous estrogens is reproduced in the case of VTED, where the comparable incidence in men and women diverges from the increased risk associated with the administration of estrogens.

Whichever the direction of the effect, it is increasingly clear that the biological mechanisms set in motion by estrogens define a system capable of regulating risk for cardiovascular clinical events. Consequently, the detailed knowledge of those mechanisms should help in regulating the whole system to reduce risk.

3. VERSATILITY OF ESTROGEN ACTION: THE SERM ALTERNATIVE

Besides the debate created in the literature on the effects of estrogens (15), the advances in the knowledge of the molecular details of estrogen action have opened an opportunity to explore other agonists that, hypothetically, might offer a better profile than estrogens. The mechanism of action of estrogens comprises a wide array of alternatives including some associated with the versatility of the estrogen receptor (ER) and others that are direct, not mediated by receptor (16-18).

In the particular case of the molecular pathways linked to ER activation there is a first step associated with the availability of two distinct receptor phenotypes, denoted ERalpha and ERbeta, which present a differential expression in different tissues along the organism (19). There are specific effects associated with each ER isoform, the ERbeta counterbalancing some actions promoted by ERalpha under certain conditions (20). The second step derives from the relative promiscuity of the ER, capable of binding molecules different enough from estrogens to generate ligand-receptor complexes with a three-dimensional conformation determining a varied range of specific cell functions. The denomination Selective Estrogen Receptor Modulators (SERMs) has been proposed to identify drugs complying with that requisite.

A substantial number of compounds, distributed in several families, has demonstrated SERM activity (21). At the present moment there is considerable information on experimental actions of a good number of SERMs but only a few, particularly tamoxifen, raloxifene, and in a lower measure toremifene, have been subjected to sufficient clinical scrutiny (Figure 1).

4. CORONARY HEART DISEASE

4.1. Atherosclerosis as pathogenetic background

The pathogenesis of the arterial forms of CVD is associated with atherosclerosis, an inflammatory process that develops at specific locations within the arterial tree. Atherosclerotic plaques tend to develop at sites where some

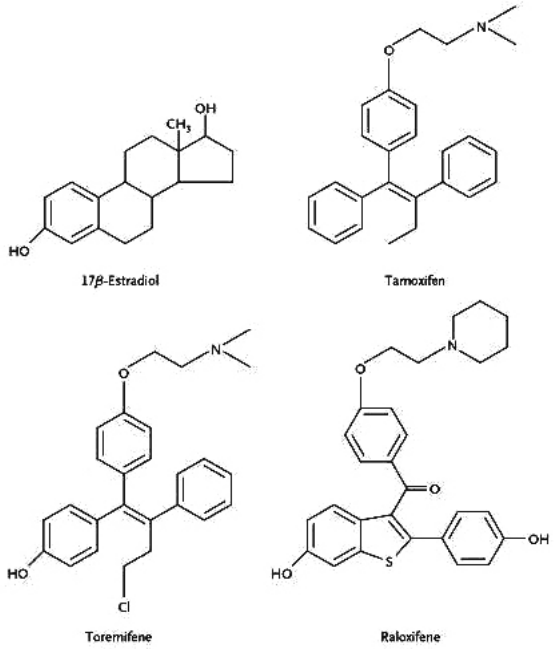

Figure 1. Chemical structure of estradiol, the principal ER agonist of natural origin, and tamoxifen, toremifene, and raloxifene, the three main SERMs approved for clinical use. Both tamoxifen and toremifene are members of the triphenylethylene family, whereas raloxifene is a member of the benzothiophene family (From Riggs BL and Hartmann LC. N Engl J Med 348: 618-629, 2003, reproduced with permission from the Massachusetts Medical Society).

determining factors, such as flow disturbances, occur preferentially. The outer wall of daughter vessels at major bifurcations or the inner wall of curved regions are consequently areas for usual plaque location.

There is, together with local changes in blood flow, a varied series of factors favoring atherogenesis (22). Whichever the inductor, the first step in the atherogenetic process affects endothelium, which develops a critical role as a result of its privileged position as both a sensor and an effector within the vascular wall. The modern conception of atherosclerosis understands the disease as an alteration in vascular wall remodeling, a process that is strongly influenced by endothelium (22,23).

The role of endothelium as an effector is conducted through the release of vasoactive substances and growth factors that regulate cellular growth and apoptosis. Endothelial dysfunction involves a rupture of the complex equilibrium of those local signals, thus leading to a well described sequence of events that begins with the adhesiveness of endothelium to leukocytes or platelets and ends in an inflammatory process presided by an atherosclerotic plaque (22,24) (Figure 2). In fact, coronary endothelial dysfunction has been shown to independently predict acute cardiovascular events in patients with and without CHD (25).

One hallmark in the evolutionary potential of atherosclerotic plaques is represented by the so-called unstable plaques. Stability has displaced thickness as the trait that becomes determinant for plaque rupture or erosion. The fibrous cap covering those plaques can be weakened by actions mediated by inflammatory molecules and proteolytic enzymes (24). The sudden release of debris and oxydized lipids that results from the permeation of the plaque cover precipitates platelet activation and local liberation of tissue factor. The generation of an occluding thrombus is then promoted (26,27).

A distinction must be made, consequently, between the mechanisms leading to plaque formation and those precipitating rupture. Research in this area has advanced drastically thanks to the availability of genetically modified animals, particularly mice that have been subjected to target deletion of the genes for apolipoprotein E (apo E) or receptors for low-density lipoprotein (LDL) or, more recently, knockout mice lacking immunoregulatory genes (28-30). This type of information has been combined with more traditional sources of data, such as cell cultures, or direct studies in humans, including research on lesions, clinical trials, or epidemiological studies.

In consonance with the distinction between mechanisms involved in plaque formation and rupture, the promoting factors for each process do not necessarily have to be the same. This notion is pivotal when considering the role of estrogens, which have been shown to differentially regulate atherosclerosis and the advent of proper clinical events.

4.1.1. Effects of estrogens

Most of the current evidences on the actions of estrogens have accumulated on effects on lipids and, more recently, on the vascular wall (Figure 3).

4.1.1.1. Lipids

In addition to changes in circulating levels that, with the exception of the increase in triglycerides, have been considered favorable when using oral estrogens (31), recent interest has concentrated in the effects of the hormone on lipid oxidation. The alteration of lipidic molecules due to oxidative processes has been associated with the pathogenesis of the disease as well as with the occurrence of clinical events (32). Only supraphysiological doses of estrogens have demonstrated potential to protect LDL particles from oxidation in the laboratory (33,34) and, although indirect evidences such as the reduction of antibodies to oxidized LDL favor protection (35), there is no general consensus on the subject (36,37). More recent research has found that estrogens reduce the levels of $F_{2\alpha}$-isoprostanes, a product of nonenzymatic, free radical catalysed peroxidation of arachidonic acid (38) that has been recognized as a stable, good biomarker of *in vivo* oxidative stress (39,40) and of atherosclerosis (41). Another area of interest is represented by myeloperoxidase (MPO), an oxidative enzyme found in phagocytes and in atherosclerotic lesions. Increased blood MPO levels are

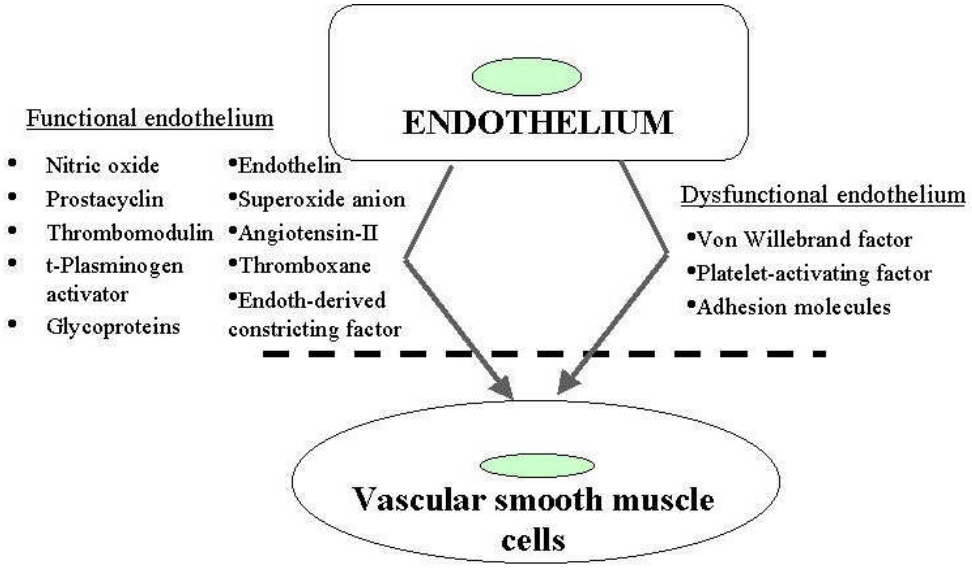

Figure 2. The vascular wall is continuously subjected to a process of remodeling where endothelium maintains a leading role. As a result of the physical or chemical signals coming from the blood, the endothelium generates a series of mediators affecting smooth muscle cells and fibroblasts. Endothelial dysfunction is followed by a change in the profile of secretion with a preeminence of mediators promoting leukocyte and platelet adhesion, an initial step in atherogenesis.

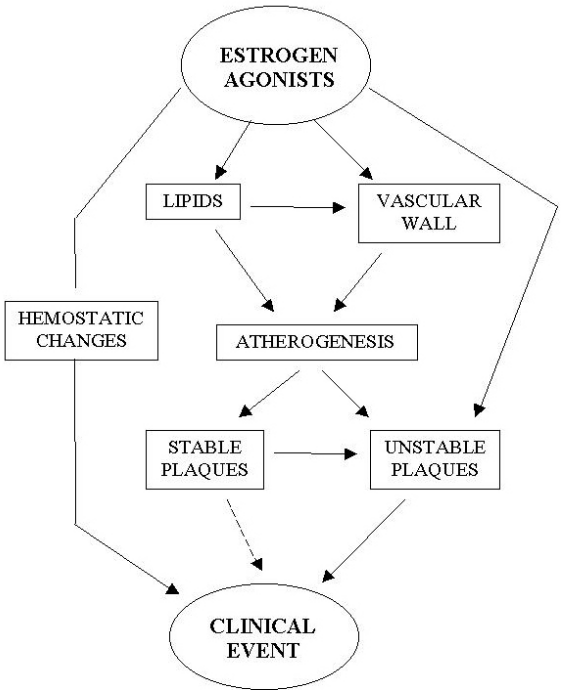

Figure 3. A schematic view of the main areas in the pathogenesis of arterial obstructive events that have exhibited estrogenic sensitivity. The process of atherogenesis may be influenced through both lipid changes and a direct action on the biologic regulation of the vascular wall. This may lead to either stable or unstable plaques, which may favor a sudden obstruction of the arterial lumen and a subsequent clinical event. The risk, however, is mainly restricted to the rupture of unstable plaques, as suggested by the discontinuous character of the arrow connecting stable plaques and clinical events. Moreover, a detrimental, direct action of some estrogen agonists, estrogens particularly, on unstable plaques has been strongly suggested by both biochemical and direct tissue evidences. There is also an action on hemostatic parameters, especially platelets, that may contribute as regulators of risk for a clinical episode.

associated with elevated incidence of CHD (42,43), whereas individuals with MPO deficiency exhibit lower risk profiles (44). The concentration of MPO in blood appears to be under some type of estrogen regulation, since oral estrogens increase (33) MPO levels, while transdermal estrogens seem to slightly decrease them (45). More recently, MPO promoter polymorphisms have been related with the long-term effect of either estrogen therapy (ET) or combined hormone therapy (HT) on atherosclerosis progression (46).

4.1.1.2. Vascular wall

The sensitivity of the vascular wall to estrogens is well endorsed by the demonstration of ER in both endothelium and vascular smooth muscle cells (VSMC) (47,48). The trophic effects of estrogens on endothelium have been advocated as crucial against initiation and promotion of atherosclerosis (22). Thus, cellular and animal models, as well as clinical observation with doppler techniques, confirm that estrogens promote vasodilation. This effect is maintained for years in menopausal women subjected to estrogen therapy (49). Nitric oxide (NO) and prostacyclin (PGI), two locally produced vasodilatory and antiaggregant mediators, have been proposed as the principal agents in this myo-relaxant effect of estrogens (50). In agreement with current concepts, their effects have been demonstrated as protective against atherosclerosis in animal models (51-53), although some debate exists as to whether it is the case in the human (54).

The expression of cell adhesion molecules at the endothelial surface is an early sign of endothelial dysfunction. Adhesion molecules facilitate leukocyte and platelet binding. Further, endothelial permeability is dependent on inter-endothelial junctions, where the participation of cadherin, a transmembrane, endothelium-specific glycoprotein, exerts an important level of control (55,56). Once expressed, adhesion molecules may be shed from the endothelial surface, and raised levels in blood have been associated with increased risk for CHD (57,58). Several studies have confirmed that estrogens reduce the circulating levels of different adhesion molecules such as E-selectin (59) or intercellular adhesion molecule-1 (ICAM-1) and vascular cell-adhesion molecule-1 (VCAM-1) (60,61). Proliferation and migration of VSMC, a step subsequent to endothelial dysfunction, has been found to be reduced by estrogens in some (62-66) but not all studies (67,68).

Concomitant with the concept of atherosclerosis as an inflammatory entity a growing interest has been developed on the action of estrogens on a series of molecules considered as vascular markers of inflammation. Among them, the C-reactive protein (CRP) seems to provide the strongest risk predictive potential for CHD in women (69,70). CRP is an acute-phase reactant that has been shown to increase in response to oral, but not transdermal, estrogens (71). A debate exists as to whether the first-pass hepatic effect of oral estrogens, the final determinant of CRP increase associated with oral estrogens, has any inference on the progression of atherosclerotic lesions. CRP has been detected in atheromatous plaques and has been shown to induce monocyte chemotactic protein-1 (MCP-1). MCP-1 is one of the best-studied members of the C-C chemokine subfamily that, according to some human and animal studies, operates as a key mediator in the recruitment of macrophages to the arterial lesion (72).

There is considerable information on the protective effect of estrogens in rabbits (73-77) and monkeys (78,79), two well-established animal models of atherogenesis. Experiments in monkeys have shown that estrogens alone, or in association with progestogens, protect against diet-induced atherosclerosis (80). There has been some discussion on whether this is the case in the human, since several studies have been unable to confirm that estrogen therapy, with or without progestogen administration, has a significant effect on the progression of the disease in women with established atherosclerosis (81-83). A different scenario might be defined in younger women with a lower prevalence or gravity of the disease (84,85).

4.1.2. Effects of SERMS

The interest for the cardiovascular potential of SERMs has emerged recently. The rationale for the attention lies in the demonstrated regulatory capacity of the estrogen-sensitive machinery on the pathogenesis of CVD that stands in the face of a deceptive behavior of estrogens in clinical trials. Research is progressing within the same tracks as those followed by estrogens in order to check similarities or differences that might be important for atherogenesis or for the advent of clinical events.

4.1.2.1. Lipids

The changes observed in the lipid profile are very similar to those linked with estrogens.

4.1.2.1.1. Experimental models

In preclinical studies on rats, new SERMs such as lasofoxifene have confirmed a powerful ability to reduce up to a 68% the increase in cholesterol associated with gonadectomy (86-88). Similar effects have been demonstrated for arzoxifene, a novel raloxifene analog (89,90).

Both tamoxifen and raloxifene have demonstrated some protection against the oxidation of LDL particles in ex-vivo experiments with isolated LDL (91,92). This effect has been also confirmed for pure antiestrogens like ICI 182780 and for other SERMs like EM 652 (93). Antioxidant properties have been also described for idoxifene, which effectively blunted the angiotensin II-induced production of reactive oxygen species on VSMC homogenates (94).

Interference of raloxifene with the action of MPO was found in a study that used isolated enzyme as well as murine peritoneal macrophages (91).

4.1.2.1.2. Clinical studies

As for estrogens, the decrease in the circulating concentration of total cholesterol and LDL was directly

related with pre-treatment levels in studies using tamoxifen, raloxifene, toremifene or droloxifene (95-100). A favorable difference from estrogens refers to the stability of triglycerides with SERMs, although slight increases have been found in women treated with raloxifene (101,102), and cases of acute triglyceridemia have been associated with tamoxifen (103,104). Only toremifene has achieved increases in the levels of high-density lipoprotein (HDL) (95).

There is fragmentary information concerning the behavior of some more recent SERMs. Whereas ospemifene showed a neutral effect (105), HMR 3339, a newly designed molecule that binds to human recombinant ER and shows selective agonistic and antagonistic activity *in vitro* and *in vivo*, rapidly decreased cholesterol and LDL in a dose-dependent manner (106).

Little evidence exists concerning alternative actions on oxidative stress, such as modulation of the circulating levels of isoprostanes or MPO. A recent study could not confirm changes in either of those markers (107).

4.1.2.2. Vascular wall

The actions of SERMs have been investigated at targets that have demonstrated importance in the process of atherosclerosis initiation or progression.

4.1.2.2.1. Endothelium

Consistent with data obtained in investigations with estrogens, both genomic and non-genomic mechanisms have been described for SERMs in endothelium. Raloxifene has been assayed most often given some indirect indications favoring a protective effect against CVD (108).

4.1.2.2.1.1. Cell and animal models

Both NO and PGI have concentrated the attention when investigating the effects of SERMs. In experiments with human umbilical vein endothelial cells (HUVEC) raloxifene has been shown to promote the induction of NOS and NO production. Furthermore, this effect occurs in seconds and involves non-genomic mechanisms where NOS is phosphorylated in a process implicating the protein kinase Akt (109). In a model of spontaneous hypertensive rats, raloxifene confirmed its ability to enhance the expression and activity of NOS (110). In agreement with this agonistic action, further experiments on HUVEC have confirmed an activation of cyclooxygenase-1 and –2 at both the protein and the gene level, leading to increased prostacyclin production (111,112). The involvement of both ERalpha and ERbeta as well as the likely participation of a mechanism distinct to the classical ER-dependent pathway, has been shown in these studies too.

Experiments with isolated vessels have confirmed the enhancing effect of raloxifene on endothelial NOS (113) with a similar behavior for tamoxifen (114).

The data with cells and isolated vessels have been corroborated in some animal models, as in ovariectomized ewes, where the vasodilating effect of raloxifene overpassed that of estrogens (115). Endothelium-dependent vasodilation was observed for rabbit coronary arteries *in vitro* (116), an effect that agrees with some vascular relaxing properties described for toremifene, tamoxifen, idoxifene, and EM 652 in rat vessels (117-120).

4.1.2.2.1.2. Studies in humans

Some studies have focused on early signs of disease, particularly endothelial dysfunction. Non-invasive physical techniques, mainly doppler or associated ultrasound systems, have been used together with measurement of circulating markers of endothelial origin. Mixed evidences have been obtained in studies of flow changes with SERMs. Raloxifene improved flow-mediated, endothelium-dependent vasodilation in postmenopausal women (121-123) to an extent similar to that of HT (122). Other investigators, however, have been unable to detect any effect of raloxifene (124,125). Flow-mediated vasodilation has been described for droloxifene (98), while a neutral effect on vascular reactivity has been described for ospemifene, a more recent SERM (105).

As demonstrated for estrogens (61), the use of raloxifene has been followed by the reduction of the circulating concentration of cell adhesion molecules (123,126,127). Information for other SERMs is both sparse and heterogeneous. Tamoxifen had a neutral effect in one study (128) whereas in another study droloxifene had a mixed effect, with a decrease in E-selectin and an increase in VCAM-1 (129).

Interestingly, there are preliminary data showing that both tamoxifen and raloxifene parallel estradiol in reducing the expression of MCP-1 in a model of endothelial cells in culture (130).

4.1.2.2.2. Vascular smooth muscle cells

There are only sparse data that, generally, support an anti-proliferative effect of SERMs. Raloxifene has been investigated in cultured cells and in animal models. In a tête-a-tête comparison with estradiol, raloxifene induced arrest and apoptosis of platelet-derived growth factor (PDGF)-stimulated cultured human VSMC (131,132). Consistent with this observation, raloxifene was equivalent to estradiol in limiting intimal thickening in a model of ovariectomized senile ewes (133). Similar protective effects have been described for tamoxifen in different studies with VSMC in culture (134-136) and for idoxifene in a study including experiments with VSCM and animal data (137).

4.1.2.3. Plaque development

The confirmation of whether the above-mentioned biological effects do truly protect against plaque development has been investigated in animal models subjected to atherogenic diet. Some experiments have been carried out in mice subjected to targeted inactivation of the apo E and LDL receptor genes. These animals respond to moderate amounts of dietary cholesterol with severe hypercholesterolemia and develop lipid-rich vascular lesions resembling human atherosclerotic plaques.

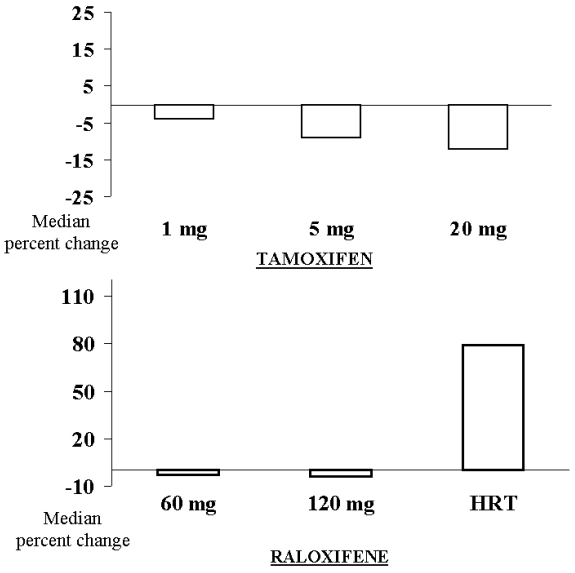

Figure 4. Changes in the circulating levels of CRP under tamoxifen (upper panel) or raloxifene (lower panel). A statistically significant linear dose–response relationship was detected for CRP in breast cancer women subjected to 1 mg, 5 mg, or 20 mg tamoxifen for 4 weeks. There was no change in the levels of CRP in healthy postmenopausal women receiving either 60 mg or 120 mg raloxifene. Instead, the use of HRT (conjugated equine estrogen (0.625 mg/day) and medroxyprogesterone acetate (2.5 mg/day) was followed by a substantial increase of CRP levels ($p<0.001$). [From ref. 100 (upper panel), and ref 145 (lower panel), with permission].

The data with SERMs are not unanimous. Together with the limitation of atherogenesis in rats previously mentioned for idoxifene (137), there is further data showing attenuating effects against atheroma development obtained with tamoxifen in apo-E null mice (138). Raloxifene has been investigated in distinct animal models. In cholesterol-fed rabbits raloxifene reduced the accumulation of cholesterol in atherosclerotic lesions in the aorta (139). In contrast, estrogens did effectively limit the development of coronary atherogenesis in a model of postmenopausal monkeys where raloxifene was not different to placebo (140).

4.1.2.4. Plaque stability

The progression of atherosclerosis may lead to either stable or unstable plaques, the latter being prone to rupture (26). In contrast with the abrupt concatenation of events occurring in the case of plaque rupture (27), the slow progression of a stable plaque may have its consequent lumen reduction limited by an adaptive response mainly based in the development of collateral circulation (141,142). This key distinction warrants the interest raised in the recent years towards a series of inflammatory factors that are increasingly considered as reliable markers of the biologic process converging into plaque rupture. Together with CRP, other markers that have also been independently associated with increased risk for CHD in women include homocysteine, interleukin-6 (IL-6), and lipoprotein (a) [Lp(a)] (143,144).

Opposed to the increase in circulating levels of CRP associated with oral estrogens, raloxifene did not modify CRP in a randomised trial on postmenopausal women (145) (Figure 4). A better profile was observed for droloxifene as well as for tamoxifen, which induced a diminution of CRP (100,129,146). Parallel, slight decreases were observed for estrogens and raloxifene when investigated for changes in homocysteine, a molecule that may have damaging effects on endothelium (145, 147-149). A reduction was found for Lp(a) as well, although in this case the decrease achieved for estrogens was of a higher magnitude in one study (-19% for estrogens vs. –7% for raloxifene) (150). Droloxifene, however, was more efficient than estrogens in reducing Lp(a) levels (98).

One interesting marker is IL-6, which participates in both atherogenesis and inflammatory processes. In one mice model that was double deficient at the apoE and IL-6 loci, animals displayed similar hypercholesterolemia compared to apoE-null mice, but disclosed larger and more calcified lesions at 1 year of age (151). Thus, IL-6 may be involved at the fibrous plaque stage of the atherosclerotic process. Moreover, IL-6 is a key factor in the generation of the hepatic acute-phase response and, consequently, participates in the increase of CRP, fibrinogen, platelet number and activity, and blood viscosity. Only raloxifene has been tested for IL-6, which did not change in one study (150), and decreased by 35% in another study after 24 months of treatment (152). Similar decreases have been found for tumor necrosis factor α (TNFα), another cytokine associated with cardiovascular risk in epidemiological trials (153), in a study comparing HT and raloxifene (150).

In conclusion, given the variable response, it is difficult to delineate a clear-cut inference. SERMs exhibit changes in inflammatory markers that do not match those found with oral estrogens, but considerable heterogeneity exists depending on the compound. Some variability exists within hormonal application, depending on the compound (estrogens or tibolone) and on the estrogen administration route (oral vs transdermal) (144). Notwithstanding the varied and heterogeneous effects, it is worthwhile to stress that despite the value of inflammatory markers as useful indicators of coronary risk, whether interventions modifying their circulating levels have a clinical influence in susceptibility to the disease is still uncertain.

4.2. Hemostasia

The rupture or erosion of a plaque requires the formation of a local thrombus to achieve an occlusive effect. Platelet activation and aggregation defines a first step in the process of thrombogenesis following the rupture of an atherosclerotic plaque. Both endothelium and mechanisms intrinsic to platelets themselves regulate platelet stabilization. The effects of estrogens are still unclear. In addition to the detrimental effects on unstable plaques estrogens exert some unfavorable actions on the mechanisms involved in platelet activation. Thus, both oral

and transdermal estradiol increased Ca^{2+} mobilisation and P-selectin expression in platelets stimulated with adenosine diphosphate or thrombin (154). Further effects of estrogens on platelet function have been identified in individuals carrying a polymorphism of the gene encoding the platelet glycoprotein IIIa, termed $P1^{A2}$, identified as a risk factor for coronary thrombosis (155). While physiologic concentrations of estrogen strongly and significantly inhibited the aggregation of $P1^{A1/A2}$ platelets, concentrations that were 1000-fold greater were required to observe the same level of inhibition with $Pl^{A1/A1}$ platelets (156). The risk for CHD in women under estrogen therapy may be influenced, consequently, by genetics. However, beneficial effects have been found for estrogens in experiments on platelet activation and aggregation with either adrenaline (157), ADP (158,159), thrombin (159), or collagen (160).

There are some studies on the actions of SERMs on platelet activation. Recent work has demonstrated that raloxifene shares with estradiol some protective effects on platelet aggregation induced by ovariectomy (161). A neutral effect on platelet aggregation has been found for tamoxifen in studies carried out in a flow chamber (162), a result that is consonant with experiments on platelets subjected to different endocrine environments where, contrary to hormonal contraceptives, tamoxifen reduced intracellular calcium and release (163).

Some attention has been given to factors that, operating in the hemostatic balance, have been attributed the role of risk markers for clinical events. Thus, increased plasma concentration of factor VII, fibrinogen, plasminogen activator inhibitor type 1 (PAI-1), and the already mentioned Lp(a) have been associated with the occurrence of CHD. Much work has been done on the modulation of these factors by estrogens (for a review see 164), and both similarities and differences have been found in the sparse literature on SERM action. Raloxifene and droloxifene were more active than ET (98) or HT (97) in decreasing the circulating levels of fibrinogen. In contrast, the effective reduction demonstrated for PAI-1 with oral estrogen, alone (98, 165) or combined with progestogen (97), has not been confirmed for raloxifene or droloxifene.

4.3. Clinical data

As previously commented, the clinical data for estrogens are provided by the strong discrepancy between the protection detected in observational studies and either the prejudice or the neutral effects found in randomised trials (for a review see 166). The only clinical study addressing the effects of SERMs is the Raloxifene Use for the Heart (RUTH) study, a randomized clinical trial specifically designed to clarify the effect of raloxifene on the risk for CHD. The study had included 10,101 women from 26 countries at the closure of the inclusion period, August 2000 (101). Although the follow-up period has recently concluded, results from the trial remain to be reported.

Apart from this study there are no other randomised clinical trials that have researched the efficacy of SERMs in either the primary or the secondary prevention of CHD. Some indications that raloxifene may give some benefit have been obtained from a *post hoc* analysis of the data from the Multiple Outcomes of Raloxifene Evaluation (MORE) study, a randomised trial designed to assess the effect of raloxifene against osteoporosis. Using the same scoring system as in the RUTH study to stratify women, a total of 1,035 women were assessed as being at significant coronary risk. When women within the group that had been randomized to raloxifene were separated from those randomized to placebo it came up that treatment was associated with protection against any clinical cardiovascular event, and that the higher the score, the higher the protection (108). The protection was not confirmed, however, when CHD events were analyzed separately. The effects of raloxifene on the combined (arterial and venous) cardiovascular risk have been analysed more recently taking the same MORE data as a basis. There was a reduced combined vascular event in women at increased risk (167). A more recent publication has reviewed the cardiovascular events detected in the MORE study and in its continuation, the Continuing Outcomes Relevant to Evista (CORE) study (168). The CORE study examined the effect of 4 additional years of raloxifene therapy in women in MORE who agreed to continue in CORE. Against the protective effect observed in the 4-year period of the MORE study for raloxifene in high-risk women (108,167), the 8-year follow-up in the MORE-CORE did not detect any benefit on coronary events for raloxifene against placebo (hazard ratio 1.23, 95% confidence intervals 0.58 to 2.58). Only 459 out of the 1,035 women at high cardiovascular risk analyzed in the MORE study continued and were subjected to scrutiny in CORE, a difference that may have influenced the contrasting results.

The effects of tamoxifen in women with and without CHD have been analyzed in the National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial (BCPT). This randomized, placebo-controlled study included 13,388 women at increased risk for breast cancer. There was no indication that tamoxifen would modify the risk for CHD in women with or without heart disease(169). The conclusions of the trial are somewhat limited by the fact that the study was designed to investigate the effect of tamoxifen as a chemopreventive for breast cancer, and not its effect on CVD risk.

5. STROKE

Cerebrovascular disease, or stroke whether the term is restricted to the abrupt impairment of brain function of vascular origin, is second, only after CHD, as the leading cause of mortality and morbidity in women in the western world (1,2). Approximately 80% of all strokes are ischemic while the remaining 20% result from hemorrhage, at either the parenchyma or the subarachnoid space. The pathogenesis is strongly associated with processes affecting the arterial tree, either atherosclerosis or obstructive emboli of cardiac origin in the case of the frequent ischemic forms, or hypertension in the forms caused by hemorrhage. Venous occlusions are much less common, and as for VTED, are usually associated with hypercoagulable states.

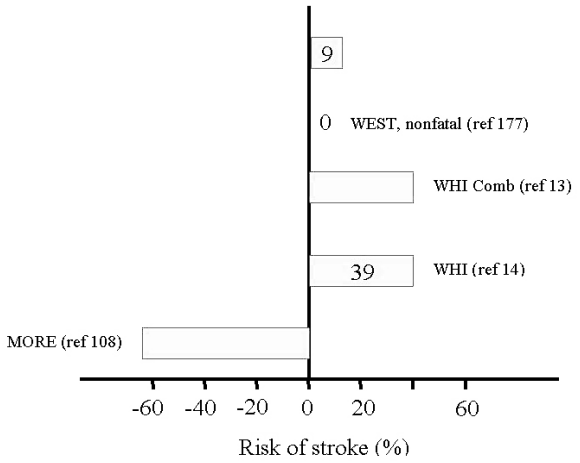

Figure 5. A schematic view of the mean percent changes observed in randomized, placebo-controlled trials on the effect of estrogen agonists on risk for stroke. Both WEST and WHI included only estrogens in the treatment arm whereas HERS and WHI combined included a estrogen-progestogen combination. The MORE trial (only the separate analysis of the subgroup of women with increased cardiovascular risk at baseline is shown in the figure), instead, was designed to discern the effect of raloxifene. Only results of nonfatal cases have been included for the WEST study, the risk for fatal stroke achieving a 290% increase. Figures in brackets at each bar title indicate the reference number from where the information has been obtained. See text for further details.

The pathophysiology of ischemic stroke is associated with atherosclerosis, and consequently, the same caveats exposed above on the effects promoted by estrogens, or compounds with some estrogen agonistic activity, may be applied in here. Concordant with this concept, CHD and stroke share a substantial number of risk factors. However, stroke presents some particular traits, as confirmed by the specific profile of the disease in aspects as important as incidence or the mortality/morbidity pattern.

5.1. Estrogens

Over the past few years it has become clear that gender differences affect several traits of stroke, including not only incidence or outcome, but also pathophysiology and recurrence. As for CHD, women suffer stroke at a later age and with lower frequency than men. However, the information on sex hormone influences in general, and of estrogens in particular, on the risk for stroke is still insufficient. Much of the problem derives from the particularities affecting the mechanisms induced by estrogens in the different tissues. In the case of stroke, these mechanisms not only affect endothelial cells, hemostasis, or inflammatory processes, but also neurons and glial cells. Moreover, we cannot discard that the vascular tree may have specific responses in the central nervous system. In fact, some mechanisms that extend beyond the increase in cerebral blood flow or antioxidant activity have been proposed for estrogens. Among them, the increased expression of the anti-apoptotic protein BCL2, preservation of mitochondrial function, or a blockade of glutamate and kainate injury, have been suggested (170). Research in the field is progressing although there is a lack of experimental models capable of reproducing human stroke because of the heterogeneity of human disease. Preclinical research is mainly based on animal models, which have been restricted to rodents. Human data have been obtained in observational studies and in clinical trials on the use of hormones in postmenopausal women.

5.1.1. Animal studies

Despite the limitations imposed by the difficulty to reproduce the complexities associated with human stroke, experiments with animals offer the advantage of analyzing particular aspects of the disease under very well defined and controlled conditions. Further, molecular components involved in estrogen action may be adequately investigated by the use of a variety of models, for example the estrogen receptor knockouts, which offer information inaccessible for studies in humans. Ischemic stroke has concentrated most of the attention given its higher prevalence. An overall neuroprotective effect is generally found in females as compared with males in models of ischemic injury (reviewed by 171). Interestingly, this gender-specific effect seems influenced by the estrous cycle, with higher level of protection during proestrus, the phase with high estrogen levels. Consistent with this estrogen-dependent protection, both ovariectomy and reproductive senescence abolish the sex differences.

5.1.2. Human studies

The use of hormones after menopause has received much of the interest since it is used in women with an age in which the prevalence of stroke starts being significant. Biological studies have produced mixed results. The effect of estrogens on endothelium has been studied in carotid arteries, where both improvement and lack of response have been observed for compliance and distensibility (172,173). Given the value of the intimal-medial thickness (IMT) as an important prognostic marker of the risk of stroke, there has been much interest on the effects of the menopause-associated lack of hormones or the hormone administration on the evolution of that indicator. In a cross-sectional assessment performed on data from the Atherosclerosis Risk in Communities (ARIC) neither menopause status nor the use of hormones, either ET or HT, was associated with a diminution in IMT (174). The effect of hormones has been more carefully analyzed in two randomized controlled trials that investigated the effects on women who were healthy (175) or at increased risk (176). ET was used in the former study whereas either ET or HT was used in the latter. Despite the different risk status of women in each study the results were equally negative.

Observational studies on clinical events have not been as unanimous for stroke as for CHD in supporting a protective effect for ET or HT (177), but have not detected increased risk either. Randomized clinical trials have not confirmed any protection associated with the use of hormones (Figure 5). The Heart and Estrogen-Progestin Replacement Study (HERS), a secondary prevention study,

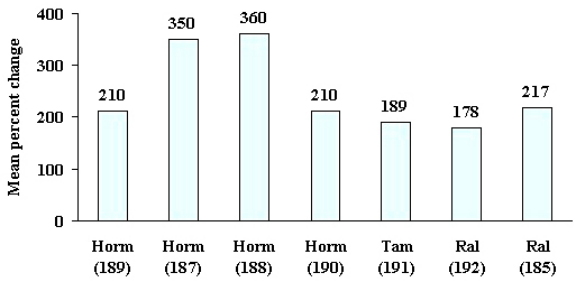

Figure 6. Mean increased risk (expressed as a percentage) for venous thromboembolic disease as observed in different studies for estrogens or SERMs. Horm denotes hormones, including either only-estrogen or estrogen-progestogen combinations; Tam denotes tamoxifen; Ral denotes raloxifene. The figure included in brackets for each title at the abscissa denotes the citation number in the reference list.

could not detect a reduction in stroke after four years of HT (12). The Women's Health Initiative (WHI) study had the advantage of being designed as a primary prevention study that, of interest, included two parallel trials examining the effect of HT and ET. Both trials were coincident in finding increased risk that averaged 40% in women taking hormones (13,14). The Women's Estrogen for Stroke Trial (WEST), which was specifically designed to assess the effect of estradiol on the risk for stroke recurrence, did not modify the risk for nonfatal stroke but confirmed an increase in fatal stroke in women receiving the steroid (178).

The strong similarity between the clinical data obtained for estrogens in trials on CHD or stroke raises the question on whether the mechanisms associated with estrogen-induced risk may be similar. It is possible that both the pro-inflammatory and the pro-thrombotic effects previously mentioned may be at work at the stroke level as well. From this point of view, the same arguments exposed to propose SERMs as an alternative with potential advantages have interest in here.

5.2. SERMs

There is information on the risk for stroke in women treated with tamoxifen because of breast cancer. The Breast Cancer Prevention Trial -1 (BCPT-P1) detected a nonsignificant 59% increase (179). The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-24, another randomized trial designed to test the efficacy of tamoxifen in intraductal breast cancer, further reinforced the suspects about a promoting effect of stroke by the drug (180). Consistent with these data, two recent meta-analyses assessed the overall risk of stroke as published in the literature on tamoxifen use for breast cancer management and prevention. A 49% increased risk was detected in the analysis of 32 randomized trials including 52,929 patients (181). The second systematic review, which assessed randomized trials published since 1980, detected an 82% increased risk of ischemic stroke and a 29% increased risk of any stroke, but the absolute increased risk for a mean follow-up period of 4,9 years was small (0.32%; number needed to harm, 313) (182).

There is debate, however, on the reliability of the previous data. The fact that the primary endpoint of those trials was not the assessment of stroke risk, together with the small number of episodes, and the difficulty in accurately identifying strokes, reduce the consistency of the conclusions. To reinforce the controversy, a recent nested case-control study that included 11,045 breast cancer women previously treated with tamoxifen could not confirm an increased risk associated with the drug (183). Mortality related with stroke was not increased in an overview of randomized trials of adjuvant tamoxifen for early breast cancer (184).

A recent review of the experience attained with toremifene for the treatment of advanced hormone-sensitive breast cancer and the adjuvant treatment of early breast cancer concluded that the risk profile for stroke was better for toremifene than for tamoxifen (185).

As for other cardiovascular data, the most comprehensive source of information regarding raloxifene is the MORE study. A secondary analysis of data from that study concluded that the incidence of cerebrovascular events, including stroke and transient ischemic attack, was not modified by raloxifene (108). Similar results were obtained when nonfatal and fatal events were analyzed separately. Of interest, the separate analysis of the subgroup of women with increased cardiovascular risk at baseline detected, as previously mentioned for CHD, a significant reduction of 62% for raloxifene when all, fatal and nonfatal, strokes were pooled together. There were no statistically significant differences in the incidence of stroke between the raloxifene and placebo groups in the CORE study (168,186,187).

As commented for tamoxifen, the fact that the MORE study was not specifically designed to discern the effect of raloxifene on stroke risk limit the reliability of these data. The RUTH study, which has CHD as a primary endpoint and stroke as one of the secondary outcomes, may help to clarify the issue.

6. VENOUS THROMBOEMBOLIC DISEASE

The low prevalence of this form of CVD, as compared with either CHD or stroke, contributes to the notion that disturbances in the venous tree are of minor relevance. However, the incidence of VTED increases substantially with age, and this property is pivotal when considering that the actual indications for use of SERMs, breast cancer and osteoporosis, affect women in the second half of their lives. Consequently, it is important to underline that most of the data collected from studies with SERMs are unanimous in reproducing an estrogen agonistic profile in venous thrombogenesis. The magnitude of the effect is very similar to that found in studies with estrogens, the relative risk oscillating between 2 and 4 (188-191) (Figure 6). A recent overview of all randomised

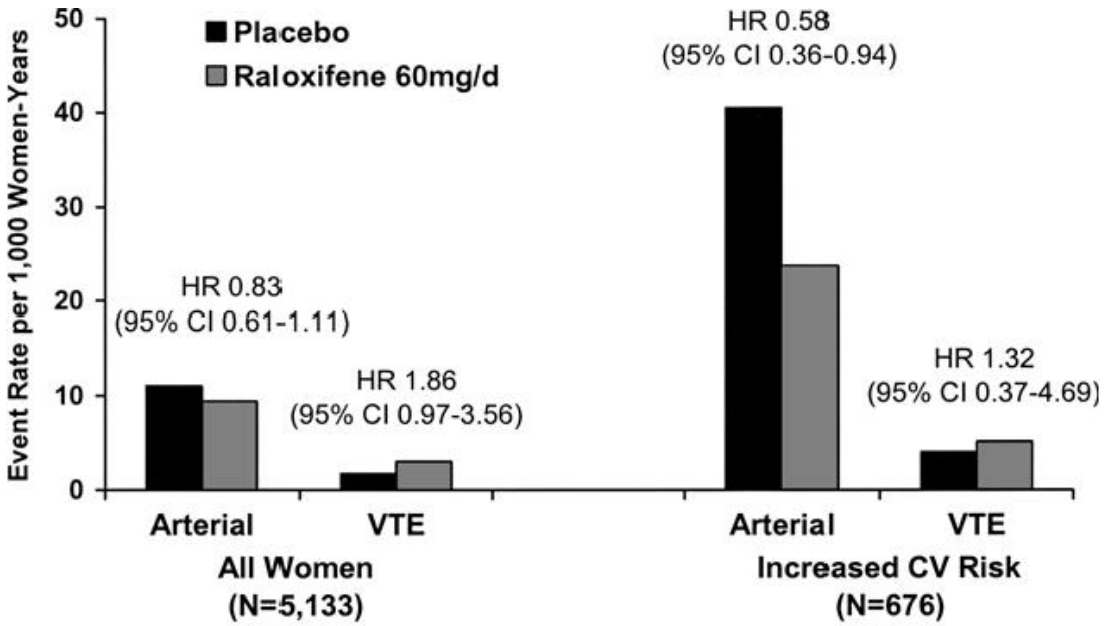

Figure 7. Arterial and venous clinical obstructive episodes per 1000 women-years in participants in the MORE study. Data corresponding to all women are presented on the left area of the X-axis whereas those corresponding to the subset of women at increased cardiovascular risk are shown on the right. (From ref 167, reproduced with permisssion from Lippincott Williams&Wilkins).

prevention trials comparing tamoxifen with placebo has reduced it slightly to 1.9 (192).

The clinical experience with tamoxifen consistently confirms an augmented risk for both deep venous thrombosis and pulmonary embolism although, again, much of the information arises from studies on women with breast cancer. Interestingly, this increase did not presuppose increased mortality in the overview of randomized trials of adjuvant tamoxifen for early breast cancer, where the one extra death per 5000 woman-years of tamoxifen attributed to pulmonary embolus was not statistically significant (184).

As for CHD or stroke, the main source of data on the effect of raloxifene on VTED risk derives from the MORE study, or its continuation, the CORE study. A 2-fold increased risk for VTED was observed through 4 years of follow-up in the MORE study (193), which was similar to the 2.17-fold increased risk detected in the CORE study (186). Concomitant with data obtained with estrogens and tamoxifen, an accumulation of events occurred during the first year of use. A recent assessment of the cardiovascular events detected in the women integrating the MORE trial was eloquent in showing the lower incidence of venous thrombotic than arterial episodes as well as the observation that the increased risk associated with raloxifene was not followed by a global increase of cardiovascular events (167) (Figure 7).

In consonance with data found for estrogens, the mechanisms set in motion by SERMs to promote risk remain obscure. The focality of venous thrombotic episodes argues in favor of some level of endothelial contribution. Moreover, there is a participation of variables affecting the local properties of venous flow, as confirmed by the observation that venous thrombogenesis seems influenced by decreased flow and local turbulence (194). The information on a direct effect of SERMs on venous endothelium is still scanty and divergent. In one study with endothelial cells, raloxifene upregulated the expression of thrombomodulin, a protein involved in the activation of protein C by thrombin (195). In contrast, tamoxifen induced a decrease in the circulating concentration of thrombomodulin in other study (196).

Much of the research, however, has focused on the action that SERMs exert on circulating parameters of the hemostatic equilibrium. The long use of tamoxifen in the prevention and treatment of breast cancer has permitted the accumulation of studies that have investigated the effect of the drug on the hemostatic balance. Most studies have detected slight changes in the anticoagulation system, particularly decreases in the levels of antithrombin (197-201), although decreases in protein S (197,200,201) have been also reported. There is some debate on the effects on protein C, with studies reporting decrease in the circularting levels (198,201,202) or no change in either the protein levels or activated protein C (APC) resistance (200). Further on the possible prothrombotic mechanisms stimulated by tamoxifen, tissue factor pathway inhibitor (TFPI), an endogenous inhibitor of factor Xa and the coagulant initiator complex tissue factor/factor VIIa, was significantly decreased by tamoxifen in a study on patients with breast cancer (196).

Raloxifene has been investigated in a recent study where it was compared with tamoxifen and with oral

equine conjugated estrogens (201). Thogether with the inhibitory effects on the anticoagulation system and certain changes on procoagulant factors, one interesting conclusion was the similar profile of changes induced by both SERMs, which kept less similarity with the changes induced by estrogens. Another study was unable to detect changes in APC resistance for raloxifene in the presence of clear changes for HT (203).

Besides the possible similitudes or differences in the mechanisms involving the role of estrogen and SERM in venous thrombogenesis, the main question is whether those changes are sufficient to explain the observed risk. The hemostatic equilibrium is a complex process where there is multiple interactions and balances that make difficult to ascertain the significance of small changes occurring in some parameters. This difficulty has favored the use of functional tests. Among the several options available the measurement of fragments 1+2 (F1+2), the amino terminus fragment split during the activation of prothrombin, has been widely considered as a first choice. The sparse information available for SERMs, however, is unclear. In one of the studies where tamoxifen was found to reduce the levels of the anticoagulant proteins antithrombin and protein C, the concurrent measurement of some functional markers did not detect any change (202). Raloxifene did not modify F1+2 fragments in one study where HT was also neutral (97). Other investigators, however, detected slight increases in F1+2 fragments for ET in another direct comparison with raloxifene (165).

7. FUTURE PERSPECTIVE

The concept that hormones exert a key role in regulating risk for CHD, stroke and VTED, is widely accepted at present. Moreover, there is a wealth of experimental data confirming the action of estrogens on several mechanisms crucial in the pathogenesis of each form of CVD. The unfavorable profile obtained for estrogens in randomised studies has raised two important questions. First, whether the biological mechanisms depending on estrogens may be powerful enough to reduce risk if adequately manipulated, and second, whether the particular modulation imposed by compounds distinct to estrogens, as it is the case of SERMs, may achieve this benefit. The data available at present are still insufficient to adequately respond to those questions. However, experimental and, in less amount, clinical data already offer a considerable body of knowledge in this regard.

The greatest amount of information has been compiled for CHD. The most widely used SERMs, tamoxifen and raloxifene, seem to behave acceptably concerning the mechanisms underlying the disruption of atherosclerotic plaques. This may be an advantage against estrogens. In contrast, estrogens seem to perform better in protection against atherogenesis. There is insufficient information on whether SERMs may offer benefit against stroke. Although only offering global data on mortality, the important overview of randomized trials of adjuvant tamoxifen could not find increased risk for the aggregate of all cardiac or vascular deaths (184). Beneficial effects have been obtained in the *post hoc* analysis of the MORE data for raloxifene in women at increased risk.

Venous thrombosis defines a field where there is a very parallel performance of estrogens and SERMs. Difficulties for improving in this area derive from the lack of reliable information on the mechanisms affected by estrogens or SERMs. There is evidence in favor of an antagonism of hormones on the anticoagulant pathway of the hemostatic equilibrium, but data obtained with functional tests of coagulation are still poor.

8. CONCLUSIONS

The modulation of estrogen action seems to offer some hope for controlling CVD risk. Additional advances in the knowledge of estrogen action as well as in the improvement in the design process of new SERMs should offer substantial progress in this area. The concomitant acquisition of clinical data, as is expected from the RUTH study, will consolidate research developments.

9. ACKNOWLEDGMENT

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