# Raloxifene: Cardiovascular Considerations

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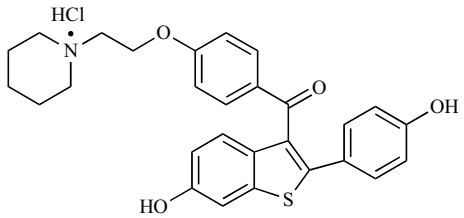
**Abstract:** Ovarian hormone deficiency status is associated with increased cardiovascular morbidity and mortality, suggesting that estrogen might exhibit a favorable cardiovascular effect. Estrogen has a multitude of beneficial biological effects on surrogate markers of cardiovascular disease that may account for this hypothesis. However, none of the randomized trials already conducted with hormone replacement therapy showed overall benefit by means of reducing clinical ischemic cardiovascular events and/or suppressing atherogenesis. Moreover, the Women's Health Initiative study (WHI) has suggested a possible detrimental effect for hormone replacement therapy including increased cardiovascular morbidity, ovarian and breast cancer. Hence, any beneficial effect of estrogen must be carefully weighed against its carcinogenic properties together with its side effects. The need for a more efficient and specific molecule led to the development of the selective estrogen receptor modulators (SERMs). This new generation of drugs mimick the effect of estrogen in some tissues while antagonize several estrogen effects in other tissues. These unique properties offer the possibility to attain the beneficial effects of estrogen while avoiding its carcinogenic effect and the accompanying adverse reactions. Here we review the different effects of raloxifene- a protype second generation SERM on the cardiovascular system. We discuss raloxifene`s role at different levels of the atherothrombotic cascade addressing each level separately; trying to clarify the net effect of raloxifene in modulating thrombosis in the arterial tree.

**Key Words:** Raloxifene, SERM's, lipids, estradiol, myocardial infarction.

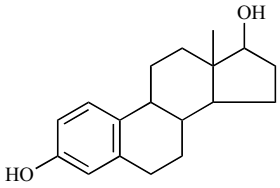
## INTRODUCTION

Estrogen has been traditionally regarded as a cardioprotective hormone. Yet, there is a puzzling discrepancy between many observational studies which suggest a protective role of postmenopausal estrogen treatment, and the results of pivotal clinical trials including the randomized, placebo-controlled hormone trial of the Women's Health Initiative (WHI) and the Heart and Estrogen/progestin Replacement Study (HERS) which found negative role for oral estrogen in primary and secondary prevention of cardiovascular events. It has been hypothesized that the beneficial effects of hormone replacement therapy on plasma lipid levels may be counteracted by an increased thrombogenicity and inflammation. The role of inflammation in atherothrombotic disorders is becoming increasingly recognized.

Raloxifene, a second generation selective estrogen receptor modulators (SERM), binds to both α and β estrogen receptors, albeit the post receptor effects are tissue specific and differ from those of estrogen. It is clear from the structure of the estrogen receptor ligand binding domain that estradiol and raloxifene bind to the same binding site. However, raloxifene contain a bulky side chain that is absent in estradiol. This chain was proved by x-ray examinations to obstruct the movement of the ligand binding domain, which prevents formation of functional activating function-2 surface (which is essential for estrogen receptor activation of gene expression by binding coregulatory proteins). The antagonistic activity of raloxifene is mediated by it's ability to bind to estrogen receptors with high affinity and competitively block the binding of estrogens and prevent the binding of coregulators. In the other hand, raloxifene exhibit agonistic action by the alternative AP-1 pathway (a transcription factor). Raloxifene is much more effective than estrogen in activating this pathway. Activating genes containing the AP-1 elements rather than the estrogen receptor elements leads to different biochemical and clinical end points. Raloxifene increases bone mass reducing the risk for subsequent vertebral fractures and it reduces the risk for breast cancer. It exerts positive effects on lipid profile and some cardiovascular inflammatory risk factors. However, as for estrogen, the net




Raloxifene




Estradiol

**Fig. (1).** Illustration of key findings in Figures.

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effect on the risk for developing cardiovascular events is still uncertain. In this review we summarize the available data about the *in vitro* and *in vivo* effects of raloxifene on cardiovascular parameters and asses its possible clinical benefits.

## BASIC SCIENCE DATA

### Smooth Muscle

Both estrogen and raloxifene significantly reduced the growth of vascular smooth muscle cells (VSMC) *via* platelet-derived growth factor (PDGF) [1]. The proportion of VSMC progression to s-phase was reduced by attenuating the increase of phosphorylated retinoblastoma protein (ppRb) which is the hallmark of the G1-S transition in the cell cycle. These effects were mediated *via* estrogen receptor α. In an isolated intralobar renal artery rings model [2] both raloxifene and estradiol caused relaxation of renal arteries with raloxifene being more potent. This effect was independent of endothelial denudation. Raloxifene mediated this effect primarily by inhibiting calcium channels influx.

### Endothelium

Simoncini *et al.* [3] demonstrated in cultured human saphenous vein endothelial cells that estradiol and raloxifene inhibit vascular cell adhesions molecule-1 (VCAM1) protein and mRNA expression in a concentration-dependent manner.

The Nitric Oxide - mediated vasodilating effect of raloxifene was demonstrated in several studies. In isolated rabbit coronary artery model [4] raloxifene induced relaxation through endothelium dependent and estrogen receptor dependent mechanism that involve nitric oxide. It also antagonized calcium at the coronary myocyte level. The net endothelial dependent relaxation effect was 100% more than estradiol.

In a sheep model of postmenopause [5] raloxifene induced more dilation of coronary arteries than estrogen with no differences in vascular remodeling.

Raloxifene reversed both the down regulation of vascular constitutive nitric oxide synthase (NOS) and increased sensitivity of the vascular tree to vasopressin in ovarectomized rats [6].

Rahimian *et al.* [7] demonstrated that chronic treatment with raloxifene in a rat model exerted a vasculoprotective effects by stimulating endothelial NOS (eNOS) expression. This was shown also in human umbilical endothelial cells (HUVEC) l [8] in which eNOS upregulation was blocked by an estrogen receptor antagonist and by PI3K (phosphatidylinositol3-kinse) inhibitor, but not by transcriptional/transitional inhibitors. Thus, it appears that raloxifene exerts non-genomic-non transcriptional effect *via* estrogen receptor rapidly activating NOS, and raloxifene is vasodilatory in human umbilical veins by both non genomic and genomic mechanism through activation and upregulation of endothelial nitric oxide synthase pathway, respectively. Following chronic treatment with raloxifene in old male and ovarectomized spontaneous hypertensive female rats, vascular relaxation was significantly restored by increasing NO bioavailability [9].

The endothelial expression of pro-inflammatory mediators was studied as well. Using human umbilical vein endothelial cells and human coronary artery endothelial cells obtained from females [10] both raloxifene and estradiol down-regulated Monocyte Chemoattractant Protein-1 (MCP1) mRNA expression.

### Neointimal Formation

Using a rat model of balloon injury Kauffman *et al.* [11] showed that raloxifene and estrogen reduce intimal thickening post vascular injury. This was mediated *via* the estrogen receptor and achieved at hormonal concentrations that do not induce positive lipid modification. A similar inhibition of intimal hyperplasia by raloxifene was observed in ovarectomized sheep after 6 months of treatment [12,13].

### Lipid Profile Modification and Atherosclerosis

Treatment of Sprague Dawley female rats with raloxifene for 4 weeks decreased cholesterol levels in a dose-dependent fashion up to 57% compared to ovariectomy and placebo [14]. Cserny [15] and co-workers used a rat model to demonstrate no effect of tamoxifen or raloxifene on the bile composition induced by ovariectomy. However, raloxifene decreased trihydroxy bile acids which might decrease the risk for gallstones formation.

The effect of raloxifene and estradiol on coronary atherosclerosis was studied in postmenopausal monkeys [16]. Both treatments exerted positive effect on lipid profile suppressing LDL cholesterol levels and elevating HDL. However, anti-atherosclerotic effect was reported just with estradiol. Bjarnason [17] and co-workers investigated the effect of raloxifene and estradiol on aortic atherosclerosis in a rabbit model. Both inhibited atherosclerosis with estradiol being more potent. Moreover, the differences in the lipid profile explained part but not all the differences between the groups suggesting an additional anti atherogenic mechanism for both estradiol and raloxifene.

In accord with this notion, in rabbits fed on cholesterol rich diet for 4 months, raloxifene reduced the aortic cholesterol content without affecting the extension of the atherosclerotic plaque [18]. Furthermore, Kallas hueb *et al.* [19] demonstrated a favorable effect of tamoxifen, raloxifene and estrogen on atherosclerotic plaque propagation in a rabbit model. This effect was achieved despite negatively modifying the lipid profile. The study by Sanjuan [20] and co-workers in a rabbit animal model reported again a positive effect of raloxifene on aortic cholesterol content with no significant effect on reducing the extension of the atherosclerotic area. Thus, it seems that in addition to modification of lipid metabolism, hormone replacement therapy hold an additional distinct mechanism by which it affect the build up of the atherosclerotic plaque.

### Coagulation Pathway

Thrombomodulin is an essential cofactor in generating activated protein-c. Raloxifene upregulates thrombomodulin in HUVEC [21] and partially blocks IL-1-induced suppression of thrombomodulin activation. In contrast, 17-β estradiol suppresses thrombomodulin expression in both unstimulated and IL-1 activated endothelial cells, promoting a prothrombotic surface on the vascular endothelium. Matrix metalloproteinase (MMP) are associated with collagen degrada-

tion and atherosclerotic plaque rupture eventually leading to acute vascular events. In human peripheral blood monocytes [22] raloxifene enhanced MMP-1 production with 2-3 fold increase, while oral estrogen increased MMP-9. Both estradiol and raloxifene down regulated monocyte chemotactic factor-1 (MCP1) expression in human coronary artery smooth muscle cells [23]; this effect may reduce macrophage recruitment inhibiting atherogenesis. On the other hand, MMP may enhance arterial remodeling in early atherosclerosis thus improving vascular compliance. In a pig model [24] both 17-β estradiol and raloxifene decreased platelets aggregation to pre-ovariectomy levels, decreased ATP secretion, reduced collagen stimulated release of PDGF-β, tended to reduce secretion of MMP2 without attaining statistical significance, and reduced mean mRNA for eNOS by 2-3 fold without significantly reducing the content of eNOS protein.

### Inflammatory Markers

In ovariectomized mice model [25] both estradiol and raloxifene induced significant involution of the thymus by reducing both weight and cellularity. Estradiol significantly suppressed delayed type hypersensitivity and granulocyte mediated olive oil induced footpad inflammation. Raloxifene appears to lack such an anti-inflammatory activity. While estradiol reduced dramatically the number of thymocytes and changed the T cell phenotypes, raloxifene had no effect on the phenotype fractions and only a minor effect on cellularity.

Using a female SD rat model Thomas *et al.* showed estrogen inducing remarkable anti inflammatory action whereas raloxifene had no significant beneficial effect [26]. This was demonstrated by means of leukocytes adhesion and transmigration, endothelial disruption and platelet activation.

In ovariectomized adult female rats, both estradiol and raloxifene attenuated tissue damage associated with paw edema and pleurisy, and both reduced tissue expression of cycloxygenase-2 (cox2) and inducible NOS [27]. Moreover they counteracted the inhibition of peroxisome proliferator-activated receptor-γ (PPAR-γ) expression caused by ovariectomy, restoring its expression to pre ovariectomy level. In addition treatment with raloxifene caused an increase of cytoprotective heat shock protein72 (HSP72) protein expression.

### Antioxidant Effect

Raloxifene inhibits *in vitro* LDL oxidation and myeloperoxidase mediated radical formation; both oxidative pathways are involved in the generation of the atherosclerotic burden [28]. In male spontaneously hypertensive rats [29] raloxifene improved endothelium dependent vasodilation by enhancing NO bioavailability through overexpression and enhanced activity of endothelial NO. It also decreased superoxide production. Raloxifene led to altered expression of vascular membrane bound rac1, an essential unit in the superoxide generating system driven by NAD(P)H oxidase. Furthermore, *in vitro* preincubation with raloxifene significantly reduced angiotensin II induced reactive oxygen species production. Using LDL isolated from plasma obtained from 12 healthy untreated postmenopausal women [30] raloxifene was more potent than estradiol or tamoxifen in reducing LDL oxidation. It also inhibited HDL oxidation [31]. In contrast, raloxifene lacks the capability to inhibit fibrinogen oxidation [32] presumably because raloxifene is unable to attack a particle without lipophilic properties.

## CLINICAL DATA

### Inflammatory Markers

C-reactive protein (CRP) is a strong risk marker for the occurrence of cardiovascular morbidity and mortality [33,34]. Several reports suggest that CRP is not a mere marker but rather a mediator of cardiovascular pathology [35,36]. In healthy postmenopausal women six months after enrollment and treatment hormone therapy (HT) increased CRP levels by 84% while raloxifene had no significant effect [37]. Walsh *et al.* also showed that hormone replacement therapy (HRT) increase while raloxifene decrease CRP levels [38]. This opposite effect was not explained by a differential effect on interleukin-6 (IL-6) or tumor necrosis factor-α (TNF-α) levels but may be due to different effects on the liver. Both treatments decreased TNF-α level, a pro-inflammatory cytokine that has a proatherogenic activity on the vessel wall including pro-adhesive effects. The effect of estradiol and raloxifene on inflammatory markers was studied on postmenopausal women [39] after one month of treatment. Estradiol again increased while raloxifene unaffected CRP. Both increased IL-6. MMP-1 which is increased in atherosclerotic plaque, was increased by both agents but significance was achieved only with estradiol. Both reduced E-selectin and intercellular adhesion molecule1 (ICAM1); vascular cell adhesion molecule-1 (VCAM-1) was reduced only by estradiol.

### Oxidative Stress\Antioxidant Effect

In *in vitro* assays of oxidation [40] raloxifene inhibited LDL oxidation and myeloperoxidase mediated tyrosyl radical formation, both oxidative pathways involved in atherosclerotic burden. In the other hand, in postmenopausal women 6 months treatments with RAL didn't modify the levels of myeloperoxidase and F2α-isoprostanase, two markers of oxidative stress believed to be reliable indicators of coronary artery disease [41].

### Coagulation Pathway

In a double blind, randomized study done with healthy postmenopausal women [42] both raloxifene and estradiol decreased the levels of fibrinogen and antithrombin. Estradiol decreased the antigen level of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) while raloxifene didn`t affect it. This demonstrates a more significant effect of estradiol on fibrinolysis. Both raloxifene and HRT significantly reduced homocysteine levels by 6-8%. Three months of raloxifene treatment were enough to achieve significant reduction in homocysteine level with maximal effect achieved after 6 months in older healthy postmenopausal women [43]. Homocysteine reduction by both was also achieved after 24 months of treatment [44].

### Endothelial Function

In a six months, double blind, randomized, placebo controlled study done in healthy postmenopausal women [45] both raloxifene and estradiol positively and significantly

affected markers of endothelial function by increasing the level of NO breakdown products, decreasing plasma endothelin levels and increasing the ratio of NO to endothelin. They also increased the flow-mediated endothelium-dependent vasodilation of the brachial artery. After 4 month treatment of healthy postmenopausal women [46] raloxifene decreased carotid blood flow resistance and endothelium-dependent vasodilation as measured by brachial artery flow-mediated dilation. The same duration of raloxifene administration was enough to demonstrate a positive modulating effect on endothelial-dependent vasodilation [47]. This effect was at least partially through reduction of endothelial damage score represented by oxidative stress indexes and plasma adhesion molecules levels; it significantly increased plasma TEAC (Trolox Equivalent Antioxidant Capacity), whereas it reduced TBARS (Thiobarbituric Acid Reactive Substances), VCAM-1, ICAM-1 and E-selectin. Raloxifene had no impact on endothelial independent vasodilation. The enhancement of endothelial dependent vasodilatation was achieved even after a shorter administration of raloxifene by 6 weeks [48]. In healthy postmenopausal women [49] estrogen but not raloxifene significantly reduced the plasma concentration of the cardiovascular risk factor asymmetrical dimethylarginine (ADMA), which is an endogenous inhibitor of NO synthase that is elevated mainly in diabetic patients. The long term effects of raloxifene and estrogen on endothelial function seemed less promising [50]: after 2 years of administration in hysterectomized otherwise healthy women they both significantly decreased endothelin and increased von Willebrand factor (vWF), but didn't significantly affect endothelium-dependent vasodilatation.

The vascular effect of raloxifene on postmenopausal women with coronary artery disease wasn't promising [51]. Eight weeks administration didn't improve brachial artery flow mediated dilation nor changed markers of vascular function such as endothelin-1, fibrinogen and urinary prostaglandins.

In postmenopausal women with increased cardiovascular risk [52] four weeks of treatment with HRT or raloxifene showed that estrogen significantly improve endothelial function and reduce endothelin-1 levels, while raloxifene lack such an effect.

Using a different approach, Soares da costa *et al.* showed that both raloxifene and estradiol improve arterial stiffness and blood pressure in postmenopausal hypertensive women [53].

Christodoulakos *et al.* studied healthy postmenopausal women given raloxifene or estradiol for 6 months and observed a decrease in VE-cadherin in both groups [54]. This is a transmembrane glycoprotein that is believed to control vascular endothelial permeability.

### Lipid Profile

The short term impact of raloxifene was studied in healthy postmenopausal women [55]. Both raloxifene and estrogen reduced LDL and total cholesterol. Positive effects on HDL and cholesterol levels were achieved by estrogen and raloxifene, respectively. The long term impact of raloxifene on lipid profile was studied on early healthy postmenopausal European women who were assigned to receive raloxifene for 3 years with resultant 7-12% decrease in LDL level [56].

Results from the EURALOX1 study [57] - a prospective, randomized, double blind study on healthy postmenopausal women, showed raloxifene affecting the lipid profile more favorably than HRT. Both reduced total and LDL cholesterol and triglycerides, raloxifene increased while HRT decreased HDL, and fibrinogen was suppressed by raloxifene while increased with HRT.

Secondary analysis of data from the Multiple Outcome of Raloxifene Evaluation trial [58] which was a 4 year follow up study on postmenopausal women showed raloxifene significantly reducing total and LDL cholesterol but not HDL. This effect was achieved after six months of treatment and was maintained thereafter.

Zheng *et al.* showed again a positive effect of raloxifene on total and LDL cholesterol after one year of treating healthy postmenopausal women. There was no effect on HDL or triglycerides [59].

In women with hypercholesterolemia and coronary artery disease [60] raloxifene again reduced total and LDL cholesterol with no effect on HDL. It also reduced Lp(a).

Hypercholesterolemic women seems to benefit more from the lipid lowering effect of raloxifene; suggesting that treatment may be better individualized to the patient [61].

### Effect on Cardiovascular Events

The year-by-year analysis of cardiovascular events in the MORE (Multiple Outcomes of Raloxifene Evaluation) trial was published in 2005 [62]. Cardiovascular safety was assessed as secondary objective. Raloxifene did not increase the risk for cardiovascular events at any year in all treatment groups. It had a neutral effect on cardiovascular events in the entire study population at 4 years. However, when retrospectively evaluating the high risk women subset raloxifene significantly reduced the incidence of cardiovascular events.

Nevertheless, the MORE trial was primarily designed to determine raloxifene effect on bone mineral density in postmenopausal women. Cardiovascular risk was evaluated retrospectively and there were few women in the high risk group, therefore a more specific data is yet to be provided. The recently reported data of the placebo-controlled Raloxifene Use for the Heart (RUTH) trial [63] came to address the affect of raloxifene on cardiovascular events in postmenopausal women with already established coronary artery disease. 10,101 women were enrolled with median follow up of 5.6 years. The study demonstrated a roughly equal total percentage of cardiovascular events in the placebo and raloxifene groups. However, only 56% of the initially assigned patients to raloxifene had ≥70% adherence. Further, the median age of the women enrolled was 67.5, reflecting a long period of hormone deprivation. This parameter might be crucial as suggested by vitale *et al.* [64].

## PERSPECTIVE

Until the publication of the HERS study [65] in 1998 which approached secondary prevention of cardiovascular events using estrogen and progestin, hormone replacement

therapy was believed to be the ultimate supplement for postmenopausal women, treating post-menopausal symptoms including osteoporosis while reducing cardiovascular morbidity. The study negated these assumptions: there was no overall cardiovascular benefit and HRT seemed to increase the risk for cardiovascular events in the first year of administration. The principal results of the women's health initiative trial [66] for primary prevention of cardiovascular diseases were even more striking and disappointing; HRT significantly increased the risk for major cardiovascular events and breast cancer. Based on these findings and the need for more efficient and safe hormone therapy the SERM`s emerged. However, despite multiple clinical and basic studies the superiority of SERM`s cannot be taken for granted. Both treatments modulate cardiovascular risk markers including TNF-α, homocysteine, E-selectin, ICAM-1, fibrinogen, antithrombin, endothelin and LDL cholesterol but RAL has a different positive effect on some inflammatory and coagulation markers including C reactive protein and thrombomodulin while estrogen positively affects others (i.e. VCAM-1, HDL). This ambiguity empathize the need for further studies to clarify the net effect *in vivo* of both treatments especially the SERM. Until such studies are completed and the data provided, caution should be taken when administrating SERM to post-menopausal women who are considered to be at high-risk for cardiovascular disease.

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