Raloxifene Improves Endothelial Dysfunction in Hypertension by Reduced Oxidative Stress and Enhanced Nitric Oxide Production

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Background—It has not been completely clarified whether selective estrogen receptor modulators (SERMs) such as raloxifene exert vasoprotective effects similar to those of estrogens.

Methods and Results—To investigate vascular effects of raloxifene, male spontaneously hypertensive rats were treated for 10 weeks with either raloxifene (10 mg · kg⁻¹ · d⁻¹) or vehicle. Raloxifene improved endothelium-dependent vasodilatation but had no effect on either endothelium-independent vasorelaxation or phenylephrine-induced vasoconstriction. Raloxifene treatment increased the release of NO from the vessel wall by enhanced expression and activity of endothelial NO synthase. Blood pressure reduction after bradykinin infusion was more pronounced in animals treated with SERMs. The production of superoxide in intact aortic segments was decreased by raloxifene treatment. Administration of raloxifene had no effect on the expression of the essential NAD(P)H oxidase subunits p22-phox and nox1 in the vasculature but reduced the activity and expression of vascular membrane-bound rac1, a GTPase required for the activation of the NAD(P)H oxidase. Finally, blood pressure levels were significantly decreased in spontaneously hypertensive rats treated with raloxifene. All SERM effects were also detected in healthy age-matched Wistar rats. In cultured rat aortic vascular smooth muscle cells, raloxifene inhibited angiotensin II–induced reactive oxygen species production dependent on estrogen receptor activation.

Conclusions—Raloxifene treatment improves hypertension-induced endothelial dysfunction by increased bioavailability of NO. This is achieved by an increased activity of endothelial NO synthase and by an estrogen receptor–dependent reduction in release of reactive oxygen species from vascular cells. These vascular effects cause a profound blood pressure reduction and lead to decreased vascular damage in male spontaneously hypertensive rats. (Circulation. 2002; 105:2083-2091.)

Key Words: estrogen ■ reactive oxygen species ■ endothelial dysfunction ■ hypertension ■ nitric oxide

The risk of cardiovascular diseases increases steeply after menopause.1,2 In this natural state of estrogen deficiency, estrogen replacement therapy potentially prevents the onset of cardiac events in postmenopausal women.3–5 Beside the possible vasoprotective effects of estrogens6 and their beneficial effects on osteogenesis,7,8 estrogens are thought to induce carcinogenesis of uterus and breast.9,10 Selective estrogen receptor modulators (SERMs) act as estrogen receptor antagonists in the breast and uterus, omitting the harmful effects of estrogens but presumably preserving the beneficial effects of estrogens on bone, lipids, and the cardiovascular system.11–13 Raloxifene, a prominent member of the SERM family, has been shown to acutely improve endothelial function and to induce endothelium-dependent and -independent vasodilatation by NO release and calcium channel blockade in rabbit coronary arteries.14 However, little is known about the long-term effects of raloxifene on vascular function.

Endothelial dysfunction is the prerequisite of atherosclerosis and is accompanied by a poor prognosis.15,16 Enhanced release of reactive oxygen species (ROS) and diminished bioavailability of NO, resulting in an impaired balance of these bioactive molecules in the vessel wall, are key events in the pathogenesis of endothelial dysfunction.17–21

The present study was conducted to evaluate the effects of raloxifene on endothelial dysfunction and the underlying oxidative stress in male spontaneously hypertensive rats (SHR). The discovery of beneficial effects of SERMs on

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vascular cells could potentially lead to novel therapeutic strategies for the treatment of atherosclerosis and hypertension.

**Methods**

**Materials**

Lucigenin, Taq DNA polymerase, nucleotides, salts, and other chemicals were purchased from Sigma Chemical. MMLV reverse transcriptase (RT), antibiotics, calf serum, and cell culture medium were obtained from GIBCO BRL. ICI 182,780 was purchased from Tocris. Nitroglycerin was obtained from Solvey. RNA clean was purchased from AGS.

**Animals**

Nine-month-old, male SHR (Aventis Pharma, Frankfurt/Main, Germany) and age-matched Wistar rats (Bayerg AG, Pharma Research Center, Wuppertal, Germany) received standard chow or standard chow supplemented with raloxifene at a dosage of 10 mg·kg body weight for 14 weeks. Body weights were identical in both groups (157±5 versus 369±7 g). Arterial blood pressure was measured in conscious animals with the tail-cuff method. Rats were euthanized by decapitation, and tissue samples were collected immediately. Animal experiments were performed in accordance with the German animal protection law.

**Cell Culture**

Vascular smooth muscle cells (VSMCs) were isolated from rat thoracic aorta and were cultured over several passages, as described previously. Experiments were performed with cells from passages 5 to 10.

**Aortic Ring Preparations and Tension Recording**

Vasodilatation and vasoconstriction of isolated aortic ring preparations were determined in organ baths filled with oxygenated modified Tyrode buffer (37°C). The reaction was within the linear exponential phase with respect to the amount of CDNAs template and number of cycles performed. Equal amounts of RT-PCR products were loaded on 1.5% agarose gels, and optical densities of ethidium bromide-stained DNA bands were quantified.

**Western Blotting**

Aortas were isolated, quickly frozen in liquid nitrogen, and homogenized with a motorized homogenizer. RNA was isolated with RNA clean, according to the manufacturer’s protocol, to obtain total cellular RNA; 1-μg aliquots were electrophoresed through 1.2% agarose/0.67% formaldehyde gels and stained with ethidium bromide to verify the quantity and quality of the RNA. One microgram of the isolated total RNA was reverse transcribed using random primers and MMLV RT for 60 minutes at 42°C and 10 minutes at 75°C. The single-stranded cDNA was amplified by polymerase chain reactions (PCRs) with Taq DNA polymerase. Sequences for sense and antisense primers, PCR conditions, cycle counts, and amplification fragment lengths were as follows. (1) eNOS: 5’TTC-CTG CTG-CCA-CCT-GAT-CCT-AA-3[sense] and 5’-AAC-ATA-TGT-CCT-TGC-TCA-AGG-CA-3[antisense]; 45 seconds at 94°C, 45 seconds at 60°C, and 90 seconds at 72°C; 31 cycles; and 340 bp. (2) p22phox: 5’-GAC-GTC-CCA-CGG-GGT-AGT-3[sense] and 5’-CAC-GAC-CTC-ATC-TGT-CAC-3[antisense]; 60 seconds at 94°C, 60 seconds at 65°C, and 90 seconds at 72°C; 30 cycles; and 485 bp. (3) nox1: 5’-CGA-AGA-GGT-ATC-CAT-CAC-3[sense] and 5’-CAT-TGT-CCC-ACA-TTG-GTC-TC-3[antisense]; 60 seconds at 94°C, 60 seconds at 65°C, and 90 seconds at 72°C; 30 cycles; and 519 bp. The same cDNA samples were used for GAPDH cDNA amplification (5’-ACC-GAT-CCA-GAC-ATC-3[sense] and 5’-CAT-TGT-CCC-ACA-TTG-GTC-TC-3[antisense]).

**Assay for rac1 GTP-Binding Activity**

Rac1 GTP-binding activity was determined by immunoprecipitation of [35S]GTPγS-labeled rac1, as described previously. Briefly, membrane proteins from aortic homogenates of control and raloxifene-treated rats were isolated. Twenty-micromgram aliquots of the proteins were incubated in a reaction buffer containing [35S]GTPγS and were then resuspended in immunoprecipitation buffer. Rac1 antiserum was added to the mixture at a final dilution of 1:75. The antibody–G protein complexes were then incubated with protein A–Sepharose, and the immunoprecipitate was collected by centrifugation. The final pellets containing the immunoprecipitated [35S]GTPγS-labeled rac1 proteins were washed and counted in a liquid scintillation counter (LS 1800, Beckman). Nonspecific activity was determined in the presence of excess unlabeled GTPγS.

**Measurement of NO Release**

Excised and prepared aortic segments were placed in oxygenated (pO2 150 mm Hg) HEPES buffer (10 mmol/L). The vessel was longitudinally opened and placed in an organ bath with the luminal face turned upward. A NO-sensitive electrode (ISO-NO electrode, WPI) was calibrated with a standardized NO solution and placed at a fixed distance of 1 mm above the aortic lumen. Substances were added at the same place in the organ bath, and NO release of the aortic segment was measured.

**Endothelial NO Synthase Activity Assay**

Vascular endothelial NO synthase (eNOS) activity was quantified by measuring the conversion of [14C]-arginine to [14C]-citrulline by use of a NO synthase assay kit (Calbiochem) in 10-μg aliquots of total protein of aortic homogenates of control and raloxifene-treated rats, as described previously.

**Measurement of ROS**

Superoxide release in intact aortic segments was determined by lucigenin chemiluminescence, as described previously. Aortic ring preparations were transferred into scintillation vials containing Krebs-HEPES buffer with 5 μmol/L lucigenin. Chemiluminescence was assessed over 10 minutes in a scintillation counter (Lumat LB 9501, Berthold) in 1-minute intervals. The vessel segments were then washed out before the next substance was added.
Results

Effect of Raloxifene on Systolic Blood Pressure Levels in SHR

Systolic arterial blood pressure was assessed in conscious animals by the tail-cuff method before and after administration of raloxifene or vehicle. Before treatment, systolic blood pressure was similar in both groups of SHR, Figure 1 shows that raloxifene treatment led to a significant blood pressure reduction in SHR. Systolic blood pressure was 169±9 mm Hg in control animals and 130±8 mm Hg in raloxifene-treated SHR (n=10 per group; P<0.05 vs vehicle-treated SHR). To compare the SERM effects in SHR with the effects in healthy control animals, 9-month-old male Wistar rats were treated with raloxifene (10 mg · kg body weight · d⁻¹) for 10 weeks. Systolic blood pressure was similar in both Wistar groups before treatment and was significantly reduced after treatment with raloxifene compared with the systolic blood pressure of control animals (141±2 mm Hg for control versus 127±2 mm Hg for raloxifene-treated rats; n=10 per group; P<0.05 vs vehicle-treated Wistar) (Figure 1).

Effect of Raloxifene on Aortic Vasorelaxation and Vasoconstriction in SHR

To investigate the underlying mechanisms, vascular function of isolated intact aortic ring preparations was assessed in organ chamber experiments. As shown in Figure 2A and 2B, treatment of SHR with raloxifene significantly improved endothelium-dependent vasorelaxation on stimulation with acetylcholine (force of contraction 42±5% versus 63±6% for control of phenylephrine-induced vasoconstriction; acetylcholine 10 μmol/L; n=10 per group; P<0.05 versus vehicle-treated SHR), whereas endothelium-independent vasorelaxation exerted by nitroglycerin was not affected. Vasoconstriction induced by phenylephrine was not modulated by raloxifene (data not shown). In raloxifene-treated Wistar rats, acetylcholine-induced vasorelaxation of isolated aortic ring preparations was significantly improved compared with that of control rats (force of contraction 28±3% versus 43±3% for control of phenylephrine-induced vasoconstriction; acetylcholine 10 μmol/L; n=6 per group; P<0.05 versus vehicle-treated Wistar), whereas endothelium-independent vasodilatation remained unchanged (Figure 2A and 2B). Co-incubation with Nω-nitro-L-arginine (L-NNA) led to an impairment of acetylcholine-mediated vasorelaxation (force of contraction 43±3% versus 139±4% for L-NNA of phenylephrine-mediated vasoconstriction; acetylcholine 10 μmol/L; n=6 per group; P<0.05 versus aortic segments without L-NNA; data not shown). Phenylephrine-mediated vasoconstriction was not altered (n=6 per group; data not shown).

Effect of Raloxifene on Aortic NO Release, eNOS Expression, and eNOS Activity in SHR

Because the improved endothelial dysfunction could be caused by increased production of NO, we measured the NO release in intact isolated aortic segments. Raloxifene enhanced the substance P-induced release of NO from the vessel wall from 3.0±1.6 to 7.5±2.0 nmol/L (n=5 per group; P<0.05 versus vehicle-treated SHR) (Figure 3A).

Next, we investigated whether raloxifene treatment led to an increased NO release by enhanced expression and activity.
of the vascular eNOS. eNOS mRNA expression was quantified by semiquantitative RT-PCR in aortic tissue of control and raloxifene-treated SHR. Figure 3B shows representative agarose gels of both vascular eNOS and GAPDH DNA fragments. The densitometric analysis of the amplified PCR fragments (Figure 3C) demonstrates that raloxifene caused an upregulation of eNOS mRNA expression to 176 ± 16% compared with that of control animals (n = 3 per group; \( P < 0.05 \) versus vehicle-treated SHR). GAPDH mRNA expression remained unchanged (data not shown).

In agreement with these data, eNOS activity, determined by measuring the conversion of \([\text{H}]\)-arginine to \([\text{H}]\)-citrulline, was increased 4-fold in raloxifene-treated animals (456 ± 12% of control; n = 4 per group; \( P < 0.05 \) versus vehicle-treated SHR) (Figure 3D).

**Effect of Raloxifene on Aortic Production of ROS in SHR**

Improved endothelial dysfunction could also be mediated by reduced vascular ROS production, leading to an increased bioavailability of NO. Therefore, the vascular production of superoxide was assessed by lucigenin chemiluminescence assays in isolated intact aortic segments of control and raloxifene-treated SHR. Figure 4A shows that treatment with raloxifene markedly decreased superoxide production in the vessel wall to 56 ± 12% of that of the control group (n = 5 per group; \( P < 0.05 \) versus vehicle-treated SHR). Preincubation of the aortic segments with the eNOS inhibitor L-NNA (100 \( \mu \text{mol/L} \)) led to an increase of superoxide release (149 ± 4% of control; 155 ± 31% of raloxifene; n = 3 per group; \( P < 0.05 \) versus aortic segments without L-NNA), indicating that vascular ROS production was not mediated by uncoupled eNOS (data not shown). This increase in superoxide production was accompanied by an impairment of acetylcholine-induced vasorelaxation after co-incubation with L-NNA (force of contraction 63 ± 6% versus 115 ± 4% for L-NNA of phenylephrine-mediated vasoconstriction; acetylcholine 10 \( \mu \text{mol/L} \); n = 4 per group; \( P < 0.05 \) versus aortic segments without L-NNA; data not shown).

**Effect of Raloxifene on Aortic NAD(P)H Oxidase Subunit Expression in SHR**

Because the NAD(P)H oxidase is thought to be the major source of ROS in the vessel wall and because decreased NAD(P)H oxidase expression results in reduced ROS production and increased bioavailability of NO, it was investigated whether raloxifene treatment led to an altered expression of essential subunits of this superoxide-generating system by RT-PCR in RNA isolated from aortic segments of control and raloxifene-treated SHR. As shown in representative immunoblots in Figure 4C, raloxifene markedly decreased superoxide production in the membrane fraction of aortic tissue of control and raloxifene-treated SHR. Treatment with raloxifene decreased membrane-associated rac1 GTP-binding activity to 38 ± 7%
arterial blood pressure was assessed. Figure 5 reveals that raloxifene treatment led to an increased reduction of systolic blood pressure on administration of bradykinin, suggesting an enhanced bioavailability of NO in these animals (n=10 per group; P<0.05 versus vehicle-treated SHR).

**Effect of Raloxifene Treatment on Oxidative Stress in Wistar rats**

Assessment of vascular ROS production with the lucigenin chemiluminescence assay revealed that superoxide release from aortic segments was significantly decreased to 63±11% of that of control in raloxifene-treated Wistar rats (n=3 per group; P<0.05 versus vehicle-treated Wistar) (Figure 6A). Preincubation with L-NNA led to an increased vascular ROS production (138±12% of control; n=3 per group; P<0.05 versus aortic segments without L-NNA; data not shown). As shown in Figure 6B, mRNA expression of GAPDH and the NAD(P)H oxidase subunits p22phox and nox1 in aortic homogenates was not significantly altered after treatment with raloxifene (n=3 per group), whereas the SERMs led to an increased vascular expression of eNOS mRNA (152±21% of control; n=3 per group; P<0.05 versus vehicle-treated Wistar) and to an enhanced vascular eNOS activity (245±21% of control; n=4 per group; P<0.05 versus vehicle-treated Wistar) (Figure 6C). Finally, raloxifene treatment induced a moderate downregulation of rac1 GTPase protein expression in the membrane fraction of aortic homogenates and decreased rac1 GTP-binding activity to 77±2% of that of control (n=3 per group; P<0.05 versus vehicle-treated Wistar) in healthy Wistar rats (Figure 6C+D).

**Expression and Activity of NAD(P)H Oxidase Subunits and eNOS in SHR and Wistar Rats**

The expression of the NAD(P)H oxidase subunits nox1 and p22phox, and the expression and activity of rac1 GTPase and eNOS in aortic homogenates were compared between SHR and Wistar rats. The mRNA expression of GAPDH and p22phox was not significantly different between SHR and Wistar rats (n=3 per group), whereas the nox1 subunit of the NAD(P)H oxidase was significantly upregulated in SHR (182±11% of Wistar; n=3 per group; P<0.05 versus Wistar; data not shown). Membrane rac1 GTPase protein expression and GTP-binding activity (136±9% of Wistar; n=3 per...
Effect of Raloxifene and Estrogen Receptor Antagonism on Production of ROS in VSMCs

To evaluate the in vitro effect of raloxifene on intracellular ROS production and the involvement of estrogen receptors in this setting, VSMCs were preincubated for 12 hours with vehicle, raloxifene (1 μmol/L), the selective estrogen receptor antagonist ICI 182,780 (1 μmol/L), or raloxifene plus ICI 182,780, followed by a 3-hour co-incubation with 1 μmol/L angiotensin II. ROS production was measured by DCF fluorescence laser microscopy. A representative microscopic scan is shown in Figure 7A, and data analysis of 8 separate experiments is illustrated in Figure 7B. Stimulation with angiotensin II led to a marked increase of ROS production (232±14% of control; *P<0.05 versus control). Preincubation with raloxifene for 12 hours significantly reduced angiotensin II–induced ROS production to 112±7% of that of control (*P<0.05 versus angiotensin II). Co-incubation with ICI 182,780 completely reversed the effect of raloxifene on angiotensin II–induced free radical production (224±10% of control; *P<0.05 versus control and versus angiotensin II plus raloxifene). Incubation with raloxifene or ICI 182,780 alone had no effect on basal ROS production.

Discussion

The early state of atherosclerosis, endothelial dysfunction, is caused by hypertension, hypercholesterolemia, diabetes mellitus, estrogen deficiency, and heart failure.24,25 Endothelial dysfunction not only is a prerequisite of atherosclerosis but also represents a pathophysiologic condition regarded as an independent predictor of a poor prognosis and cardiovascular events.15,16 One of the underlying mechanisms of endothelial dysfunction appears to be an impaired balance between endogenously formed NO and harmful ROS, such as superoxide.17 Increased oxidative stress by enhanced production of ROS and diminished release and bioavailability of NO results in impaired vasodilatation and the initiation of atherosclerotic lesions in the vessel wall.18

Estrogens act vasoprotective by improvement of endothelial function.6 It is well established that estrogens enhance the release of NO and reduce oxidative stress by decreased production of free radicals.6,26–28 These properties could explain the putatively beneficial vascular effects of estrogen replacement therapy. This is supported by the epidemiologic findings that menopause is associated with an increased cardiovascular risk and endothelial dysfunction.1–6 Unfortunately, unimposed estrogen replacement causes an enhanced incidence of breast and uterus malignancies.9,10

SERMs such as raloxifene that act as estrogen antagonists in breast and uterus may be an attractive alternative with respect to cardiovascular risk reduction, provided that these compounds exert vasoprotective effects comparable to those of estrogens. Indeed, raloxifene has been investigated in recent studies. First, raloxifene shares the lipid-lowering properties of estrogens.13,29 Second, raloxifene inhibits the accumulation of cholesterol in ovariectomized cholesterol-fed rabbits and inhibits macrophage lipid oxidation.30,31 Third, raloxifene acutely dilates rabbit coronary arteries via NO- and calcium influx–dependent pathways and increases uterine and coronary blood flow in ovariectomized ewes.14,32 However, it has not yet been elucidated whether long-term treatment with raloxifene leads to reduced blood pressure and improved vascular function in hypertensive males.
The presented data provide evidence that raloxifene treatment exerts vasoprotection in male SHR. Raloxifene profoundly reduces blood pressure levels and improves endothelial dysfunction in these animals. There are at least 2 underlying mechanisms. First, long-term treatment with raloxifene increases the release of NO from the vessel wall. This is caused by raloxifene-induced enhancement of vascular eNOS expression and activity. Second, raloxifene reduces the vascular production of ROS. This is induced by a direct effect of raloxifene on the membrane-bound NAD(P)H oxidase, which is known to represent a major source of free radicals in the vascular wall.33,34 The NAD(P)H oxidase is an enzymatic system composed of various subunits, such as p22phox, nox1, and rac1.35-38 The GTPase rac1 seems to be crucial for the agonist-mediated activation of this enzyme. Activation of the NAD(P)H oxidase leading to enhanced ROS production requires translocation of rac1 from the cytosol to the cell membrane,39,40 and superoxide is produced on assembly of all subunits. Raloxifene treatment induces downregulation of rac1 expression in vascular cell membranes and furthermore reduces the activity of rac1 GTPase, thereby leading to a decreased activity of the whole NAD(P)H oxidase enzyme. The expression of the essential NAD(P)H oxidase subunits p22phox and nox1, however, is not altered by raloxifene.

Raloxifene acts as an estrogen receptor agonist in the vasculature, and it has been shown that raloxifene leads to acute NO-dependent vasodilatation via stimulation of estrogen receptors.13,14 The presented effects of raloxifene have been evaluated in male animals that express both estrogen α and β receptors in the vasculature (RT-PCR experiments in aortic homogenates of male SHR; data not shown). Our data demonstrate that the effect of raloxifene on cellular ROS production can be completely blocked by the estrogen receptor antagonist ICI 182,780, indicating that the antioxidant effect of raloxifene is estrogen receptor-mediated.

Dysfunctional eNOS may contribute to oxidative stress in SHR by producing superoxide.41-43 In our study, however, co-incubation of aortic preparations with the eNOS inhibitor L-NNA led to an increase of superoxide release and impairment of endothelium-dependent vasorelaxation, indicating that vascular ROS production was not mediated by uncoupled eNOS in our model. These contrasting data may be related to different SHR strains (eg, stroke prone) or to different ages at the experimental period.
The SHR used in our study display hypertension (systolic blood pressure \( \approx 170 \text{ mm Hg} \)), which leads to profound endothelial dysfunction as indicated by impaired endothelium-dependent vasorelaxation (\( \approx 35\% \) vasorelaxation on maximal stimulation with acetylcholine after preconstriction with phenylephrine). It is known that increased oxidative stress, mediated by decreased bioavailability of NO because of enhanced superoxide production and reduced eNOS activity, is involved in the pathology of SHR.\(^{44,45}\) Our findings indicate that compared with healthy age-matched Wistar rats, SHR used in our study display a higher membrane rac1 GTPase expression and activity and increased nox1 expression, leading to an increased activation of the NAD(P)H oxidase, and a lower eNOS expression and activity in aortic homogenates. This higher level of activation in SHR is normalized by treatment with raloxifene, because expression and activity of rac1 GTPase and vascular superoxide production are profoundly decreased, expression and activity of eNOS are enhanced, and, finally, endothelial function is significantly improved after treatment with SERMs. Interestingly, nox1 expression is not altered by raloxifene treatment. However, quantitatively the effects of raloxifene are beyond the normalization of the level of activation in SHR compared with Wistar rats. Moreover, raloxifene exerts the same vasoprotective effects in healthy normotensive Wistar rats, although the effects are not as pronounced as in SHR. Interestingly, endothelial function is also improved in the Wistar rats (\( \approx 60\% \) versus 70\% vasorelaxation after treatment) and systolic blood pressure is decreased by 14 \text{ mm Hg}. Given the fact that raloxifene causes reduction of oxidative stress in normotensive control animals and through direct interactions with vascular cells, it is unlikely that the reduction of oxidative stress is secondary to the detected blood pressure decrease in SHR. In contrast, it is reasonable to assume that the reduction of oxidative stress and enhancement of NO release results in an increased bioavailability of NO, which ultimately restores vascular function and profoundly lowers blood pressure in SHR.

The presented findings of beneficial vascular properties of raloxifene in animals suffering from vascular dysfunction caused by hypertension may have important implications. From the mechanical point of view, it provides novel actions of SERMs on the vasculature, namely, reduction of free radical release, induction of the eNOS/NO system, improvement of endothelial dysfunction, and lowering of blood pressure. Clinically, the discovery of these mechanisms of vasoprotection by raloxifene may allow considerations of innovative treatment regimes in atherosclerosis and hypertension in both women and men.

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**References**


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