Estrogen Replacement Reduces Age-Associated Remodeling in Rat Mesenteric Arteries

Yunlong Zhang, Ken G. Stewart, Sandra T. Davidge

Abstract—Estrogen replacement therapy significantly decreases the incidence of cardiovascular disease in postmenopausal women. In aging, there is an increase in vascular stiffness along with a decrease in matrix metalloproteinase (MMP) activity. Our hypothesis was that estrogen replacement would increase MMPs and therefore reduce the vascular stiffness that is associated with aging. Female Sprague-Dawley rats were implanted with a placebo or 17β-estradiol–containing pellet (0.5 mg/pellet, 60-day release) at 10 months of age (n=6, each). Six young rats (3 months old) were also studied. After a 2-month exposure to the pellet, mesenteric arteries were studied on a pressurized arteriograph system. Distensibility and wall thickness were measured in response to stepwise increases in intraluminal pressure in Ca2+-free physiological saline solution buffer with papaverine (10-4 mol/L). In response to increasing pressure, aged placebo rats exhibited a significant decrease in distensibility compared with young rats (P<0.05) that was accompanied by an increase in wall thickness (P<0.05). Conversely, estrogen replacement increased distensibility and decreased wall thickness in aged rats (old estrogen-replaced versus old placebo, P<0.05). Zymography data indicated that MMP-2 activity decreased in aging but was increased by estrogen replacement. In summary, estrogen replacement in aging female rats reduces age-associated vascular remodeling. (Hypertension. 2000;36:970-974.)

Key Words: estrogen • aging • vasculature • remodeling

Cardiovascular disease is the leading cause of death among postmenopausal women. Decreased estrogen levels and aging are both risk factors that contribute to the heightened incidence of cardiovascular disease in aging women. Aging is associated with several adaptations, including altered structural properties of the arterial wall in a variety of vascular beds. In addition to vasoactive pathways, the passive properties of arteries significantly contribute to the determination of vessel tone and therefore cardiovascular homeostasis. Although the effects of estrogen on vasoactive pathways have been studied extensively, relatively little work has been done to address how passive properties are influenced.

Media thickness and collagen/elastin content are factors that contribute to arterial wall compliance. Vessels with low compliance, defined as the change in volume for a given change in pressure, less effectively dampen the pulsatile flow of blood by stretching and contracting in response to the systolic and diastolic cardiac phases, respectively. As well, vessels with low compliance result in increased mean arterial blood pressure that can have a variety of detrimental effects, including left ventricular hypertrophy and damage to organs and the vascular endothelium.

The process of vascular remodeling is mediated by matrix metalloproteinases (MMPs), a family of enzymes capable of degrading components of the extracellular matrix. MMP-2 digests collagen, an extracellular matrix protein that is associated with decreased arterial compliance and intimal thickening. Considering that aging is associated with increased collagen content as well as decreased MMP activity, there is a potential link between MMPs and decreased arterial compliance in aging.

Recent evidence suggests that a portion of the vasoprotective effect of estrogen is through increased vessel compliance. Studies, including our own, have found that estrogen replacement increases arterial compliance in young ovariectomized rats. Radial artery distensibility fluctuates in accordance with estrogen levels during menstrual cycles, and age-associated increases in common carotid artery stiffness are reduced by estrogen replacement. In agreement with the above data, estrogen has been found to decrease collagen synthesis and increase MMP-2 release from vascular smooth muscle cells in a dose-dependent manner. However, very little is known about the effect of estrogen on resistance artery remodeling in aging. Therefore, the objective of this study was to determine the effect of estrogen replacement on arterial compliance, wall thickness, and MMP-2 expression/activity in a model of aging.

Methods

Animal Model
Female Sprague-Dawley rats (2 months of age) were obtained from Biological Sciences (University of Alberta, Canada) and housed in...
the facilities at the University of Alberta. One group of rats was 3 months of age (n=6). Two other groups of rats were 10 months of age and were provided with a subcutaneous 60-day release pellet containing 0.5 mg 17β-estradiol (n=6) or placebo pellet (n=6) for 2 months. The concentration of estrogen pellet was selected on the basis of our previous studies in which physiological levels of estradiol were achieved in ovariectomized rats.15 On the day of the experiment, rats were killed under light anesthesia with methohexitol sodium (50 mg/kg body wt). The animal protocols were examined by the University of Alberta Animal Welfare Committee and found to be in compliance with the guidelines issued by the Canada Council on Animal Care.

Vessel Preparation
A section of the mesentery 5 to 10 cm distal to the pylorus was rapidly removed and placed in ice-cold N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES)-buffered physiological saline solution (HEPES-PSS). Arteries were transferred to a dual-chamber arteriograph (Living Systems Instrumentation), and each vessel was mounted on 2 microcannulas. The cannulas rested in 3-mL organ baths with HEPES-PSS solution kept at 37°C. Arteries were initially tied to the proximal cannula. Residual blood was then flushed from the lumen followed by the distal cannula being inserted into the artery. After the arteries were tied to the microcannulas, the arteriograph was placed on the stage of a compound microscope. The proximal cannula was joined in series with a pressure transducer connected to a servo-controlled peristaltic pump. This allows a desired intraluminal pressure to be set. A video camera on the microscope provided an image of the artery on a video monitor; measurements of lumen diameter and wall thickness were made with a video dimension analyzer and direct observations with a filar eyepiece. This vessel system has been described in detail elsewhere.16

Passive Mechanics
To compare distensibility of arteries between the young, placebo aged, and estrogen-replaced aged rats, the active contractile activity must be eliminated. In this study, vascular smooth muscle activity was deactivated by papaverine (0.1 mmol/L) and studies were performed in calcium-free buffer containing 0.1 mmol/L ethylene glycol-bis (β-aminoethyl ether)- N, N, N', N'-tetraacetic acid to remove the effect of extracellular calcium. Inactivation of smooth muscle was confirmed by the lack of contraction to potassium chloride (124 mmol/L). Lumen diameter and wall thickness were measured at 11 pressures ranging from 0 to 150 mm Hg. Passive pressure-diameter and wall thickness relations were determined for the arteries. Distensibility was defined as the relative change in diameter per unit change in pressure. To obtain the relative change in diameter, the internal diameter of a vessel at each pressure was normalized to an initial diameter observed at 3 mm Hg. This reference diameter of 3 mm Hg was used because it was not possible to reliably measure arterial diameter at 0 mm Hg. The stress-strain relation was also compared to further evaluate the passive mechanical properties of the arteries. These parameters were normalized for wall thickness and therefore characterize the stiffness of the components that comprise the vascular wall. Stress was defined as the force exerted on the vascular wall per unit of tissue and was calculated by the following equation: Stress=(P×D)/2T, where P is pressure in dynes per square centimeter, D is diameter, and T is wall thickness. Circumferential strain represents the response of an artery to force or intraluminal pressure. Strain was calculated with the following equation: Strain=(D1−D)/D1, where D1 is the initial diameter at a pressure of 3 mm Hg and D2 is the diameter at the new pressure.

Western Immunoblot and Zymography
To address a potential mechanism for the age-dependent increase in mesenteric artery wall thickness and the estrogen-dependent reduction in wall thickness in the estrogen-replaced aged group, we measured α-actin content, pro–MMP-2 activity, and MMP-2 activity in rat mesenteric arteries. As previously described,17 Western immunoblot was performed for α-actin with monoclonal antibodies (Boehringer Mannheim). Gelatin zymography of MMP-2 from rat mesenteric arteries was performed as previously described.18

Statistics
Data are summarized as mean±SEM. A 2-way ANOVA with repeated measures was applied for analysis of passive mechanics. A 1-way ANOVA was used for evaluation of protein quantity and enzyme activity. Where appropriate, post hoc analysis was performed with a Tukey test. Tests with a value of P<0.05 were considered significantly different.

Results
The wall thickness of mesenteric arteries was measured as intraluminal pressure was increased in a stepwise manner. At each level of intraluminal pressure, wall thickness in the placebo aged group was significantly greater than that of the young. However, the wall thickness of mesenteric arteries measured α-actin content, pro–MMP-2 activity, and MMP-2 activity in rat mesenteric arteries.

![Figure 1. A. Relative change in wall thickness per unit change in pressure was significantly blunted in mesenteric arteries from aged placebo rats (○; n=6) compared with arteries from aged rats treated with 0.5 mg/pellet estradiol (▼; n=6) and young rats (●; n=6) (P<0.001, ANOVA). Data points are mean±SEM. B, Distensibility, or relative change in diameter per unit change in pressure, of mesenteric arteries was significantly greater in young rats (●; n=6) and aged rats treated with 0.5 mg/pellet estradiol (▼; n=6) compared with arteries from aged placebo rats (○; n=6) (P<0.001, ANOVA). Data points are mean±SEM.](http://hyper.ahajournals.org/Download)
from the estrogen-replaced aged group was similar to that of the young (Figure 1A).

Artery distensibility was determined by calculating the normalized change in vessel diameter relative to intraluminal pressure. Arteries in the placebo aged group were significantly less distensible in comparison to young arteries. Estrogen replacement restored vessel wall distensibility to that of the young (Figure 1B).

The passive properties of the mesenteric arteries were further assessed by plotting the stress-strain relation. This calculation is a normalized representation of arterial wall distensibility that accounts for differences in vessel wall thickness. The stress-strain curve for the placebo aged group was shifted to the left relative to both the young and estrogen-replaced group (Figure 2), further demonstrating that the altered structural properties of the artery wall decrease compliance in the placebo aged group.

Western immunoblot data indicate that the difference in distensibility is not due to smooth muscle hypertrophy because arteries in the 3 groups contained similar amounts of α-actin. Activity of the pro and active forms of MMP-2, an enzyme responsible for vascular wall remodeling through collagen cleavage, was measured by zymography (Figure 3A). Pro–MMP-2 activity decreased in both the placebo and estrogen-replaced aged groups compared with arteries from the young group (Figure 3B). Although aging also decreased MMP-2 activity in the placebo group, estrogen replacement in aged rats restored MMP-2 activity similar to that in the young group (Figure 3B).

**Discussion**

In resistance-sized mesenteric arteries, estrogen replacement reversed age-associated alterations in the passive properties of the vascular wall. Aging significantly increased arterial wall thickness, decreased distensibility, shifted the stress-strain curve to the left, and suppressed MMP-2 activity. However, estrogen replacement increased MMP-2 activity and restored the passive properties of aged arteries similar to that of the young group. These effects probably represent another mechanism whereby estrogen reduces the incidence of cardiovascular disease among postmenopausal women.

Aging is associated with a variety of deleterious changes to both vasoactive pathways and passive properties of the vasculature. Previous studies have reported age-associated changes such as arterial hypertrophy, decreased distensibility, and thickening of the subendothelial layer with increased protein and lipid deposition. Noncompliant arteries convey greater resistance to the systemic vasculature and therefore induce elevated blood pressure and pulse pressure. This in turn increases the incidence of damage to organs and the vascular endothelium. Elevated blood pressure has also been correlated with overexpression of the potent vasoconstrictor endothelin. Furthermore, chronically elevated intravascular pressure is associated with smooth muscle hypertrophy and increased deposition of collagen in the media. Compared with stiff arteries, compliant and thin-walled vessels reduce the afterload in which the left ventricle pumps against, thereby decreasing left ventricular hypertrophy and a variety of associated complications. Thus, the ability of estrogen to restore arterial distensibility and reduce wall thickness in aging is significant.

MMP-2 is a key modulator of vascular remodeling and probably contributed to the decreased vessel thickness and

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**Figure 2.** Stress-strain relation in rat mesenteric arteries. In placebo group, aging shifted stress-strain curve left (○; n=6) compared with aged rats treated with 0.5 mg/pellet estradiol (▲; n=6) and young rats (●; n=6). Data points are mean±SEM.

**Figure 3.** Pro–MMP-2 and MMP-2 activity. A, Zymogram of pro–MMP-2 and MMP-2 activity in mesenteric arteries from young rats (lanes 1 to 3), aged rats treated with 0.5 mg/pellet estradiol (lanes 4 and 5), and aged placebo rats (lanes 6 and 7). B, Mesenteric arteries from aged rats treated with either 0.5 mg/pellet estradiol (n=4) or placebo (n=4) demonstrated significantly lower pro–MMP-2 activity (solid bars) compared with young group (n=6) (*P<0.01, ANOVA). MMP-2 activity (open bars) in placebo aged group was significantly lower compared with both young and aged group treated with 0.5 mg/pellet estradiol (#P<0.05, ANOVA). Values represent mean±SEM.
increased distensibility in the estrogen-replaced group. Zymography data indicate that MMP-2 activity was increased in the estrogen-replaced group compared with the placebo aged group. These data are in accordance with previous literature that demonstrate estrogen increases the release of MMP-2 in vascular smooth muscle cells, increases the production of 60K gelatinase in mammary tissue, and decreases mRNA expression of the MMP inhibitor TIMP-1 in endometrial tissue.

Estrogen may affect MMP activity by altering intracellular calcium concentrations because it is known to reduce calcium influx in smooth muscle cells, and calcium channel blockers have been shown to increase MMP-2 activity by 2-fold. It is also possible to speculate that estrogen modulates the expression of MMPs, as the promoter region for the MMP-2 gene in humans is known to contain 3 half-palindromic estrogen response elements. Estrogen receptor antagonists such as tamoxifen and ICI 182,780 block or attenuate estrogen-mediated increases in pro–MMP-2 release in various tissues as well. Finally, the highly estrogenic state of pregnancy induces a marked increase in arterial distensibility to accommodate the rise in blood volume and cardiac output. Hence, estrogen probably contributes to the maintenance of arterial compliance in aging by facilitating MMP-mediated degradation of the extracellular matrix.

Arterial wall structure is also regulated by a variety of vasoactive compounds such as angiotensin II, catecholamines, endothelins, prostaglandins, and nitric oxide (NO), which stimulate or suppress vascular wall thickening. In addition to its potent vasodilatory effects, NO exerts a substantial influence on vascular remodeling. Several studies have established the ability of NO to regulate vessel thickness and cellular composition. Models of chronic NO synthase inhibition have demonstrated a decrease in lumen diameter, an increase in vascular smooth muscle cell and endothelial cell proliferation, and thickening of the adventitia. Given that we have previously demonstrated decreased NO-mediated relaxation in this model of aging, it is reasonable to speculate NO availability decreased with age and consequently contributed to the vascular remodeling in the placebo aged group. Conversely, the preservation of mesenteric artery wall structure in the estrogen-replaced aged rats may be due in part to an increase in NO availability.

In summary, estrogen replacement in aged rats restored several of the parameters used to assess passive properties of arteries to that of the young group. A portion of the vasoprotective effects of estrogen probably can be attributed to shifting the stress-strain relation curve to the right, increasing arterial distensibility, decreasing arterial wall thickness, and increasing MMP-2 activity in aged arteries. Thus, estrogen attenuates age-associated changes in the structure of resistance arteries.

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References

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