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ORIGINAL ARTICLE

Estrogen Deficiency Modifies Matrix Metalloproteinases Activity and Vascular Function of Mesenteric Arteries in Female Rats

Hsin-Hsueh Shen[1], Mei-Hui Liao[1], Yu-Hui Chang[1], Yen-Mei Lee[1]

[1]*Department and Institute of Pharmacology, National Defense Medical Center, Taipei, Taiwan, Republic of China*

Objective: Estrogen possesses vasoprotective effects and its deficiency has been implicated in the pathogenesis of postmenopausal hypertension. Reduced matrix metalloproteinases (MMPs) activity is accompanied with extracellular matrix (ECM) accumulation in large arteries, leading to the hypertensive arterial remodeling in resistance arteries. In the present study, we investigate whether estrogen deficiency induces alteration of MMP-mediated cleavage of vascular collagen in rats with ovariectomy (Ovx). **Materials and Methods:** Adult female rats were ovariectomized bilaterally to induce estrogen deficiency. Evolution of systolic blood pressure, diastolic blood pressure (DBP), and mean blood pressure (MBP) was monitored weekly until 12 weeks after Ovx in conscious rats. The vascular reactivity and time-course changes of collagen type I, MMP-2, membrane type 1-MMP (MT1-MMP), and tissue inhibitor of metalloproteinase-2 (TIMP-2) protein expression in mesenteric arteries were evaluated. **Results:** Compared with sham group, DBP and MBP significantly increased 5 weeks after Ovx, lasting to at least 12 weeks. Acetylcholine-induced vasodilatation of precontracted mesenteric rings significantly reduced 9 weeks after Ovx. Collagen type I accumulation in mesenteric arteries appeared 6 weeks after Ovx, which persisted till 12 weeks. The levels of latent and active MMP-2 did not show significant change until 12 weeks after Ovx. Moreover, MT1-MMP significantly downregulated during 1–4 weeks and soon recovered to normal levels. TIMP-2 reduced at 4th week and gradually returned to normal levels during 6–12 weeks. **Conclusion:** Long-term estrogen deficiency results in a shift in ECM profiles and diminished MMPs activities, leading to remodeling of small arteries, which may be associated with postmenopausal hypertension. This study provides new insight into the pathophysiology of vascular remodeling in estrogen-deficient conditions.

Key words: Estrogen, matrix metalloproteinases, vascular remodeling, postmenopausal hypertension

INTRODUCTION

Postmenopausal status with estrogen deficiency seems to play an important role in the prevalence of the hypertension, which is one of the leading causes for cardiovascular diseases (CVDs).[1] The previous report demonstrated the incidences of CVD are much lower in premenopausal women as compared to men of the same age, and this sexual advantage disappears after menopause, suggesting estrogen possesses beneficial effects in cardiovascular system.[2,3] Loss of estrogen triggers impairment in endothelium-dependent vasodilation that may contribute to increases in hypertension and CVD in postmenopausal women.[4] Furthermore, vessels with lower compliance result in increased arterial blood pressure.[5] Estrogen alters arterial wall remodeling by elevating elastin production and decreasing collagen deposition in human arteries that have been linked to lower arterial stiffness.[6] Protective effects of estrogen may also result from limited activation of the renin-angiotensin system as a result of prevention of hypertension.[7]

The stability and compliance of the arterial wall are maintained by a well-regulated balance of extracellular matrix (ECM) proteins, including collagen and elastin. The alteration of ECM contents has been proposed as major determinants of the arterial compliance and vascular remodeling.[8] The process of vascular remodeling is regulated by balance of matrix metalloproteinases (MMPs), their

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Corresponding Author: Prof. Yen-Mei Lee, No. 161, Section 6, Min-Chuan East Road, Taipei 114, Taiwan, Republic of China. Tel: +886-2-87927877; Fax: +886-2-87927877. E-mail: ymlee@mail.ndmctsgh.edu.tw

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activators membrane type-membrane type (MT) MMPs, and their inhibitors tissue inhibitors of MMP (tissue inhibitor of metalloproteinases [TIMPs]). They are a family of zinc-containing enzymes capable of degrading components of ECM and connective tissues.[9,10] MMP-2 is the most commonly expressed enzyme to degrade collagen, elastin, and fibronectin.[11] Clinical studies revealed that the attenuation of MMP-2 expression was observed in plasma from patients with hypertension.[12] Animal investigations from adult spontaneously hypertensive rats (SHRs) also indicated that pro-MMP2 and activated MMP-2 activities were diminished in mesenteric arteries, contributing to accumulation of collagen and fibronectin.[13] The remodeling of large and small arteries may be the major causes of the development of ECM accumulation and hypertension.[14]

Pro-MMP-2 is a zymogen (also called gelatinase A) and its activation involves forming a complex on the cell surface with MT1-MMP, and TIMP-2, in which a neighboring MT1-MMP cleaves proMMP-2 at the pro-domain.[15] Imbalance among MMPs, MT-MMP and TIMPs result in alterations in MMP activity and contribute to pathological deposition in vascular collagen as well as remodeling of resistance arteries in hypertension.[16] Aging and estrogen loss induce changes in MMPs as well as TIMP concentrations that mainly contributes to ECM remodeling.[17,18] Estrogen also exerts antifibrotic effects by both inhibiting collagen synthesis and enhancing its degradation. In our previous report, estrogen deficiency caused by Ovx in female rats reduces active MMP2 expression in large artery (aorta) which is associated with collagen type I accumulation and MT1-MMP induction.[19] However, little is known about the effect of estrogen deficiency in small artery remodeling. Thus, the objective of this study is to determine time-course changes of MMP-2 and MMP-2-related modulators, MT1-MMP, and TIMP-2 expression and vascular function in mesenteric arteries of Ovx-induced estrogen deficiency in female rats.

MATERIALS AND METHODS

Animal preparation

Female Sprague-Dawley rats (8-week-old, 250–380 g) were purchased from National Laboratory Animal Breeding and Research Center of Ministry of Science and Technology, Taiwan. Handling of the animals was in accordance with the guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1996). This study was approved by the National Defense Medical Center Institutional Animal Care and Use Committee, Taiwan. All animals were housed at an ambient temperature of 22 ± 1°C and humidity of 55% ±5%. To induce the estrogen-deficient condition, female rats were anesthetized with sodium pentobarbital (50 mg/kg) by intraperitoneal and underwent bilateral ovariectomy (Ovx). Small incisions were made bilaterally on the sides of their backs to expose the ovaries retroperitoneally. The ovaries were clamped and removed and the uterine tubes were ligated, and finally, the muscle and skin were sutured. The sham procedure consisted of anesthesia, visualization of the ovaries through incisions into the abdominal cavity, and closure of the wounds. The rats were divided into two groups: (i) sham group: rats were undergone sham operations (n = 21); (ii) Ovx group: rats were ovariectomized bilaterally (n = 18).

Plasma E_2 assay

Blood samples (1 ml) were withdrawn from the abdominal aorta in heparinized syringes under anesthesia at 12 weeks after Ovx and centrifuged for 10 min at 4°C. Plasma concentration of E_2 was determined by 125I radioimmunoassay using a commercially available kit (Diagnostic Products, Los Angeles, CA, USA). The protocol provided by the manufacturer was strictly followed. All samples and standards were measured in duplicate and repeated twice.

Measurements of blood pressure

The systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP) was determined in conscious rats by a tail-cuff method using an automatic blood pressure monitoring system (MK-2000; Muromachi Kikai, Tokyo, Japan). In brief, conscious rats were placed in restraints, and an occlusion cuff containing a piezoelectric pulse sensor was placed around the tail. The blood pressure was measured after 15 min of acclimatization. A minimum of 10 serial measurements were made, and the average value was calculated.

Measurement of mesenteric vascular reactivity *in vitro*

The preparation of mesenteric artery rings and vascular reactivity assessment were performed as described previously.[20] Nine weeks after Ovx or sham operation, rats were reanesthetized, and the mesentery arteries quickly removed and placed in Krebs buffer (mM: NaCl 118.0, $NaHCO_3$ 25.0, KCl 3.6, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.0, $CaCl_2$ 2.5) at room temperature. A segment (2 mm in length) of the mesentery was carefully cleared of adherent tissue and mounted in Krebs buffer at 37°C in a small vessel myograph (model 400A, Danish Myotechnology, Denmark). The rings were allowed to equilibrate for 30 min under an optimal resting tension of 1 g and the experimental protocols begun once the mesenteric artery reached a steady basal resting tension. NE (1 μM) and

acetylcholine (Ach) (1 μM) were applied to establish control responsiveness. Then, concentration-response curves to NE and ACh (1–10 μM) were obtained to evaluate the *in vitro* vascular reactivity in each group.

Western blot analysis of collagen type I, matrix metalloproteinase-2, membrane type 1-matrix metalloproteinase, and tissue inhibitor of metalloproteinase-2 protein expression in the mesenteric artery

Among the different collagen types described until now, type I collagen is the major fibrillary collagen detectable in vessels.[21] It is the common substrates of MMP-2. Therefore, we chose collagen type I as the representative to examine the possible role of vascular MMP-2 in the E_2-deficiency condition in rats. Mesenteric artery samples were collected 1, 2, 4, 6, 8, and 12 weeks after Ovx and ground in a mortar containing liquid nitrogen and pulverized using a pestle whereas the tissue was kept frozen by the addition of liquid nitrogen. The powdered tissue was then suspended in 1 ml of lysis buffer (5 mM ethylenediaminetetraacetic acid, 50 mM NaCl, and 50 mM HEPES, pH 7.5) containing protease inhibitors (10 μg/ml aprotinin, 1 mM phenylmethanesulfonyl fluoride, and 10 μg/ml leupeptin). Samples containing equal amounts of protein were subjected to electrophoresis in a 10% sodium dodecyl sulfate-polyacrylamide gel for 1 h at 100 V. The separated proteins were then transferred electrophoretically to nitrocellulose membranes (Millipore, Bedford, MA, USA). Membranes were blocked with Tris-buffered saline, pH 7.4, containing 0.1% Tween 20 and 5% skim milk and then incubated overnight at 4°C with primary antibodies, including mouse collagen type I (1:1000 dilution), MT1-MMP (1:2000 dilution), and TIMP-2 (1:2000 dilution), and MMP-2 (1:2000 dilution; above all are purchased from Calibiochem, Darmstadt, Germany). The membrane was washed and incubated for 1 h at room temperature with horseradish peroxidase conjugated secondary antibody (1:2000 dilution; Cell signaling Technology), followed by enhanced chemiluminescent substrate (Pierce, Rockford, IL, USA). A bioimaging analyzer (Pierce, Rockford, IL, USA) was used for visualization and band densities were quantified using Image-Pro software (Media Cybernetics, Inc., Bethesda, MD, USA).

Statistical analysis

The data are expressed as mean ± standard error of the mean. Statistical evaluation was performed with Student's *t*-test and one-way ANOVA followed by the *post hoc* Student–Newman–Keuls test to identify significant differences. A two-way ANOVA and least significant difference *post hoc* comparison test were used to compare the means of arterial blood pressure between the groups and time. $P < 0.05$ was accepted as indicating statistical significance.

RESULTS

Plasma E_2 concentration

Nine weeks after sham operation and Ovx, the plasma E_2 concentration averaged 120 ± 4.7 and 38 ± 6.1 pg/mL, respectively, which showed a significant difference between group ($P < 0.05$, $n = 3$ in each group) [Figure 1].

Time-course changes of blood pressure in conscious sham-operated and ovariectomy rats

No marked changes in SBP, DBP, and MBP were observed in sham group during the long-term observation to 12 weeks after Ovx. There were no significant different in time-course changes of SBP between sham and Ovx group, except at 6 weeks after Ovx [Figure 2a]. In Ovx group, DBP increased progressively and exhibited a marked increase at 5 weeks after Ovx ($P < 0.05$), which was significantly higher than that of sham group and maintained at high levels till 12 weeks after Ovx [Figure 2b]. As shown in Figure 2c, MBP of Ovx group gradually elevated and appeared a dramatic increase at 5 weeks as compared with sham group and lasted to the end of experimental period.

Effects of E_2 deficiency on vascular reactivity *in vitro*

As shown in Figure 3a, the mesenteric arterial rings were contracted by cumulative concentrations of NE (1–10 μM) 9 weeks after Ovx or sham operation, in which vasoconstriction between sham and Ovx groups is not significantly different.

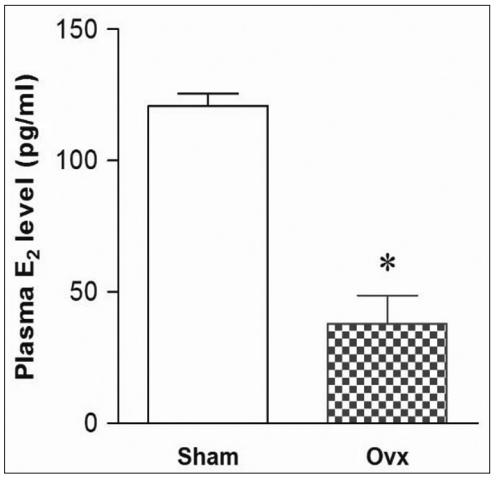

Figure 1: Plasma levels of E_2 at 9 weeks after ovariectomy. Data are given as mean ± standard error of the mean ($n = 3$). *$P < 0.05$ versus sham

Endothelium-dependent vasodilatation elicited by ACh was conducted to evaluate endothelial function. The ACh (1 and 10 μM)-induced vasodilatation of precontracted mesenteric rings in the Ovx group was significantly less than that of sham group ($P < 0.05$) [Figure 3b].

Time-course changes in mesenteric collagen type I, matrix metalloproteinase-2, membrane type 1-matrix metalloproteinase, and tissue inhibitor of metalloproteinase-2 protein expression after ovariectomy

The levels of collagen type I, pro- and active form of MMP-2, MT1-MMP, and TIMP-2 protein expression in mesenteric arteries of sham-operated rats were not significantly changed at the time points evaluated (1–12 weeks; data not shown). As shown in Figure 4, the level of collagen type I was significantly increased 6 weeks after Ovx when compared to sham group ($P < 0.05$) and lasted at high level till 12 weeks. To evaluate whether collagen type I deposition was associated with alterations of MMPs, we measured time-course changes of MMP-2, MT1-MMP, and TIMP protein expression. As shown in Figure 5, the levels of pro- and active-MMP-2 protein expression were not significantly changed before 12 weeks of Ovx. At 12 weeks of Ovx, the levels of pro- and active-MMP-2 significantly decreased when compared to sham group ($P < 0.05$). Figure 6 demonstrated the level of MT1-MMP expression was also attenuated by Ovx. During 1–4 weeks after Ovx, MT1-MMP was significantly lower than that of sham group. However, no significant changes in MT1-MMP were observed during 6–12 weeks after Ovx. Compared with sham group, the levels of TIMP-2 in Ovx rats significantly decreased at 4 weeks after Ovx ($P < 0.05$). No significant changes were observed in other time points after Ovx [Figure 7].

DISCUSSION

In the present study, we demonstrated that long-term estrogen deficiency caused by Ovx resulted in elevated blood pressure and reduced endothelium-dependent vasodilatation as well as remodeling of mesenteric arteries evidenced by increased collagen deposition and diminished MMP-2 expression. These results suggested that estrogen modulated MMPs activity to facilitate collagen turnover in small arteries that might be associated with postmenopausal hypertension.

Abnormalities of resistance arteries may play a pivotal role in the pathogenesis and pathophysiology of hypertension. Remodeling of resistance arteries results from the deposition of collagen and other components of the

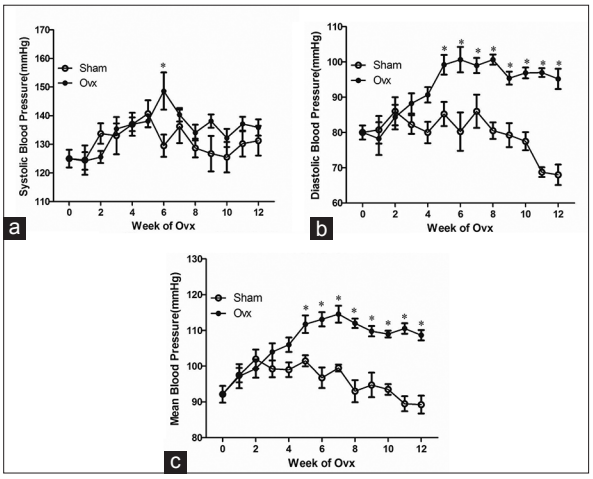

Figure 2: Time-course changes of (a) systolic blood pressure, (b) diastolic blood pressure, and (c) mean blood pressure were measured by the tail-cuff method in conscious rats after ovariectomy (1–12 weeks). Data are given as mean ± standard error of the mean (ovariectomy, $n = 20$; Sham, $n = 8$). *$P < 0.05$ versus sham

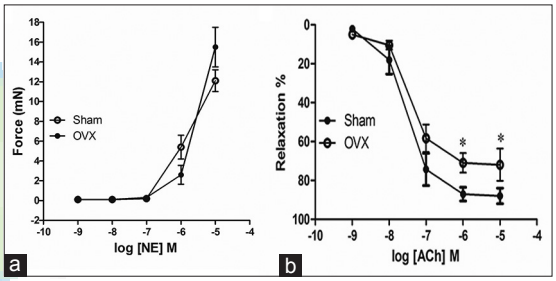

Figure 3: Concentration-responsive curves of NE (1–10 μM)-induced force (a) and acetylcholine (1–10 μM)-induced relaxation (b) in the mesenteric arteries at 9 weeks after ovariectomy. Data are given as mean ± standard error of the mean ($n = 5$). *$P < 0.05$ versus sham

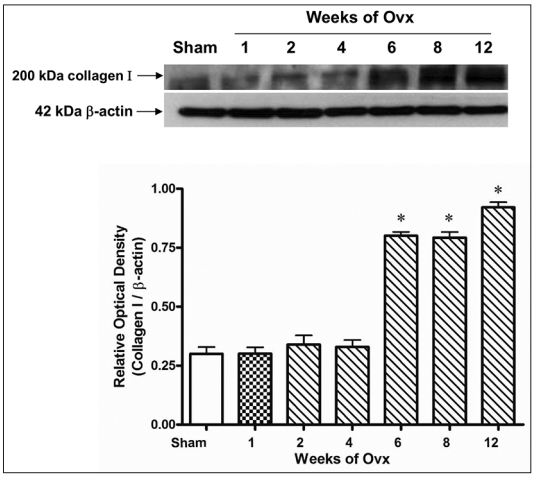

Figure 4: Time-course changes in collagen type I protein expression in the mesenteric arteries after ovariectomy in rats. Data are given as mean ± standard error of the mean ($n = 3$ for each time point). *$P < 0.05$ versus sham

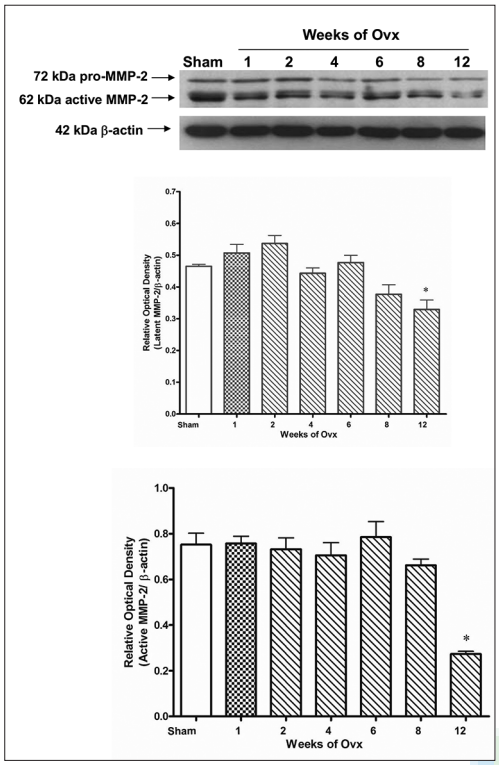

Figure 5: Time-course changes in latent and active matrix metalloproteinase-2 protein expression in the mesenteric arteries after ovariectomy in rats. Data are given as mean ± standard error of the mean ($n = 3$ for each time point). *$P < 0.05$ versus sham. matrix metalloproteinase-2, matrix metalloproteinase-2

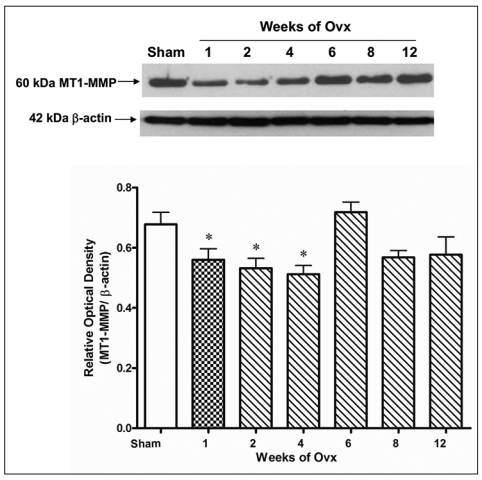

Figure 6: Time-course changes in membrane type 1-matrix metalloproteinase protein expression in the mesenteric arteries 1–12 weeks after ovariectomy in rats. Data are given as mean ± standard error of the mean ($n = 3$ for each time point). *$P < 0.05$ versus sham. membrane type 1-matrix metalloproteinase, membrane type-1-matrix metalloproteinase

ECM which contributes to the alterations of arterial structure and impairments of endothelium-dependent relaxation, leading to the development of hypertension.[22,23] In essential hypertension, peripheral vascular resistance is increased due to abnormal vascular reactivity, and decreased lumen diameter may originate from functional and structural changes of blood vessels.[8] The vasculature is the site of critical changes with estrogen loss, including increased atherosclerosis and abnormal function (relaxation and constriction). Estrogen has key actions in the vasculature enhancing relaxation through upregulation of NO production.[24] In this study, ACh-induced vasodilation was damaged 9 weeks after Ovx, indicating the NO production by endothelium of mesentery artery was attenuated, which might be a consequence of high blood pressure elicited by chronic estrogen deficiency. Furthermore, reduced endothelium-dependent vasodilation in mesenteric artery caused by Ovx can result from decreased endothelium-dependent hyperpolarization.[25] Therefore, the prevalence of hypertension could be related to endothelial dysfunction caused by endogenous estrogen deficiency.

Besides vascular endothelial dysfunction, alterations of ECM composition in small arteries are involved in the elevated cardiovascular morbidity and mortality associated with hypertension.[26] Progressive increases in collagen deposition may enhance the stiffness of wall components, and result in narrowing of small arteries and decreasing compliance. MMPs have long been recognized as key regulators of tissue remodeling in a variety of pathophysiological conditions of hypertension, due to their ability to degrade ECM.[27,28] In this study, collagen type I deposition occurred at 6 weeks and lasted to 12 weeks after Ovx, but the reduction of MMP-2 activity occurred till 12 weeks. This result implicates that besides MMP-2, there are other types of MMPs involved in collagen type I degradation, which may lose function earlier than MMP-2. MMP-9 (known as gelatinase B) shares the structural similarity with MMP-2 and has been widely investigated the ability to degrade native collagen types I and IV.[29] The absence of MMP-9 activity results in collagen accumulation, vessel stiffness as well as increased pulse pressure, eventually leading to the progression of hypertensive vascular disease *in vivo*.[30] Estrogen treatment increased the expression and activity of MMP-9 in in mesangial cells isolated from glomerulosclerosis-resistant mice, predominantly through estrogen receptor (ER) α activation.[31] Hence, we suggest that MMP-9 activity reduced after Ovx, which may participate in the collagen deposition in this experiment. MMP-2 is the most constitutively expressed enzyme by vascular cells which plays a key role in the regulation of vascular remodeling and contributes to the decreased vessel thickness and increased arterial compliance.[32] Altered MMP-2 activity in the mesenteric artery of estrogen-deficient rodent is believed to drive the chronic structural remodeling associated with hypertensive

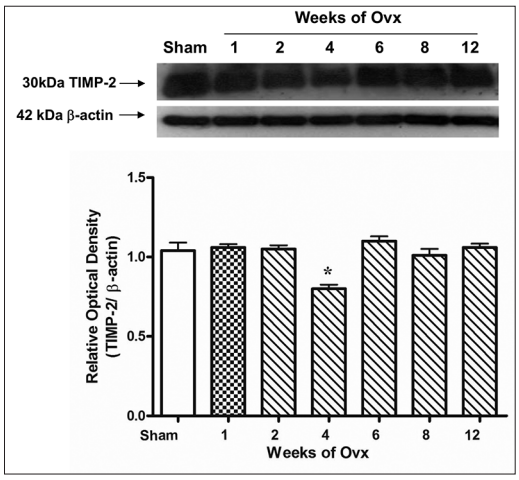

Figure 7: Time-course changes in tissue inhibitor of metalloproteinase-2 protein expression in the mesenteric arteries 1–12 weeks after ovariectomy in rats. Data are given as mean ± standard error of the mean ($n = 3$ for each time point). *$P < 0.05$ versus sham. tissue inhibitor of metalloproteinase-2, tissue inhibitor of metalloproteinase-2

vascular disease.[33] It has been shown that mesenteric MMP-2 activity was downregulated in aged rats while estrogen replacement reversed MMP-2 activity, accompanying with decreased arterial wall thickness.[34] Evidence has also showed MMP-2 activity was inhibited in mesenteric artery from adult SHR, leading to accumulation of collagen and fibronectin.[13] Estrogen has been found to decrease collagen synthesis and increase MMP-2 release from vascular smooth muscle cells in a dose-dependent manner.[35] As an endogenous antioxidant, estrogen possesses genomic and nongenomic regulation to reduce oxidative stress on the cardiovascular system through ER-dependent and -independent mechanisms. Estrogen stimulates MMP-2 activity in a dose-dependent manner which is mediated by upregulation of the transcription factor activator protein-2 through extracellular signal-regulated kinase/mitogen-activated protein kinase signaling cascade.[36] These findings support the evidence that estrogen plays an important role on maintaining vascular integrity.

MT1-MMP mediates cell surface activation of proMMP-2 and possesses collagenase activity on type I, II, and III collagens.[37] By forming a trimolecular complex with MMP-2 and TIMP-2, MT-1MMP plays a crucial role in the activation of pro-MMP. TIMP-2 has dual functions of activating pro-MMP-2 and when in excess it also inhibits active MMP-2.[38,39] The present results show that estrogen deficiency causes reduction of MT1-MMP protein expression in early phase (1–4 weeks after Ovx), accompanied a normal level of active form MMP-2 and collagen type I, suggesting the level of MT1-MMP in this period is still enough to activate MMP-2 to prevent collagen accumulation. In the late phase (6–12 weeks after Ovx), MT1-MMP rebounds to normal levels, but the collagen deposition still not be prevented. Because MT1-MMP also can degrade type I collagen, this indicates that MT1-MMP may not play an important role in the prevention of vascular remodeling in small arteries in estrogen-deficient status. However, in aorta, collagen deposition, reduction of MMP-2 and elevation of MT1-MMP appeared 1 week after Ovx[19] demonstrating different characteristics in conduct arteries and small arteries.

Oxidative stress increases in postmenopausal women[40] and Ovx rats.[41] Our previous report revealed superoxide anion formation in the aortas of Ovx rats is significantly higher than that of age-matched sham-operated rats.[42] Long-term treatment of estrogen and tempol (an antioxidant) significantly reduced the accumulation of collagen type I and reversed the reduction of active MMP-2 protein in aorta of Ovx rats.[19] Based on the evidence, increased oxidative stress caused by estrogen deficiency may also diminish MMPs activities leading to remodeling of small arteries.

CONCLUSION

Long-term estrogen deficiency results in a shift in ECM profiles and diminished MMPs activities, leading to remodeling of small arteries that are associated with postmenopausal hypertension. Although the mechanisms responsible for increases in blood pressure in postmenopausal women remain to be elucidated. Estrogen deficiency-induced collagen accumulation and impaired MMP-2 activation in resistance arteries may play a crucial role for postmenopausal hypertension. Our study hence unravels the time-course modulation of MMP-2, MT1-MMP, and TIMP-2 expression in small arteries and provides new insight into the pathophysiology of vascular remodeling in estrogen-deficient conditions. For clinical perspective, hormone replacement therapy may ameliorate the arterial stiffness by an increase in MMP-2 activity to prevent postmenopausal hypertension.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. JAMA 1991;265:1861-7.
2. Bittner V. Menopause, age, and cardiovascular risk: A complex relationship. J Am Coll Cardiol 2009;54:2374-5.

3. Reckelhoff JF, Maric C. Sex and gender differences in cardiovascular-renal physiology and pathophysiology. Steroids 2010;75:745-6.
4. Taddei S, Virdis A, Ghiadoni L, Mattei P, Sudano I, Bernini G, *et al.* Menopause is associated with endothelial dysfunction in women. Hypertension 1996;28:576-82.
5. Laurent S, Lacolley P, Girerd X, Boutouyrie P, Bezie Y, Safar M. Arterial stiffening: Opposing effects of age- and hypertension-associated structural changes. Can J Physiol Pharmacol 1996;74:842-9.
6. Natoli AK, Medley TL, Ahimastos AA, Drew BG, Thearle DJ, Dilley RJ, *et al.* Sex steroids modulate human aortic smooth muscle cell matrix protein deposition and matrix metalloproteinase expression. Hypertension 2005;46:1129-34.
7. Hinojosa-Laborde C, Craig T, Zheng W, Ji H, Haywood JR, Sandberg K. Ovariectomy augments hypertension in aging female Dahl salt-sensitive rats. Hypertension 2004;44:405-9.
8. Intengan HD, Schiffrin EL. Structure and mechanical properties of resistance arteries in hypertension: Role of adhesion molecules and extracellular matrix determinants. Hypertension 2000;36:312-8.
9. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. Circ Res 2003;92:827-39.
10. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: The good, the bad, and the ugly. Circ Res 2002;90:251-62.
11. Woessner JF Jr. The family of matrix metalloproteinases. Ann N Y Acad Sci 1994;732:11-21.
12. Derosa G, D'Angelo A, Ciccarelli L, Piccinni MN, Pricolo F, Salvadeo S, *et al.* Matrix metalloproteinase-2, -9, and tissue inhibitor of metalloproteinase-1 in patients with hypertension. Endothelium 2006;13:227-31.
13. Kim S, Ohta K, Hamaguchi A, Omura T, Yukimura T, Miura K, *et al.* Angiotensin II type I receptor antagonist inhibits the gene expression of transforming growth factor-beta 1 and extracellular matrix in cardiac and vascular tissues of hypertensive rats. J Pharmacol Exp Ther 1995;273:509-15.
14. Laurent S, Boutouyrie P. The structural factor of hypertension: Large and small artery alterations. Circ Res 2015;116:1007-21.
15. Ellerbroek SM, Wu YI, Overall CM, Stack MS. Functional interplay between type I collagen and cell surface matrix metalloproteinase activity. J Biol Chem 2001;276:24833-42.
16. Zervoudaki A, Economou E, Pitsavos C, Vasiliadou K, Aggeli C, Tsioufis K, *et al.* The effect of Ca2+channel antagonists on plasma concentrations of matrix metalloproteinase-2 and -9 in essential hypertension. Am J Hypertens 2004;17:273-6.
17. Lindsey ML, Goshorn DK, Squires CE, Escobar GP, Hendrick JW, Mingoia JT, *et al.* Age-dependent changes in myocardial matrix metalloproteinase/tissue inhibitor of metalloproteinase profiles and fibroblast function. Cardiovasc Res 2005;66:410-9.
18. Xu Y, Arenas IA, Armstrong SJ, Davidge ST. Estrogen modulation of left ventricular remodeling in the aged heart. Cardiovasc Res 2003;57:388-94.
19. Lam KK, Cheng PY, Hsiao G, Chen SY, Shen HH, Yen MH, *et al.* Estrogen deficiency-induced alterations of vascular MMP-2, MT1-MMP, and TIMP-2 in ovariectomized rats. Am J Hypertens 2009;22:27-34.
20. Chen SJ, Wu CC, Yen MH. Exercise training activates large-conductance calcium-activated K(+) channels and enhances nitric oxide production in rat mesenteric artery and thoracic aorta. J Biomed Sci 2001;8:248-55.
21. Mayne R. Collagenous proteins of blood vessels. Arteriosclerosis 1986;6:585-93.
22. Intengan HD, Schiffrin EL. Vascular remodeling in hypertension: Roles of apoptosis, inflammation, and fibrosis. Hypertension 2001;38:581-7.
23. Schiffrin EL. Reactivity of small blood vessels in hypertension: Relation with structural changes. State of the art lecture. Hypertension 1992;19 2 Suppl: II1-9.
24. Knowlton AA, Lee AR. Estrogen and the cardiovascular system. Pharmacol Ther 2012;135:54-70.
25. Yap FC, Taylor MS, Lin MT. Ovariectomy-induced reductions in endothelial SK3 channel activity and endothelium-dependent vasorelaxation in murine mesenteric arteries. PLoS One 2014;9:e104686.
26. Tran ED, Yang M, Chen A, Delano FA, Murfee WL, Schmid-Schönbein GW. Matrix metalloproteinase activity causes VEGFR-2 cleavage and microvascular rarefaction in rat mesentery. Microcirculation 2011;18:228-37.
27. Rizzoni D, Paiardi S, Rodella L, Porteri E, De Ciuceis C, Rezzani R, *et al.* Changes in extracellular matrix in subcutaneous small resistance arteries of patients with primary aldosteronism. J Clin Endocrinol Metab 2006;91:2638-42.
28. Briones AM, Xavier FE, Arribas SM, González MC, Rossoni LV, Alonso MJ, *et al.* Alterations in structure and mechanics of resistance arteries from ouabain-induced hypertensive rats. Am J Physiol Heart Circ Physiol 2006;291:H193-201.
29. Bigg HF, Rowan AD, Barker MD, Cawston TE. Activity of matrix metalloproteinase-9 against native collagen types I and III. FEBS J 2007;274:1246-55.
30. Flamant M, Placier S, Dubroca C, Esposito B,

Lopes I, Chatziantoniou C, *et al.* Role of matrix metalloproteinases in early hypertensive vascular remodeling. Hypertension 2007;50:212-8.
31. Suzuki h, kondo k. Chronic kidney disease in postmenopausal women. Hypertens res 2012;35:142-7.
32. Ponticos M, Smith BD. Extracellular matrix synthesis in vascular disease: Hypertension, and atherosclerosis. J Biomed Res 2014;28:25-39.
33. Lekontseva O, Jiang Y, Davidge ST. Estrogen replacement increases matrix metalloproteinase contribution to vasoconstriction in a rat model of menopause. J Hypertens 2009;27:1602-8.
34. Zhang Y, Stewart KG, Davidge ST. Estrogen replacement reduces age-associated remodeling in rat mesenteric arteries. Hypertension 2000;36:970-4.
35. Wingrove CS, Garr E, Godsland IF, Stevenson JC. 17beta-oestradiol enhances release of matrix metalloproteinase-2 from human vascular smooth muscle cells. Biochim Biophys Acta 1998;1406:169-74.
36. Guccione M, Silbiger S, Lei J, Neugarten J. Estradiol upregulates mesangial cell MMP-2 activity via the transcription factor AP-2. Am J Physiol Renal Physiol 2002;282:F164-9.
37. Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y. Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. J Biol Chem 1997;272:2446-51.
38. Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. J Biol Chem 1995;270:5331-8.
39. Butler GS, Butler MJ, Atkinson SJ, Will H, Tamura T, Schade van Westrum S, *et al.* The TIMP2 membrane type 1 metalloproteinase "receptor" regulates the concentration and efficient activation of progelatinase A. A kinetic study. J Biol Chem 1998;273:871-80.
40. Reckelhoff JF. Gender differences in the regulation of blood pressure. Hypertension 2001;37:1199-208.
41. Lee YM, Cheng PY, Hong SF, Chen SY, Lam KK, Sheu JR, *et al.* Oxidative stress induces vascular heme oxygenase-1 expression in ovariectomized rats. Free Radic Biol Med 2005;39:108-17.
42. Lam KK, Ho ST, Yen MH. Tetrahydrobiopterin improves vascular endothelial function in ovariectomized rats. J Biomed Sci 2002;9:119-25.