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Preventive effects of raloxifene, a selective estrogen receptor modulator, on monocrotaline-induced pulmonary hypertension in intact and ovariectomized female rats

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ABSTRACT

We investigated whether the chronic treatment with raloxifene, a selective estrogen receptor modulator, prevents the development of monocrotaline-induced pulmonary hypertension in ovary-intact and ovariectomized female rats. Four weeks after a single subcutaneous injection of monocrotaline (60 mg/kg), right ventricular systolic pressure, right ventricle-to-left ventricle plus septal weight ratio, pulmonary arterial medial thickening and endothelin-1 levels in right ventricular tissue increased significantly in both female rats, compared with saline-treated control rats. These monocrotaline-induced alterations were much greater in ovariectomized rats than the changes in intact females. Daily oral administration of raloxifene (10 mg/kg/day for 4 weeks) significantly attenuated the increase in right ventricular systolic pressure to the same levels in both groups of animals, but raloxifene suppressed the increases in right ventricle-to-left ventricle plus septal weight ratio and pulmonary arterial medial thickness more efficiently in ovariectomized females than the case with intact females. In addition, raloxifene completely suppressed the increase in right ventricular endothelin-1 levels in ovariectomized rats, but not in intact females. These data suggest that chronic treatment with raloxifene effectively prevents the development of monocrotaline-induced pulmonary hypertension in ovariectomized female rats than in intact females, at least in part, by suppressing right ventricular endothelin-1 overproduction.

1. Introduction

Pulmonary hypertension is characterized by elevated pulmonary arterial pressure, pulmonary arterial remodeling, and right ventricular hypertrophy (Simonneau et al., 2004). Although estrogen seems to play an important role in the progression of various pulmonary diseases such as acute lung injury and chronic obstructive pulmonary disease, the role of estrogen in the pathogenesis and progression of pulmonary hypertension is controversial (Scorza et al., 2002; Simonneau et al., 2004; Beretta et al., 2006; De Marco, 2006; Lahm et al., 2008). The incidence of idiopathic pulmonary hypertension is twice as more frequent in young women than age-matched men, but the reason for this predominance remains unclear (De Marco, 2006). In contrast, postmenopausal women have an increased risk for the development of pulmonary hypertension that is associated with scleroderma; and hormone replacement therapy may prevent the development of pulmonary hypertension in these patients (Scorza et al., 2002; Beretta et al., 2006). However, experimental studies using a chronic hypoxia- or monocrotaline-induced pulmonary hypertension model have consistently demonstrated that endogenous and exogenous administration of estrogen or its metabolites exert protective effects on the progression of pulmonary hypertension, pulmonary arterial remodeling, and right ventricular hypertrophy (Goldenthal et al., 1964; Rabinovitch et al., 1981; Farhat et al., 1993; Kasahara et al., 1997; Resta et al., 2001; Ahn et al., 2003; Packer et al., 2003).

Raloxifene, a selective estrogen receptor modulator, has estrogen agonistic properties towards the bone and the cardiovascular system, but it exerts estrogen antagonistic actions in the breast and uterus (Black et al., 1994; Leung et al., 2007). Clinical and animal studies suggest that raloxifene, as well as estrogen, has multiple cardiovascular effects (Leung et al., 2007). A daily administration of raloxifene improves endothelial function by increasing plasma nitric oxide (NO) concentrations and decreasing endothelin-1 levels in healthy postmenopausal women (Saitta et al., 2001). In rat isolated vessel preparations, raloxifene acutely relaxes pulmonary arteries and veins (Chan et al., 2005). Chronic treatment with raloxifene to spontaneously hypertensive rats improves pulmonary vascular function by

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increasing basal NO release, reducing vascular smooth muscle tone, and improving the effect of NO on vascular smooth muscle (Chan et al., 2007). A chronic 4-week administration of raloxifene to transverse aortic-banded mice significantly prevents cardiac hypertrophy and dysfunction (Ogita et al., 2004); however, it remains to be determined whether chronic treatment with raloxifene exerts protective effects on the progression of pulmonary hypertension and right ventricular hypertrophy.

Endothelin-1 plays an important role in the progression of pulmonary hypertension, and both selective endothelin ET_A and nonselective endothelin ET_A/ET_B receptor antagonists are currently available for the treatment of pulmonary hypertension in clinical level. In monocrotaline-treated rat pulmonary hypertension models, endothelin-1 mRNA expression and endothelin-1 peptide levels are elevated in right ventricule as well as in plasma (Miyauchi et al., 1993; Jasmin et al., 2003; Nishida et al., 2004b). Elevated endothelin-1 levels in right ventricular tissue are considered to be induced mainly by pressure overload to the heart rather than the primary causal factor of pulmonary hypertension (Miyauchi et al., 1993); however, since endothelin-1 is a potent growth factor of myocardial cells (Shubeita et al., 1990), excess endothelin-1 expression in right ventricular tissue seems to promote the progression of right ventricular hypertrophy in pulmonary hypertension. In fact, endothelin ET_A receptor antagonism or endothelin ET_A/ET_B receptor antagonism efficiently attenuated the progression of right ventricular hypertrophy and dysfunction in monocrotaline-induced pulmonary hypertensive rats (Ichikawa et al., 1996; Hill et al., 1997; Jasmin et al., 2003; Nishida et al., 2004a). Although raloxifene has been reported to decrease the plasma endothelin-1 concentration in postmenopausal women (Saitta et al., 2001), little is known about the effect of chronic raloxifene treatment on the endothelin-1 expression in the cardiac tissue.

In the present study, we investigated whether chronic treatment with raloxifene prevents the development of monocrotaline-induced pulmonary hypertension, pulmonary arterial remodeling, and right ventricular hypertrophy using ovary-intact and ovariectomized female rats. Furthermore, we examined the effects of raloxifene on the right ventricular endothelin-1 level, which is one of the causal factors for cardiac hypertrophy and dysfunction in pulmonary hypertension.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats (160–180 g, 7 weeks old, Japan SLC, Shizuoka, Japan) were used in this study. The animals were housed in a light-controlled room with a 12-hour light/dark cycle and were allowed access to food and water ad libitum. The animals were maintained at the departmental Animal Care Facility of the Osaka University of Pharmaceutical Sciences in accordance with the recommendations of the Declaration of Helsinki. Experimental protocols and animal care methods were approved by the Experimental Animal Research Committee of the Osaka University of Pharmaceutical Sciences. Female rats were randomly divided into the following 6 groups: (1) sham-operated saline-treated with vehicle (intact-control, $n=9$), (2) sham-operated monocrotaline-treated with vehicle (intact-monocrotaline + vehicle, $n=9$), (3) sham-operated monocrotaline-treated with raloxifene (intact-monocrotaline + raloxifene, $n=9$), (4) ovariectomized saline-treated with vehicle (ovariectomized control, $n=8$), (5) ovariectomized monocrotaline-treated with vehicle (ovariectomized monocrotaline + vehicle, $n=9$), and (6) ovariectomized monocrotaline-treated with raloxifene (ovariectomized monocrotaline + raloxifene, $n=7$). A bilateral ovariectomy or sham operation was performed under sodium pentobarbital (40 mg/kg, i.p.) anesthesia. After a 1-week recovery period, animals received a single subcutaneous injection of monocrotaline (60 mg/kg) or saline at same volume as the monocrotaline. Monocrotaline-treated animals were gavaged once a day with raloxifene (10 mg/kg/day) (Wassmann et al., 2002) or vehicle (20% hydroxypropyl-β-cyclodextrin in distilled water) for 4 weeks starting on the day of monocrotaline injection.

2.2. Experimental protocol

Four weeks after the injection of monocrotaline or saline, each rat was artificially ventilated under anesthesia with sodium pentobarbital (40 mg/kg, i.p.). A polyethylene catheter, connected to a pressure transducer was inserted into the right carotid artery to measure mean arterial blood pressure recorded by a polygraph system (RM 6000, Nihon Koden, Tokyo, Japan). Another polyethylene catheter was inserted into the right jugular vein to measure right ventricular systolic pressure. The heart and lungs were excised, weighed, and used for morphometric analysis. A portion of right ventricle was frozen separately to determine the endothelin-1 content.

2.3. Histologic studies

Excised left lungs were processed for light microscopic observation, according to standard procedures (Nishida et al., 2004a). The lungs were then preserved in phosphate-buffered 10% formalin, chopped into small pieces, embedded in paraffin, cut into 3-μm slices, and stained using the Elastica-van-Gieson technique. The pulmonary arteries were identified as vessels with two clearly defined elastic laminae, with a layer of smooth muscle cells between the two laminae. The external diameter and medial wall thickness were measured for 15 to 20 muscular arteries (in size ranges of 50–100 and 100–150 μm in external diameter) per lung section at ×400 magnification. For each artery, the percent wall thickness was calculated by using the following formula: percent wall thickness = (medial thickness × 2) / (external diameter) × 100. The wall thickness was determined by using an image analyzer (AE-6905C, ATTO, Tokyo, Japan). One lung section was obtained from one rat, values from 15 to 20 arteries were determined, and the average was calculated. Evaluations were made in a blind manner.

2.4. Endothelin-1 measurement

Endothelin-1 was extracted from the right ventricle, as described elsewhere (Nishida et al., 2004b). Briefly, right ventricular tissue was weighed and homogenized for 60 s in 4 ml of ice-cold organic solution (chloroform/methanol, 2:1, including 1 mM N-ethylmaleimide). The homogenates were left overnight at 4 °C and then 0.4 ml of 0.09% trifluoroacetic acid was added to the homogenates. Homogenates were centrifuged at 3000 rpm for 30 min and the supernatant was stored. Aliquots of the supernatant were diluted 1/10 with a 0.09% trifluoroacetic acid solution and applied to Sep-Pak C18 cartridges. The samples were eluted with 3 ml of 63.3% acetonitrile and 0.1% trifluoroacetic acid. Eluates were dried in a centrifugal concentrator, and the dried residue was reconstituted in assay buffer for radioimmunoassay. The clear solution was subjected to radioimmunoassay. The recovery of endothelin-1 was approximately 80%. Radioimmunoassay for tissue endothelin-1 was performed, as described elsewhere (Fujita et al., 1995), using endothelin-1 antiserum (a generous gift from Dr. Marvin R. Brown, Department of Medicine, University of California, San Diego, CA, USA). This serum does not cross-react with big endothelin-1 (Matsumura et al., 1990).

2.5. Drugs

Monocrotaline was obtained from Sigma Chemicals (St. Louis, MO, USA). Raloxifene was purchased from LKT Laboratories, Inc. (St. Paul,

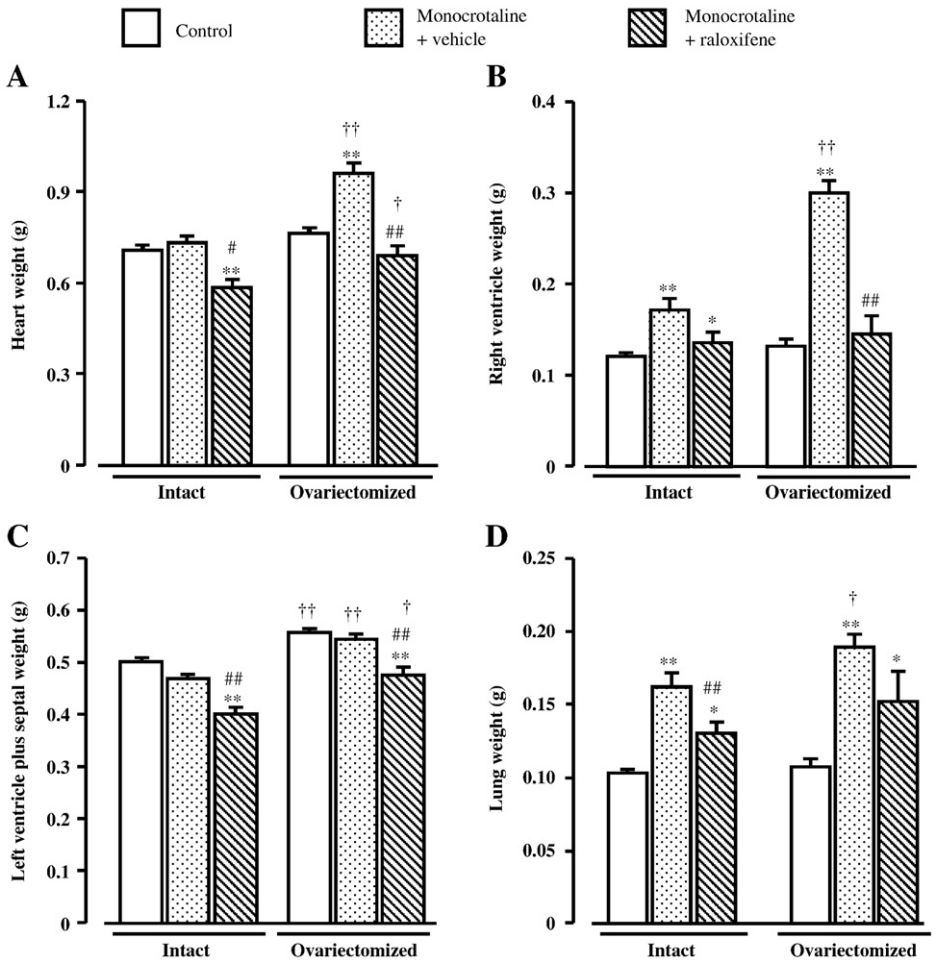

Fig. 1. Effects of raloxifene on the heart (A), right ventricle (B), left ventricle plus septal (C), and lung weight (D) in monocrotaline-treated intact and ovariectomized female rats 4 weeks after the monocrotaline injection. Each column and bar represent the mean ± S.E.M. ($n = 7–9$). *$P<0.05$, **$P<0.01$, compared with corresponding control rats; #$P<0.05$, ##$P<0.01$, compared with corresponding monocrotaline rats; ††$P<0.01$, compared with corresponding intact female rats.

MN, USA). All other chemicals were purchased from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical (Osaka, Japan).

2.6. Statistical analysis

Each value represents the mean ± S.E.M. Comparisons among groups of intact and ovariectomized female rats were performed with a one-way ANOVA followed by Tukey–Kramer post-hoc test. Differences between intact and ovariectomized female rats were compared using unpaired Student's *t* test. Differences were considered significant at $P<0.05$.

3. Results

3.1. Body, heart, and lung weight, and systemic hemodynamics

Results of the body, heart, lung weights and systemic hemodynamics are shown in Fig. 1 and Table 1. Body weight gain in saline-treated ovariectomized female rats was significantly greater than that in ovary-intact female rats. There were no significant differences in mean arterial pressure and the ratio of heart weight-, right ventricular weight-, and left ventricle and septal weight-, and lung weight-to-body weight between intact and ovariectomized control females, but

Table 1
Comparative data on body, heart, and lung weight, and systemic hemodynamics.

	Intact female			Ovariectomized female		
	Control ($n=9$)	Monocrotaline + vehicle ($n=9$)	Monocrotaline + raloxifene ($n=9$)	Control ($n=8$)	Monocrotaline + vehicle ($n=9$)	Monocrotaline + raloxifene ($n=7$)
Body weight	242 ± 7	218 ± 2	198 ± 10[b]	276 ± 7[f]	237 ± 9[b]	221 ± 5[b]
Hear rate (bpm)	434 ± 8	443 ± 8	414 ± 24	464 ± 12[e]	377 ± 14[b]	410 ± 15[a]
MAP (mm Hg)	130 ± 5	116 ± 4	125 ± 9	129 ± 4	114 ± 4[a]	127 ± 3[c]
HW/BW (mg/g)	2.94 ± 0.06	3.37 ± 0.09[b]	2.96 ± 0.10[d]	2.77 ± 0.05	4.07 ± 0.10[bf]	3.11 ± 0.10[ad]
RVW/BW (mg/g)	0.50 ± 0.02	0.78 ± 0.05[b]	0.69 ± 0.05[b]	0.48 ± 0.02	1.27 ± 0.07[bf]	0.64 ± 0.08[d]
(LV+S)W/BW (mg/g)	2.06 ± 0.06	2.14 ± 0.04	2.03 ± 0.04	2.01 ± 0.03	2.29 ± 0.07[b]	2.13 ± 0.06
LungW/BW (mg/g)	0.43 ± 0.01	0.74 ± 0.04[b]	0.67 ± 0.05[b]	0.39 ± 0.02	0.81 ± 0.05[b]	0.68 ± 0.08[b]

Each value represents the mean ± S.E.M. [a]$P<0.05$, [b]$P<0.01$, compared with corresponding control rats; [c]$P<0.05$, [d]$P<0.01$, compared with corresponding monocrotaline rats. [e]$P<0.05$, [f]$P<0.01$, compared with corresponding intact females. BW, body weight; MAP, mean arterial pressure; HW, heart weight; RV, right ventricle; LV+S, left ventricle plus septum; LungW, lung weight.

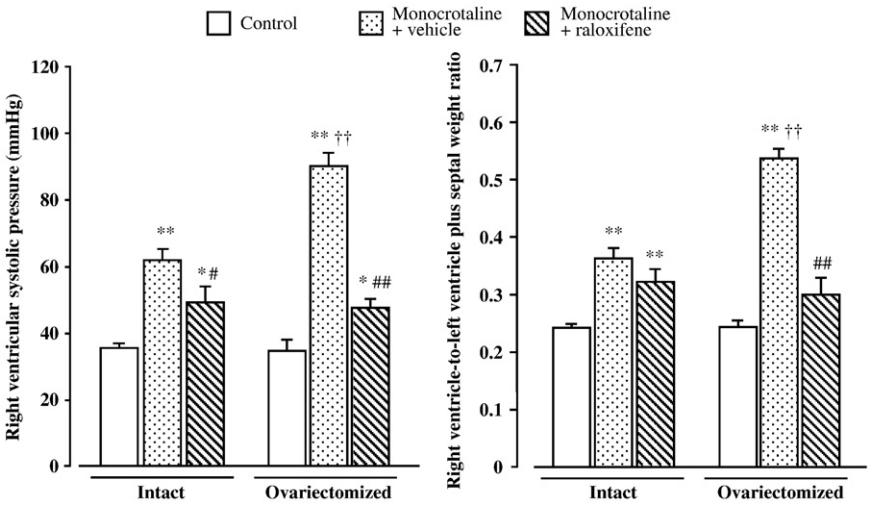

Fig. 2. Effects of raloxifene on right ventricular systolic pressure and right ventricle-to-left ventricle plus septal weight ratio in monocrotaline-treated intact and ovariectomized female rats 4 weeks after the monocrotaline injection. Each column and bar represent the mean ± S.E.M. ($n = 7–9$). *$P<0.05$, **$P<0.01$, compared with corresponding control rats; #$P<0.05$, ##$P<0.01$, compared with corresponding monocrotaline rats; ††$P<0.01$, compared with corresponding intact female rats.

heart rate was higher in ovariectomized rats than in intact females. Monocrotaline treatment significantly decreased mean arterial pressure ($P<0.01$) and heart rate ($P<0.05$) in ovariectomized females compared with control animals. In intact females, monocrotaline treatment did not affect heart rate. In both intact and ovariectomized females, monocrotaline injection produced significant increases in the heart weight- and right ventricular weight-to-body weight ratio, compared with each control animal, but the magnitudes of these increases in ovariectomized females were significantly higher than those in intact females ($P<0.01$). Furthermore, left ventricle plus septal weight-to-body weight ratio was significantly increased in monocrotaline-treated ovariectomized rats, but not in intact females, as compared with that in control animals. These changes in heart weight in ovariectomized females were efficiently suppressed by a daily oral administration of raloxifene ($P<0.01$). In contrast, in intact females, raloxifene treatment significantly suppressed the increase in heart weight-to-body weight ratio, but failed to suppress the right ventricular weight-to-body weight ratio. The lung weight-to-body weight ratio, which is an index of pulmonary edema and fibrosis, increased significantly and similarly in intact and ovariectomized females. Chronic treatment with raloxifene did not significantly suppress the increases in the lung weight-to-body weight ratio. Uterine weight was markedly reduced in ovariectomized females compared with ovary-intact animals, and chronic treatment with raloxifene did not affect the uterine weights (data not shown), as reported by others (Black et al., 1994).

3.2. Right ventricular systolic pressure and right ventricular weight-to-left ventricle plus septal weight ratio

Monocrotaline-induced right ventricular hypertrophy and pulmonary hypertension were further evaluated by measuring right ventricular systolic pressure and the right ventricle-to-left ventricle plus septal weight ratio. Monocrotaline treatment produced significant increases in right ventricular systolic pressure (intact, 62 ± 4 vs. 35 ± 2 mm Hg; ovariectomized, 90 ± 4 vs. 35 ± 2 mm Hg) and the

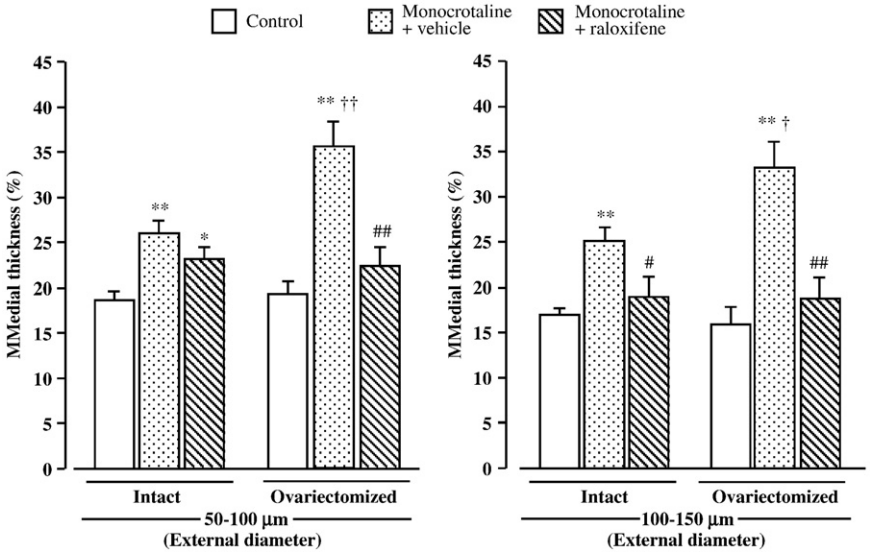

Fig. 3. Effects of raloxifene on the medial wall thickness (%) of small pulmonary arteries in monocrotaline-treated intact and ovariectomized female rats 4 weeks after the monocrotaline injection. Each column and bar represent the mean ± S.E.M. ($n = 7–9$). *$P<0.05$, **$P<0.01$, compared with corresponding control rats; #$P<0.05$, ##$P<0.01$, compared with corresponding monocrotaline rats; †$P<0.05$, ††$P<0.01$, compared with corresponding intact female rats.

right ventricle-to-left ventricle plus septal weight ratio (intact, 0.36 ± 0.02 vs. 0.24 ± 0.01; ovariectomized, 0.54 ± 0.02 vs. 0.24 ± 0.01) in both intact and ovariectomized female rats, compared with saline-treated control rats (Fig. 2; $P<0.01$, respectively). These monocrotaline-induced changes in right ventricular systolic pressure and the right ventricle-to-left ventricle plus septal weight ratio were greater in ovariectomized females than in intact females ($P<0.01$). The daily administration of raloxifene for 4 weeks significantly attenuated the increases in right ventricular systolic pressure to the same levels in both animals (intact, 49 ± 4 mm Hg; ovariectomized, 47 ± 3 mm Hg). In contrast, raloxifene tended to attenuate the monocrotaline-induced increase in right ventricle-to-left ventricle plus septal weight ratio (0.34 ± 0.02) in intact female rats, whereas the increase in right ventricle-to-left ventricle plus septal weight ratio was efficiently suppressed by raloxifene in ovariectomized female rats to the same level as in control animals (0.30 ± 0.03, $P<0.01$).

3.3. Lung vascular morphology

The lung vascular morphology of monocrotaline-treated intact and ovariectomized female rats revealed significant increases in the medial thickness of pulmonary arteries with diameters that ranged from 50 to 100 μm and 100 to 150 μm compared with the lungs of saline-treated control animals. The monocrotaline-induced increases in pulmonary medial thickness were greater in ovariectomized females than those in intact animals ($P<0.05$). Monocrotaline induced an increase in the medial thickness of the pulmonary arteries with diameters that ranged from 50 to 100 μm that was significantly suppressed by raloxifene treatment in ovariectomized females ($P<0.01$), but not in intact females; however, the increased medial thickness in the intact females administered raloxifene was still statistically significant compared with control animals ($P<0.05$). In contrast, raloxifene efficiently suppressed the increase in medial thickness of the pulmonary arteries with diameters between 100 and 150 μm in both intact and ovariectomized animals to the same levels as the control animals (Fig. 3).

3.4. Right ventricular endothelin-1 levels

Endothelin-1 levels in the right ventricular tissues are shown in Fig. 4. Four weeks after the monocrotaline injection, there were marked increases in right ventricular endothelin-1 levels in both intact and ovariectomized female rats, and the levels in ovariectomized rats were significantly higher than those in intact females ($P<0.05$). Daily administration of raloxifene for 4 weeks completely suppressed the increase in right ventricular endothelin-1 levels of ovariectomized female rats, however, treatment of intact female monocrotaline rats with raloxifene resulted in only slight and non-significant decreases in right ventricular endothelin-1 levels. When compared with monocrotaline-treated intact animals that received raloxifene, raloxifene-treated ovariectomized females showed lower levels of right ventricular endothelin-1 ($P<0.05$).

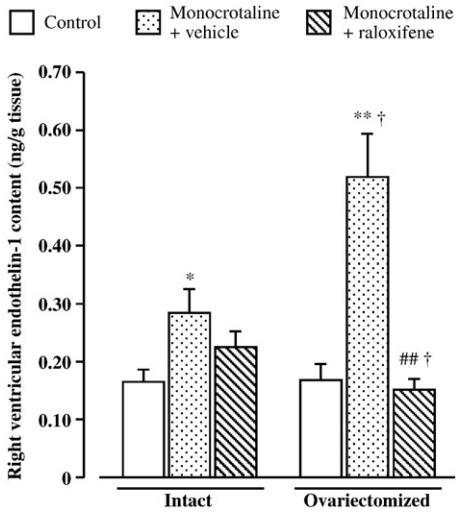

Fig. 4. Effects of raloxifene on right ventricular endothelin-1 content of monocrotaline-treated intact and ovariectomized female rats 4 weeks after the monocrotaline injection. Each column and bar represent the mean ± S.E.M. ($n=7$–9). $^{*}P<0.05$, $^{**}P<0.01$, compared with corresponding control rats; $^{\#\#}P<0.01$, compared with corresponding monocrotaline rats; $^{\dagger}P<0.05$, compared with corresponding intact female rats.

4. Discussion

Ovariectomized female rats showed exaggerated increases in right ventricular systolic pressure, pulmonary arterial medial thickness, and right ventricular hypertrophy 4 weeks after monocrotaline injection compared with ovary-intact female rats. These findings are in agreement with previous studies (Ahn et al., 2003; Packer et al., 2003; Tofovic et al., 2006). In the current study, we originally demonstrated that chronic administration of raloxifene, a selective estrogen modulator, effectively attenuated the progression of monocrotaline-induced pulmonary hypertension in both intact and ovariectomized females. Furthermore, when compared with ovary-intact female rats, raloxifene exhibited beneficial effects on the progression of monocrotaline-induced right ventricular hypertrophy and pulmonary arterial medial thickening more efficiently in ovariectomized and the effect seemed to, at least in part, result from suppression of right ventricular endothelin-1 overproduction.

Although the role of estrogens in the pathogenesis and progression of pulmonary hypertension is controversial (Scorza et al., 2002; Simonneau et al., 2004; Beretta et al., 2006; De Marco, 2006; Lahm et al., 2008), experimental studies using a chronic hypoxia- or monocrotaline-induced pulmonary hypertension model have consistently indicated that estrogens produce the beneficial effects in the progression of pulmonary hypertension (Goldenthal et al., 1964; Rabinovitch et al., 1981; Farhat et al., 1993; Kasahara et al., 1997; Resta et al., 2001; Ahn et al., 2003; Packer et al., 2003). Packer et al. (2003) have demonstrated that acetylcholine-induced vasodilation in pulmonary arterial ring preparation from monocrotaline-treated female rats is greater than that in male rats. Chronic treatment with 17β-estradiol in male rats efficiently attenuated monocrotaline-induced right ventricular hypertrophy and pulmonary vascular remodeling (Farhat et al., 1993). Tofovic et al. (2006) reported that chronic treatment with 2-methoxyestradiol, a major metabolite of estrogen, significantly prevents monocrotaline-induced cardiopulmonary alterations in ovariectomized female rats. Our findings showed that monocrotaline-induced increases in right ventricular systolic pressure, pulmonary arterial medial thickness, right ventricle-to-/(left ventricle and septum) weight ratio, all of which are indices of the development of pulmonary hypertension, were exaggerated in ovariectomized female rats, and these results are in agreement with above reports.

The present study demonstrated that monocrotaline-treated ovariectomized female rats showed a marked elevation of right ventricular endothelin-1 levels compared with the case of intact females. A close relationship between monocrotaline-induced pulmonary hypertension and enhanced right ventricular endothelin-1 production has been shown (Miyauchi et al., 1993; Jasmin et al., 2003; Nishida et al., 2004b). Recent evidence demonstrating that endothelin-1 overexpression is associated with myocardial inflammation and a dilated cardiomyopathy (Yang et al., 2004) and that cardiac-directed suppression of endothelin-1 attenuates thyroxine-induced cardiac hypertrophy (Shohet et al., 2004) suggest the importance of endothelin-1 as a pathological factor in cardiac hypertrophy and dysfunction. Moreover, it has been reported that antagonism of the endothelin ET_A or endothelin ET_A/ET_B receptor efficiently attenuates

the progression of right ventricular hypertrophy and dysfunction in monocrotaline-induced pulmonary hypertensive rats (Ichikawa et al., 1996; Hill et al., 1997; Jasmin et al., 2003; Nishida et al., 2004a). In the present study, ovariectomized female rats showed significant decreases in mean arterial pressure and heart rate 4 weeks after the administration of monocrotaline compared with saline-treated control animals, which may reflect decreased cardiac function. It is well known that estrogen modulates both endothelin-1 gene expression and production (Tostes et al., 2008). However, further studies are required to determine whether estrogen deficiency in ovariectomized female rats directly affects cardiac endothelin-1 levels in monocrotaline-induced pulmonary hypertensive models.

To our knowledge, this is the first report demonstrating that chronic treatment with raloxifene attenuates the progression of monocrotaline-induced pulmonary hypertension in ovary-intact and ovariectomized females. In the present study, precise mechanisms by which raloxifene suppresses the monocrotaline-induced pulmonary hypertension are unclear. However, raloxifene attenuated the progression of monocrotaline-induced right ventricular hypertrophy and pulmonary arterial medial thickening more efficiently in ovariectomized animals compared with ovary-intact female rats. Beneficial effects of raloxifene would be expected to be more pronounced in ovary-intact females than in ovariectomized rats in the light of beneficial effects of endogenous estrogens as described above. The reason for the contradictory results remains unclear, but the differences in selectivity and affinity to estrogen receptor-α and -β between raloxifene and estrogens may explain our findings (Serock et al., 2008). Ball et al. (2009) recently have demonstrated that raloxifene activates estrogen receptor-β more effectively than estrogen receptor-α while 17β-estradiol and tamoxifene modulates both receptors equally. Moreover, they also showed that raloxifene produced opposite effects with estrogen receptor-β to the effects of 17β-estradiol (Ball et al., 2009). Indeed, cardiovascular responses to chronic treatment with 17β-estradiol and raloxifene in ovariectomized animals were not comparable (Zoma et al., 2006). Although further studies using estrogen receptor knockout animals and selective estrogen receptor agonists are necessary to determine the roles of estrogen receptors (Arias-Loza et al., 2008), it is conceivable that endogenous estrogens could not enhance the protective effects of raloxifene. In addition, fluctuation and/or depletion of circulating ovary hormones have been reported to influence expression of estrogen receptors (Jazbutyte et al., 2006; Esqueda et al., 2007; Paquette et al., 2007; Miller and Duckles, 2008; Serock et al., 2008) and vascular responsiveness to estrogens or raloxifene (Crews and Khalil, 1999; Sudhir and Komesaroff, 1999; Shaw et al., 2001; Bracamonte et al., 2002; Lahm et al., 2007). It has been reported that chronic activation of estrogen receptor-β using 8β-VE2, but not estrogen receptor-α, efficiently decreased blood pressure in ovariectomized spontaneously hypertensive rats with increased expression of estrogen receptor-β in aorta (Jazbutyte et al., 2008). On the other hand, it has been reported that Ca^{2+} entry into vascular smooth muscle was enhanced in male and ovariectomized female rats when compared with the case in ovary-intact females and that estrogen inhibited Ca^{2+} influx through voltage-sensitive Ca^{2+} channels more efficiently in the absence of ovary hormone (Crews and Khalil, 1999). In this context, raloxifene-induced relaxations of pulmonary arteries and veins, which are also through inhibiting voltage-sensitive Ca^{2+} channels, were greater in male than the case in female rats (Chan et al., 2005). Thus, the difference in effectiveness of raloxifene may be attributed to the differences in the expression of estrogen receptors and vascular reactivity between ovary-intact and ovariectomized female rats. Although precise mechanisms underlying protective effects of raloxifene in the pulmonary hypertension remain to be elucidated, it is important to note that efficacy of estrogen receptor modulators may be affected by hormonal status considering a broad age range of patients with pulmonary hypertension.

Interestingly, in the present study, effectiveness of raloxifene on right ventricular hypertrophy and endothelin-1 levels in right ventricle tissue was much greater in ovariectomized females than in intact females despite the exaggerated monocrotaline-induced alterations in ovariectomized females. Although alterations in right ventricular endothelin-1 expression can be largely explained by the pressure overload caused by pulmonary hypertension (Miyauchi et al., 1993; Yamazaki et al., 1996), we cannot exclude the possibility that raloxifene directly affected the right ventricular endothelin-1 production independent of hemodynamic changes. Monocrotaline-treated ovariectomized female rats with raloxifene displayed completely suppressed endothelin-1 expression in right ventricle to basal levels in control rats, despite showing moderate but significant increase in right ventricular systolic pressure. We recently demonstrated using cultured pulmonary artery endothelial cells that raloxifene significantly decreases endothelin-1 mRNA expression (Ohkuma et al., 2008). Further studies are required to determine whether raloxifene exerts direct inhibitory effects on endothelin-1 expression in right ventricle tissue. However, considering that an enhanced endothelin-1 level in cardiac tissue leads to further deterioration of cardiac hypertrophy and dysfunction (Shohet et al., 2004; Yang et al., 2004), the efficient suppressive effects on right ventricular endothelin-1 levels may contribute to the effectiveness of raloxifene in ovariectomized female compared with intact female rats.

We cannot rule out the possibility that NO and/or prostacyclin, which are well known as a protective factor against the progression of pulmonary hypertension, may also be involved in the beneficial effects of raloxifene. It has been reported that chronic treatment with raloxifene enhances NO and/or prostacyclin production in endothelial cells (Oviedo et al., 2004; Smith et al., 2006; Chan et al., 2007).

In conclusion, chronic treatment with raloxifene exerts preventive effects on the development of monocrotaline-induced pulmonary hypertension, pulmonary arterial remodeling, and right ventricular hypertrophy more effectively in ovariectomized female rats than in ovary-intact female rats, at least in part, via the suppression of right ventricular endothelin-1 overproduction.

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