

Estrogen and Enalapril attenuate the Development of Right Ventricular Hypertrophy induced by Monocrotaline in Ovariectomized Rats

The present study evaluated the importance of ovarian functions and the renin-angiotensin system in the progression of the right ventricular (RV) hypertrophy. Female Sprague-Dawley rats were bilaterally ovariectomized (Ovx) and injected with monocrotaline (MCT, 60 mg/kg, sc). Four weeks after MCT-treatment, only the male and Ovx female rats showed marked RV hypertrophy. The hypertrophied RV of the male-MCT and Ovx-MCT rats exhibited remarkably elevated renin mRNA levels. Gene expression levels of angiotensinogen, TGF- β_1 , and endothelin-1 in the hypertrophied RV also increased, but to the less degree than did the renin mRNA. To investigate beneficial effects of estrogen or enalapril on progression of the pulmonary hypertension and RV hypertrophy, histological changes of the lung and heart were examined. Sham-MCT female rats showed histological changes indicating pulmonary hypertension without RV hypertrophy. In contrast, Ovx-MCT rats showed marked RV hypertrophy with pathological changes, denoting severe pulmonary and myocardial injuries. Estrogen- or enalapril-treated Ovx-MCT rats did not show RV hypertrophy, and showed remarkably ameliorated ultrastructural changes in the lung and RV. These results from this rat model suggest that both estrogen and inhibition of the renin-angiotensin system have protective functions against the development of the pulmonary hypertension and cardiac remodeling.

Key Words : Sex Difference; Cardiac Remodeling, Ventricular; Hypertension, Pulmonary; Renin-Angiotensin System

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INTRODUCTION

Gender differences in the development of cardiovascular diseases have been documented in both human and animal studies. The rate of incidence of cardiovascular disease is lower in premenopausal women than in men, but increases sharply in postmenopausal women (1). Among the clinical consequences of postmenopausal estrogen deficiency, the deaths associated with cardiovascular diseases represent the largest concern in public health. Many studies have demonstrated that estrogen replacement therapy reduces the risk of cardiovascular disease in postmenopausal women (2). Animal studies also demonstrate gender differences in the development of cardiovascular diseases (3-5). Thus, circulating endogenous estrogen is proposed to protect against cardiovascular disease. However, mechanisms by which estrogen induces its protective effects are not fully understood.

Various vasoactive substances and growth factors have been implicated in cardiac and vascular remodeling, as well as further degenerative transformation of these tissues (6). A critical role of the cardiac renin-angiotensin system (RAS), among others, has been recognized (7, 8). Increased activity of the

cardiac RAS has been confirmed both in humans (9) and animal models of cardiac failure (10, 11), whereas inhibition of the RAS decreased ventricular remodeling and improved cardiac function (12, 13).

Cardiac hypertrophy is known to be one of the most critical risk factors of heart diseases. It has been demonstrated that monocrotaline (MCT) treatment produces pulmonary hypertension and right ventricular (RV) hypertrophy in male rats but not in female rats (4, 14). We previously found enhanced gene expressions of the cardiac RAS in the hypertrophied RV of male rats (15). The present study evaluated 1) the importance of the ovarian function and the RAS in the progression of the pulmonary hypertension, and 2) RV hypertrophy in MCT treated rats. We examined the cardiac expression of genes that contribute to the pathogenesis of cardiac hypertrophy, such as the RAS components, TGF- β_1 , and endothelin-1 as well as histological changes in the lung and heart. Although this MCT rat model has no human equivalent, as a study design, we used a system of MCT-induced cardiopulmonary dysfunction to investigate possible beneficial effects of estrogen and inhibition of angiotensin converting enzyme.

MATERIALS AND METHODS

Ovariectomy and Development of RV Hypertrophy

Thirteen-week-old male and female Sprague-Dawley rats were used. Female rats were randomly divided, and a bilateral ovariectomy or sham operation was performed using pentobarbital anesthesia (40 mg/kg, intraperitoneal, ip). After two weeks, the ovariectomized (Ovx) and the sham-operated female rats, as well as the intact male rats, were given a single subcutaneous injection of 60 mg MCT/kg (Sigma, St. Louis, MO, U.S.A.). MCT was dissolved in phosphate-buffered saline (PBS) and the pH adjusted to 7.4 with 0.5 N HCl. The control rats were injected with saline.

Four weeks after the MCT treatment, all the rats were anesthetized with ether, and the heart and lungs were removed from each rat. The heart was divided into the RV and left ventricle plus septum (LV+S), and each portion was separately weighed. RV samples were rapidly frozen in liquid nitrogen and then stored at -80°C until total RNA isolation. The experiment was carried out following the guidelines for animal care provided by Kyungpook National University in Korea.

RNA Extraction and RT-PCR

Total RNA was extracted according to the method of Chomczynski and Sacchi (16) with slight modifications as described previously (17). Samples of RNA were stored at -80°C as a suspension in 70% ethanol. RNA was spectrophotometrically quantified by measuring the optical density of samples at 260/280 nm.

The nucleotide sequence of the primers were used as previously indicated (15). Total RNA (20 μg) was primed with oligo (dT) primers, and the first strand cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, U.S.A.) in a 50 μL of reaction volume for 90 min at 37°C . PCR cycles were performed in a DNA thermal cycler (PTC-100, M.J. Research, Watertown, MA, U.S.A.) with the following profile: denaturation for 45 sec at 94°C , annealing for 45 sec at 56°C for GAPDH, renin, AT_{1A}, AT_{1B} and TGF- β_1 primers and 1 min extension step at 72°C ; denaturation for 45 sec at 94°C , annealing for 45 sec at 58°C for angiotensinogen primers and 1 min extension step at 72°C ; denaturation for 45 sec at 94°C , annealing for 45 sec at 56°C for ET-1 primers and for 90 sec extension step at 72°C . After the end of the PCR, one-tenth reaction mixture was separated on a 1% agarose gel, containing 0.5 $\mu\text{g}/\text{mL}$ of ethidium bromide.

Densitometric Analysis

Polaroid film was scanned using an Epson (GT-9500) scanner with a resolution of 72 DPI. The resulting image was analyzed using the NIH-Image analysis program (NIH, Bethe-

da, MD, U.S.A.). The scale of each band was expressed by multiplying the values of mean density and total area of the band. The resulting scale was used to quantify each band.

Histological Evaluation

Eighteen female rats (13 week old) were randomly divided into six groups, and were either sham-operated or ovariectomized. Two weeks after the operation, MCT (60 mg/kg, subcutaneous, s.c.) or vehicle was given to the Ovx and sham-operated rats, and 17β -estradiol (50 μg s.c., twice/week) or enalapril (250 mg/L drinking water) was treated in Ovx-MCT rats for 4 weeks. Then the heart and lungs were removed and weighed, and small blocks of the lung and RV were fixed for morphological examination.

RV walls were fixed by immersion in cold 2.5% glutaraldehyde solution (pH 7.4). After fixation, samples were rinsed and postfixed with 1% osmium tetroxide in 0.1 M PBS for 2 hr at room temperature, dehydrated through a graded series of ethanol to propylene oxide and embedded in epoxy resin. Block sections, 60-70 nm thick, were cut, and then stained with uranyl acetate and lead citrate.

Lung tissues were cut into small sizes and fixed by 10% neutral buffered formalin. Then air was removed from the tissue by capping the sample vial and by applying a small vacuum with a 50 mL syringe. After fixation, samples were washed in running tap water, dehydrated through a graded series of ethanol to xylene, and embedded in paraffin. Paraffin block sections, 4 μm thick, were cut and then stained with hematoxylin and eosin.

Statistical Analysis

Data are expressed as mean \pm SE. The data were analyzed statistically using the SPSS for Windows statistical program. Comparisons between the control and MCT-treated male rats were made with an unpaired Student's *t*-test. One-way ANOVA with Tukey's multiple test was used to compare the four female groups. Differences were considered statistically significant at a value of $p < 0.05$.

RESULTS

Effect of Ovariectomy on the Development of RV Hypertrophy and Gene Expressions of the Cardiac RAS, TGF- β_1 and Endothelin-1

The results of the body, heart, and lung weights are shown in Table 1. The body weight (BW) of the Ovx rats was significantly greater than that of the sham-operated rats throughout the experimental period. Treatment with MCT at a dose of 60 mg/kg caused significantly lower body weights in both the male and the Ovx female groups, compared with their

Table 1. Body weight (BW), the wet weights of hearts and lungs of the control and monocrotaline (MCT)-injected male rats, as well as the ovariectomized (Ovx) and the sham-operated female rats

	Male		Female			
	Control	MCT	Sham	Ovx-control	Sham-MCT	Ovx-MCT
Initial BW, g	298±7.5	295±7.6*	225±9.6	232±5.8	236±6.8	232±8.6
Final BW, g	410±14.6	354±8.7*	292±18 ^{bc}	355±14.1 ^a	280±4.5 ^c	314±13.6 ^b
RV/BW, × 10 ⁻⁴	6.8±0.2	13.5±1.6*	5.9±0.4 ^{bc}	5.5±0.2 ^c	7.0±0.7 ^b	15.1±1.1 ^a
(LV+S)/BW, × 10 ⁻⁴	20.5±1.1	20.6±0.3	22.0±1.2	20.0±0.7	22.1±0.5	22.5±0.9
RV/(LV+S)	0.33±0.01	0.65±0.07*	0.27±0.01 ^b	0.28±0.01 ^b	0.32±0.04 ^b	0.67±0.04 ^a
Lung/BW, × 10 ⁻³	4.1±0.34	6.6±0.37*	4.6±0.24 ^b	4.0±0.18 ^b	6.9±0.60 ^a	7.5±0.72 ^a

Values are mean±SE of six rats in each group. * $p<0.01$, vs. control in male rats by t-test. Different superscript letters in female rats show significant differences at $p<0.05$ by Tukey's test. RV, right ventricle; LV, left ventricle; S, septum.

Table 2. Ratios of the densitometry readings of the RT-PCR amplification of renin, angiotensinogen (Aogen), angiotensin II receptor subtypes AT_{1A}, AT_{1B}, TGF- β_1 and endothelin-1 and GAPDH mRNAs in the right ventricle from the control and monocrotaline (MCT)-injected male rats, as well as the ovariectomized (Ovx) and the sham-operated female rats

	Male		Female			
	Control	MCT	Sham	Ovx-control	Sham-MCT	Ovx-MCT
Renin	1	6.1±0.07**	1 ^c	3.8±0.02 ^c	7.8±0.03 ^b	35.9±0.12 ^a
Aogen	1	2.0±0.12*	1	1.2±0.11	0.9±0.07	1.3±0.03
AT _{1A}	1	1.4±0.09	1 ^b	1.0±0.10 ^b	0.9±0.15 ^{ab}	1.4±0.02 ^a
AT _{1B}	1	1.0±0.01	1 ^b	0.6±0.07 ^b	0.6±0.07 ^b	1.5±0.03 ^a
TGF- β_1	1	3.1±0.04**	1 ^c	1.4±0.02 ^{bc}	1.5±0.02 ^b	2.3±0.11 ^a
ET-1	1	2.3±0.07**	1 ^b	0.6±0.08 ^b	0.7±0.14 ^b	1.3±0.01 ^a

Values are mean±SE of six rats in each group. * $p<0.05$, ** $p<0.01$, vs. control in male rats and sham in female rats. Different superscript letters in female rats show significant differences at $p<0.05$ by Tukey's test.

respective control groups. The lung and RV weights were similar between the sham and the Ovx female groups. However, the MCT-treated males and the Ovx females showed significantly higher weight ratios of lung/BW, RV/BW and RV/(LV+S) than did their respective controls. The weight ratio of (LV+S)/BW in the MCT-treated males and the Ovx females was not different from their respective controls. Interestingly, the sham-MCT females showed a significantly increased lung weight without significant RV hypertrophy.

Changes in gene expressions of the cardiac RAS components, TGF- β_1 , and endothelin-1 are shown in Table 2. Renin mRNA level both in RV and LV was very low in a basal state (Fig. 1), but ovariectomy increased RV renin mRNA level 3.8-fold. The Ovx-MCT group showed 9.4-fold higher RV renin mRNA level than the Ovx-control group. The male- and sham-MCT groups showed approximately 6~8-fold higher RV renin mRNA levels than their respective control groups. LV renin mRNA expression in the MCT-treated male and Ovx rats also increased approximately 2-fold, but the increase was markedly lower than the RV level (data not shown). Neither the Ovx-control nor the sham-MCT group showed alteration in LV renin mRNA expression.

RV angiotensinogen mRNA expression increased two-fold in the male-MCT group, but was not significantly increased in MCT treated female groups. LV angiotensinogen mRNA expression was not significantly changed either in the MCT

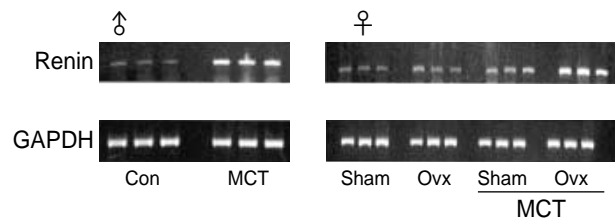


Fig. 1. Representative photographs of the RT-PCR products for renin mRNA in the right ventricle of the control and monocrotaline (MCT)-treated male rats, and the ovariectomized (Ovx) and the sham-operated female rats. GAPDH mRNA was amplified in parallel for the internal control.

treated male, sham or Ovx female groups (data not shown). Gene expression of Ang II-AT_{1A} and AT_{1B} receptors in RV increased slightly but significantly only in the Ovx-MCT group.

Four-week treatment of MCT elevated RV TGF- β_1 mRNA expression 3-fold in male rats, but lesser degree in the sham and the Ovx groups. MCT-treatment slightly elevated TGF- β_1 mRNA expression in LV to the lesser degree than that in the RV of the male and the Ovx rats (data not shown). The Ovx-control rats did not show significant increase in TGF- β_1 mRNA level either in RV or LV. Endothelin-1 mRNA levels increased significantly in the RV of male- and Ovx-MCT rats, but not in the Ovx or sham-MCT rats.

Table 3. Body weight gain, and heart and lung weights of the sham-operated or the ovariectomized (Ovx) female rats treated with monocrotaline (MCT) with or without administration of estrogen or enalapril

	Sham	Sham-MCT	Ovx	Ovx-MCT		
				-	Estrogen	Enalapril
Weight gain, g	65±5.1	37±2.5	125±9.7	67±3.8	60±4.2	110±10.7
RV/(LV+S)	0.38±0.05	0.42±0.04	0.34±0.05	0.68±0.03	0.34±0.04	0.50±0.08
Lung/BW, × 10 ³	4.4±0.78	7.6±0.58	4.6±0.67	8.3±0.28	7.9±0.60	7.7±0.72

Values are mean ±SE of 3 rats in each group.

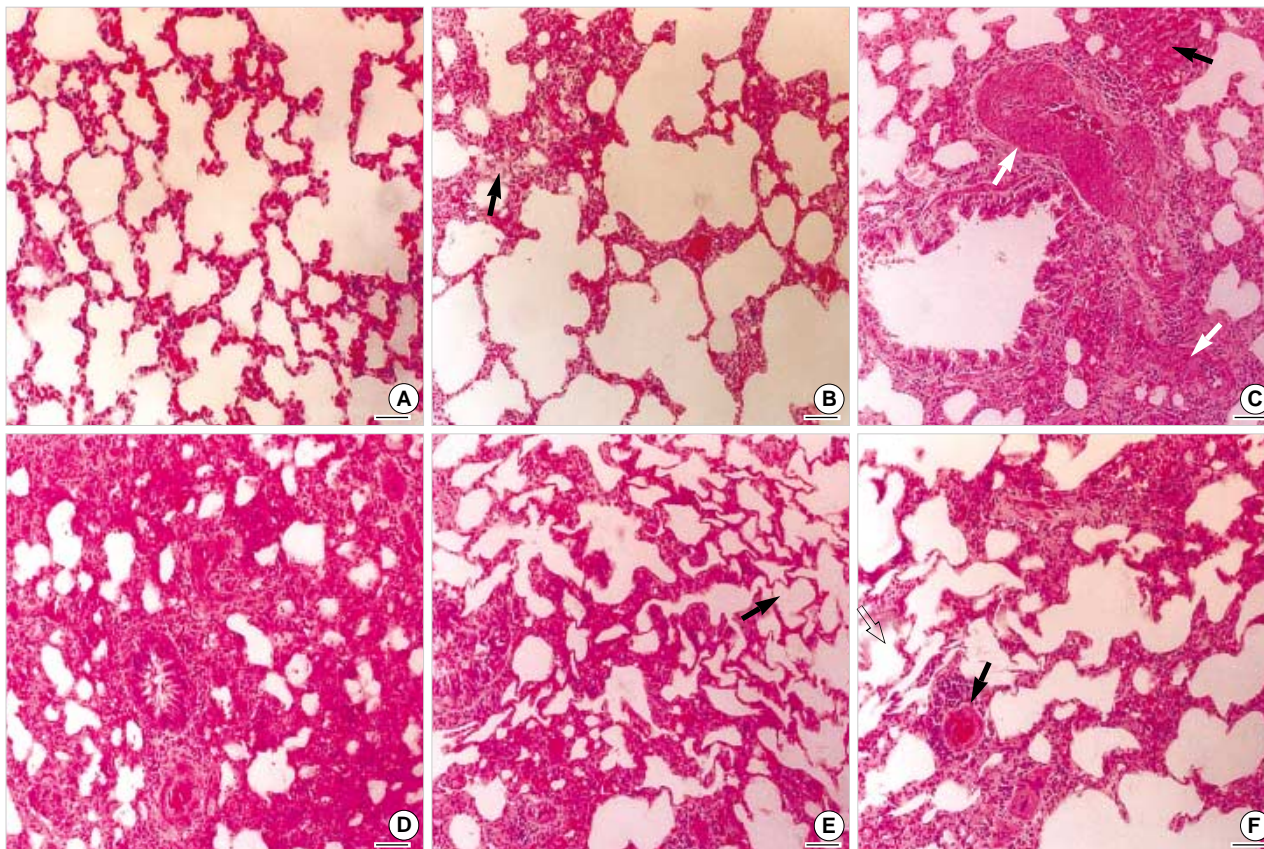


Fig. 2. Light microscopic findings of the lung tissues of the sham-operated (A) or the ovariectomized (Ovx) female rats (B) treated with or without monocrotaline (MCT). Hematoxylin and eosin stain, scale bar=50 μ m. The Ovx rats (B) show histological changes such as increased alveolar size, inflammatory cells (arrows), and mild bronchial obstruction. The sham-MCT rats (C) show hypertrophy of small arteries (white arrows) and atelectasis (arrow), denoting pulmonary hypertension. The Ovx-MCT (D) rats show severe pathological changes such as dif-fused atelectasis, fibrosis and hypertrophy of vascular smooth muscle. In contrast, estrogen (E) or enalapril (F) treatment to the Ovx-MCT rats markedly ameliorate the pathological findings, i.e., patent alveoli, and less prominent hypertrophy of vascular wall.

Effect of Estrogen and Enalapril on the Progression of the Pulmonary Hypertension and RV Hypertrophy: Histological Evaluation

To substantiate the notion of beneficial effects of estrogen and inhibition of the renin-angiotensin system on the progression of the RV hypertrophy, histological changes of the lung and RV were examined in female rats. RV hypertrophy was prominent in the Ovx-MCT rats, but not in the sham-MCT rats and estrogen or enalapril treated Ovx-MCT rats (Table

3). However, lung weight increased in all of the sham-MCT and the Ovx-MCT rats treated with or without estrogen and enalapril.

Histological changes of the lung and RV are shown in Fig. 2 and 3, respectively. Compared to the sham-operated female rats, the lungs of the Ovx rats showed increased alveolar size, partial atelectasis, thickened interalveolar septa, and slightly hypertrophied walls of the small arteries. Lungs of the sham-MCT rats showed prominent atelectasis and hypertrophied walls of the small arteries, denoting developed pulmonary hyper-

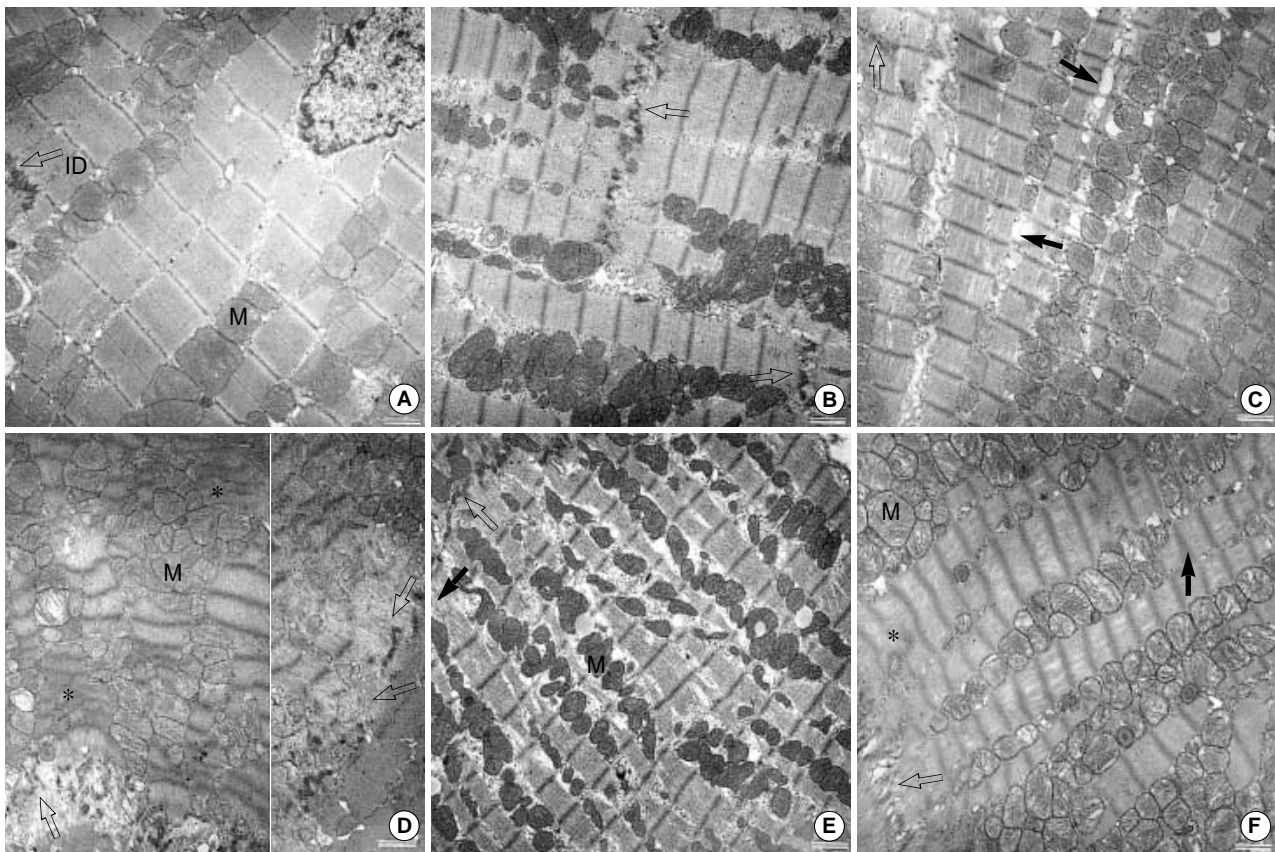


Fig. 3. Electron microscopic findings of the right ventricular wall of the sham-operated (A) or the ovariectomized (Ovx) female rats (B) treated with or without monocrotaline (MCT). Uranyl acetate and lead citrate stain, scale bar=2 μ m. The sham-MCT rats (C) show intact intercalated disk (line arrow) and striated pattern, but dilated intramembranous spaces of the diad system of T-tubule and sarcoplasmic reticulum (arrows). The Ovx-MCT rats (D) show severe myocardial injuries such as disruption of the intercalated disk (line arrow), formation of a hypercontraction band (asterisk), an irregular pattern of cross-striation, and an aggregation of enlarged mitochondria (M). The right side shows the disoriented myofilaments of the degenerated cardiac myocyte at the intercalated disk. Estrogen (E) or enalapril (F) treatment to the Ovx-MCT rats ameliorate the ultrastructure of the RV striated patterns, intercalated disc and mitochondria.

tension. The Ovx-MCT rats, in comparison with the sham-MCT rats, showed very severe pathological changes such as obliteration of most alveoli, prominent fibrosis, infiltration of inflammatory cells, bronchial obstruction, and hypertrophied vessel walls. The histological findings were similar between the Ovx-MCT and the male-MCT rats (data not shown). However, the treatment by using estrogen or enalapril to the Ovx-MCT rats markedly ameliorated the pathological changes of the lungs; patent and almost intact alveoli, and less prominent hypertrophy of blood vessels.

By the electron microscopy examination of the RV, the sham-MCT rats did not reveal abnormal findings of the cardiac myocytes, i.e., an intact intercalated disk and striated pattern, but slightly dilated intramembranous spaces of the diad system of the T-tubule. In contrast, the Ovx-MCT rats showed very severe injuries of the hypertrophied RV tissue, i.e., disruption of the intercalated disk, irregular pattern of cross-striation, formation of hypercontraction band, fraying of myofibrils, and aggregation of enlarged mitochondria. Treatment of estrogen

or enalapril to the Ovx-MCT rats remarkably ameliorated the ultrastructures of the RV myocytes toward the normal.

DISCUSSION

The present study shows that RV hypertrophy was not induced by MCT treatment in female rats, not like in male rats, as reported previously (4, 14), but was exacerbated after ovariectomy. Although the MCT-treated female rats did not show RV hypertrophy, they showed histological changes denoting moderate pulmonary hypertension. The Ovx-MCT rats showed remarkable RV hypertrophy with very severe pathological changes in the lung and RV, and enhanced gene expressions of the RV RAS. Treatment by using estrogen or enalapril to the Ovx-MCT rats prevented the RV hypertrophy, and remarkably ameliorated the ultrastructural changes in the lung and RV. These findings from this rat model suggest that estrogen and the inhibition of the renin-angiotensin system have pro-

protective functions against the development of the pulmonary hypertension and cardiac remodeling.

The protective influences of estrogen on the development of RV hypertrophy in the present study could potentially result from both direct and indirect actions on the heart. Estrogen has been recognized to directly influence cardiac as well as vascular cells (18). Indeed, functional estrogen α - and β -receptors have been demonstrated in the cardiac myocytes and fibroblasts as well as vascular endothelial and smooth muscle cells (19, 20). Several months after ovariectomy, abnormal cardiac mass, function, and biochemistry were produced in rats (21, 22). Estrogen treatment after ovariectomy prevented left ventricular hypertrophy in rats with spontaneously hypertensive heart failure (21), and improved cardiac performance following global ischemia and reperfusion (23, 24). Estrogen also attenuated the cardiac hypertrophic response to pressure overload by transverse aortic constriction in ovariectomized mice (25). The antihypertrophic effect was not mediated by the blood pressure-lowering effect of estrogen. Therefore, these results support the notion that estrogen has direct protecting effects on cardiac myocytes and heart.

Considering ultrastructural findings of the lung and RV in the present study, additional possibility is that the less degree RV hypertrophy in the sham-MCT female and the E_2 -treated Ovx-MCT rats is secondary to lower pulmonary hypertension. Although pulmonary arterial pressure was not measured directly in the present study, more severe and prevalent hypertrophy of the pulmonary arterial wall of the Ovx-MCT rats may suggest higher pulmonary hypertension. Considering the severe atelectasis and lung injury of the Ovx-MCT rats, it is possible that hypoxia also contributed to the observed arterial remodeling and pulmonary hypertension. Several studies suggested that ovariectomy augmented chronic hypoxia-induced pulmonary hypertension, RV hypertrophy and arterial remodeling (26), whereas estrogen attenuated the development of pulmonary hypertension induced either by chronic hypoxia (26) or MCT (27) in rats. In hypoxia-induced pulmonary hypertension, the protective effects of estrogen appear to be mediated in part by reduced pulmonary ET-1 expression (28) and associated arterial modeling (26), and decreased polycythemia (29). Direct beneficial effects of estrogen on the lung tissue have been reported that ovariectomy decreased gas-exchange surface area and increased alveolar size, whereas treatment of estrogen prevented the changes (30). Taken together, estrogen may attenuate the MCT-induced RV hypertrophy secondary to diminished pulmonary hypertension. To differentiate relative importance of direct and indirect cardioprotective roles for estrogen in this animal model, the development of RV hypertrophy should be compared at similar levels of pulmonary hypertension.

Myocardial hypertrophy is the compensatory response to a chronic elevation in mechanical workload. It is a complex process that involves cardiac cell growth and accumulation

of extracellular matrix proteins (6). In cultured cardiac fibroblast, estrogen inhibited cell growth, and collagen synthesis (31). In the pressure-overloaded heart, several vasoactive substances and growth factors synthesized and released from the heart may be involved in the process of cardiac remodeling (6). A critical role of the cardiac RAS, among others, has been recognized (7). Actually, beneficial effects of an inhibition of RAS has been shown to decrease ventricular remodeling induced by chronic hypoxia (32, 33) and myocardial infarction (34, 35).

The present study showed enhanced gene expression of the renin-angiotensin system components in the hypertrophied RV of the male MCT rats, as we previously reported (15). Interestingly, although the sham-MCT female rats did not show RV hypertrophy, they showed a similar degree of increase in RV renin mRNA level as the male MCT rats. However, the Ovx-MCT rats showed remarkably greater increase in renin mRNA level in the hypertrophied RV. This result may suggest significant interactions between estrogen and cardiac RAS, and raise a question whether estrogen inhibit cardiac renin expression. Angiotensinogen mRNA level was also increased in the hypertrophied RV of the Ovx-MCT rats, but to a less degree. Although ACE mRNA was not measured in the present study, we previously reported increased ACE mRNA in the hypertrophied RV of MCT-male rats (15). Increased ACE activity and expression in the hypertrophied RV (36), and plasma levels of renin, ACE and angiotensin II (32) have also been demonstrated in rats with hypoxic pulmonary hypertension. In contrast, AT1 receptors do not seem to be changed in the hypertrophied RV. Adamy *et al.* (37) also showed no change in AT1 and AT2 receptors in RV and LV of the MCT-treated male rats.

Elevated cardiac renin-angiotensin system suggests possible contribution to the MCT-induced RV hypertrophy. This hypothesis was supported by the present result that treatment of enalapril, an ACE inhibitor, prevented RV hypertrophy and profoundly ameliorated ultrastructural changes of the lung and RV in the Ovx-MCT rats. Enalapril and estrogen produced very similar effects. Previous studies also demonstrated the benefit of ACE inhibitors and AT1 receptor antagonists against the development of MCT (38)- and hypoxia (32, 33)-induced pulmonary hypertension and RV hypertrophy in male rats. Inhibitory effects of enalapril against the development of RV hypertrophy in the present study may also be consequences of direct cardiac action and secondary to diminished pulmonary hypertension. In addition, further studies are needed to evaluate possible influence of enalapril-induced decrease in systemic blood pressure on the MCT-induced cardiopulmonary pathogenesis.

Increased gene expression of TGF- β_1 and endothelin-1 in the hypertrophied RV of the male-MCT and the Ovx-MCT rats in the present study confirms our previous findings (15). Both TGF- β_1 and endothelin-1 are known to stimulate the production and accumulation of extracellular matrix proteins

in myocardial hypertrophy and fibrosis (39, 40). A blockade of endothelin-1 prevented MCT-induced pulmonary hypertension and RV hypertrophy (41, 42) suggesting that endogenous endothelin-1 contributes to the progression of cardiopulmonary alterations. On the other hand, TGF- β_1 mRNA expression was slightly but significantly increased even in the unhypertrophied RV of the Ovx control and the sham-MCT female rats, but increased further in the hypertrophied RV of the Ovx-MCT rats. The result from this rat model may suggest a suppressive effect of estrogen on cardiac TGF- β_1 expression and cardiac remodeling.

In summary, our results demonstrate gender differences and beneficial effects of the ovarian function and the inhibition of the renin-angiotensin system on the development of MCT-induced pulmonary hypertension and RV hypertrophy. Enhanced gene expression of the cardiac renin-angiotensin system, TGF- β_1 and endothelin-1 in the hypertrophied RV of the Ovx-MCT rats may suggest the interaction of these cardiac vasoactive substances with estrogen. However, further investigations are required to understand underlying mechanisms of the interactions in the pathogenesis of heart disease.

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