Role of Chymase-Dependent Angiotensin II Formation in Monocrotaline-Induced Pulmonary Hypertensive Rats

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ABSTRACT: Angiotensin II-forming chymase is expressed in the pulmonary arteries of the monocrotaline-induced pulmonary hypertensive rats, but its actual role is unclear. We studied chymase-dependent angiotensin II formation in the pulmonary arteries of the monocrotaline-induced pulmonary hypertensive rats and observed the effects of an angiotensin II receptor blocker on vascular remodeling. Four weeks after the administration of monocrotaline (60 mg/kg, s.q.), echocardiographic, hemodynamic, morphometric and biochemical analyses were performed. Age-matched rats were used as controls. To evaluate the effects of an angiotensin II receptor blocker, 2 wk after beginning of monocrotaline treatment, the rats were given candesartan (10 mg/kg per day) or placebo for 2 wk. In the monocrotaline-induced pulmonary hypertensive rats, the elevated systolic pulmonary arterial pressure and right ventricular hypertrophy were observed. Medial hypertrophy of lung arterioles was also observed. Chymase activity and angiotensin II concentration, but not angiotensin-converting enzyme activity, were significantly increased in the lung. In the angiotensin II receptor blocker-treated group, both systolic pulmonary arterial pressure and right ventricular hypertrophy were significantly reduced, and arteriolar hypertrophy was also prevented. Thus, angiotensin II-forming chymase may play a role in the proliferation of the medial layer in the lung arterioles of monocrotaline-induced pulmonary hypertensive rats. (*Pediatr Res* **60: 77–82, 2006**)

Chymase is a chymotrypsin-like serine protease that is thought to be present in the secretory granules of mast cells. Chymase is able to cleave the bonds of angiotensin I between phenylalanine and histidine (1,2). Chymase purified from human cardiovascular tissues easily converts angiotensin I to angiotensin II (1,2), and chymase-dependent angiotensin II may have a role in human cardiovascular tissue function. For example, in human heart extracts, over 75% of total angiotensin II-forming activity depended on chymase, and the remaining depended on angiotensin-converting enzyme (ACE) (1). In human vascular tissue extracts, about 90% of total angiotensin II-forming activity was inhibited by a chymase inhibitor, and the remaining activity was inhibited by an ACE inhibitor (3). These findings suggest that chymase pri-

marily produces angiotensin II compared with ACE in human cardiovascular tissues. However, both ACE inhibitor and angiotensin II receptor blocker (ARB) lower systemic blood pressure, suggesting that ACE plays a dominant role in regulating angiotensin II formation. Although chymase converts angiotensin I to angiotensin II in the extracts of hearts and arteries, chymase-dependent angiotensin II formation may be irrelevant for the regulation of blood pressure *in vivo* (4). Chymase is synthesized as an inactive prochymase, and dipeptidylpeptidase I (DPPI) is necessary for chymase activation in the secretory granules (5). DPPI is a thiol proteinase with a pH optimum of 6.0 and the pH optimum of DPPI is consistent with its proposed function as a prochymase-activating enzyme, since the pH within the granule is regulated at pH 5.5 (5). On the other hand, the optimum pH of chymase in human vascular tissues is between 7 and 9, and chymase has no enzymatic activity at pH 5.5 (2). Chymase shows enzymatic activity immediately upon release into the interstitial tissues after strong stimulation in cardiovascular tissues (4). Therefore, chymase may be released and have enzymatic activity only after the activation of mast cells in cardiovascular tissues.

Two rat chymases, rat mast cell protease (RMCP) I and RMCP II, both of which are expressed in mast cells, are known. Since these chymases only weakly convert angiotensin I to angiotensin II (6), it has been thought that rat cardiovascular tissues hardly contain angiotensin II-forming chymases. However, recently, Guo *et al.* (7) cloned a novel chymase, rat vascular chymase (RVCH) from rat vascular smooth muscle cells (VSMCs). The cDNA encodes 247 amino acids and exhibits 74% and 80% homology for the amino acid sequence of RMCP I and RMCP II, respectively. RVCH, like human chymase, converts angiotensin I to angiotensin II (7). Over-expression of RVCH in VSMCs of transgenic mice induces systemic hypertension and vascular remodeling (8). In monocrotaline-induced pulmonary hypertensive (PH) rats, RVCH mRNA and protein are increased in the pulmonary VSMCs, but it is unclear whether RVCH-dependent angio-

Abbreviations: ACE, angiotensin-converting enzyme; **ARB,** angiotensin II receptor blocker; **DPPI,** dipeptidylpeptidase I; **LV,** left ventricle; **MWT,** medial wall thickness; **PH rats,** pulmonary hypertensive rats; **RMCP,** rat mast cell protease; **RVCH,** rat vascular chymase; **RV,** right ventricle; **VSMCs,** vascular smooth muscle cells

tensin II formation plays a pathophysiological role in vascular remodeling. In this study, we clarified the pathophysiological role of chymase-dependent angiotensin II formation in monocrotaline-induced PH rats.

METHODS

Materials. Monocrotaline were purchased from Sigma Chemical Co. (St. Louis, MO). An ARB candesartan was kindly provided by Takeda Chemical Industries (Osaka, Japan). An ACE inhibitor trandolaprilat was gifted from Hoechst Marion Roussel (Romainville, France). A chymase inhibitor NK3201 was gifted from Nippon Kayaku (Tokyo, Japan). NK3201 inhibited human, dog, rat and hamster chymases by IC_{50} at concentrations of 2.5, 1.2, 19 and 28 nM, respectively, but has no inhibitory activity to ACE, tryptase, thrombin, elastase, plasmin and plasminogen activator (9).

Hemodynamics and medial thickness index. A total of 34 male Sprague-Dawley rats aged 6 wk, weighing 268 ± 4 g at the beginning of the experiment, were obtained from Japan SLC Inc. (Shizuoka, Japan). The rats were fed a regular hamster chow diet, had free access to tap water, and were housed in a temperature-, humidity-, and light-controlled room. The experimental procedures were done in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

We used two protocols. With the first protocol, we analyzed the pathophysiological role of chymase in the monocrotaline-induced PH rats; with the second, we evaluated the effect of an ARB in monocrotaline-induced PH rats.

To analyze the pathophysiological role of chymase in monocrotaline-induced PH rats, 8 rats were given monocrotaline (60 mg/kg, s.q.) (10). Four weeks after the injection, echocardiography and hemodynamic measurements were done under anesthesia. The rats were then killed with an overdose of sodium pentobarbital, and biochemical and histologic analyses were done. Six-age-matched rats received saline (s.q.) and were used as controls.

To evaluate the effect of an ARB in monocrotaline-induced PH rats, candesartan (10 mg/kg per day) or placebo (each group, $n = 10$) was given orally for 2 wk, starting 2 wk after the monocrotaline injection.

Echocardiographic study. Four weeks after the injection of monocrotaline, echocardiography was done using an echocardiographic system (Nemio 30, Toshiba, Japan) according to methods described previously (11). In brief, after IP injection of ketamine HCl (25 to 50 mg/kg) and xylazine (5 to 10 mg/kg), measurements of the pulmonary acceleration time were taken and the Tei index was calculated using the pulsed Doppler mode (12,13). The pulmonary acceleration time was measured from the time of onset of systolic flow to the time of onset of the peak pulmonary outflow velocity; a decrease in the acceleration time has been observed in monocrotaline-induced PH rats (12). The Tei index allows the evaluation of right ventricle (RV) systolic and diastolic functions and generally increases with PH, indicating RV dysfunction (13).

Hemodynamic measurement and tissue preparation. Four weeks after the injection of monocrotaline, the trachea was intubated for subsequent mechanical ventilation under anesthesia with ketamine HCl (25 to 50 mg/kg) and xylazine (5 to 10 mg/kg). A left thoracotomy was performed through an incision in the fourth intercostal space. The pulmonary artery was isolated, and the pulmonary artery pressure was obtained by direct needle puncture of the pulmonary artery using a 22 gauge needle connected to a pressure transducer (TP-200T, Nihon Koden, Tokyo, Japan). A PE-10 catheter (Clay Adams, Parsippany, NJ) was inserted into the left femoral artery and was connected to a pressure transducer (TP-200T) to measure the systemic arterial blood pressure.

The rats were killed with an overdose of sodium pentobarbital (100 mg/kg) *via* the femoral artery. After the trachea and lungs were removed, the left lung was ligated, separated, weighed, and immediately frozen at $-80°C$ for use in the biochemical analysis. The right lung was preserved with 4% formaldehyde solution by airway gravity fixation and then used for histologic analysis. The RV, left ventricle (LV), and septum were weighed separately to determine the ratio of the RV weight to the LV weight plus the septum weight as an index of RV hypertrophy (14).

Histologic analysis. The right lung lobes were fixed *via* the trachea using a 4% formaldehyde solution. Next, the whole lobe was cut into 5 sections for sampling. Each section was immersed in fixative overnight at 4°C, and then processed and embedded in paraffin. Four-μm-thick serial sections were stained with van Gieson stain. To assess pulmonary artery remodeling, the medial wall thickness (MWT) was calculated as $(2 \times$ medial thickness/external diameter) $\times$ 100% (14). For all muscular arteries with an external diameter of 50 to 100 μm, the wall thickness of the media (that is, the distance between external and internal elastic laminae) was measured along the

shortest curvature, and the MWT of the pulmonary arteries was measured in 10 muscular arteries per each section by two investigators who were blinded to treatment allocation.

Measurements of ace and chymase activities. ACE activities were measured by incubating the plasma or the tissue extracts for 30 min at 37°C with 5 mM hippuril-His-Leu (Peptide Institute, Minoh, Japan) as the substrate when measuring ACE activity in 100 mM phosphate buffer, pH 8.3, containing 300 mM NaCl (15). The enzyme reaction was terminated by the addition of 3% metaphosphoric acid (wt/vol). After centrifugation of the mixture, the supernatant was applied to an ODS reversed-phase column (4.6 mm $\times$ 25 cm; Tosoh, Tokyo, Japan). Hippuric acid was detected by UV absorbance at 228 nm. One unit of ACE activity was defined as the amount of enzyme that cleaved 1 μmol hippuric acid/min.

Chymase activity was measured by incubating the tissue extracts for 30 min at 37°C with 5 mM Suc-Ala-Ala-Pro-Phe-4-methylcoumaryl-7-amide (Peptide Institute) as a substrate for the measurement of chymase activity in 100 mM Tris-HCl buffer, pH 8.5, containing 200 mM NaCl (15). The enzyme reaction was terminated by the addition of 3% metaphosphoric acid (wt/vol). After centrifugation of the mixture, 7-amino-4methylcoumarin was measured by fluorophotometric determination (excitation 380 nm; emission, 460 nm). One unit of chymase activity was defined as the amount of enzyme that cleaved 1 μmol 7-amino-4methylcoumarin/min.

Protein concentration was assayed with BCA Protein Assay Reagents (Pierce, Rockford, IL), using BSA as the standard.

Angiotensin II-forming activity and angiotensin ii concentration. Aliquots of the extract were incubated for 30 min at 37°C with 5 mM angiotensin I in 10 mM phosphate buffer, pH 7.4, containing 150 mM NaCl (15). To study the effects of enzyme inhibitors, an ACE inhibitor, trandolaprilat (1 μM), or a chymase inhibitor, NK3201 (1 μM), was added, and pre-incubated for 10 min at 37°C, followed by incubation for 30 min at 37°C with 5 mM angiotensin I in 10 mM phosphate buffer, pH 7.4, containing 150 mM NaCl (15). At these concentrations, trandolaprilat and NK3201 completely inhibited ACE and chymase, respectively (9). The reaction was terminated by the addition of 15% trichloroacetic acid (wt/vol), followed by centrifuging at 20,000 g. The supernatant was applied to an ODS reversed-phase column (4.6 mm $\times$ 25 cm; Tosoh). Angiotensin II was detected by UV absorbance at 218 nm. One unit of angiotensin II-forming activity was defined as the amount of enzyme that formed 1 μmol angiotensin II/min.

To measure the concentration of angiotensin II, the lungs were minced into small pieces, and then incubated overnight in ice-cold ethanol to extract angiotensin II (16). After the incubation, samples were centrifuged, and the supernatant was evaporated to remove ethanol. The extracts were then treated by shaking with diethylether, and the supernatant was again evaporated. Finally, the dried powder was dissolved in distilled water, and the angiotensin II concentration was determined by a high sensitivity enzyme immunoassay (Peninsula Laboratories, Inc., Belmont, CA, USA).

Reverse transcription (RT)-Pcr. The total RNA of the lung was extracted using TRIzol reagent (Life Technologies, Inc., Rockville, MD) and dissolved in 0.1% diethyl pyrocarbonate-treated water. RT to cDNA was accomplished by analyzing 5 μg of the total RNA sample with SuperScript II reverse transcriptase and oligo(dT)$_{12-18}$ primer (Invitrogen, Carlsbad, CA). The reaction was carried out in the presence of first-strand buffer, 1 mM dNTPs, and 20 M DTT, at 42°C for 50 min. The PCR mixture contained 1 μL of the cDNA reaction mixture, 20 pM primers, PCR buffer, 0.4 mM dNTPs, and 2.5 U *Taq* polymerase. The reaction was carried out with a RoboCycler (Stratagene, La Jolla, CA). The sequences of the oligonucleotide primers for PCR were as follows: RVCH sense primer, 5′-GAGGCCTGTAAAATC-TATAGAC-3′, and anti-sense primer, 5′-TGTGTATCTTTGAGAGCCT-CAA-3′, were used for the amplification of RVCH; ACE sense primer, 5′-CAGCTTCATCATCCAGTTCC-3′ and anti-sense primer, 5′-CTAG-GAAGAGCAGCACCCAC-3′; and β-actin sense primer, 5′-CCAAGGC-CAACCGCGAGAAGATGAC-3′, and anti-sense primer, 5′-AGGGTACAT-GGTGGTGCCGCCAGAC-3′, were used for the amplification of β-actin to calibrate sample loading (7,17,18). The PCR products were separated by electrophoresis on 2% agarose gel stained with ethidium bromide and the samples were then visualized by UV trans-illumination. Polaroid photographs were scanned using Epson GT-9500 (Seiko Corp, Nagano, Japan), and the electrophoresis bands stained with ethidium bromide were analyzed using the public domain image analysis program from the National Institutes of Health Image software (version 1.61 available at rsb.info.nih.gov/nih-image). The density of an individual band was normalized to that of β-actin to correct for differences in samples.

Statistical methods. Data are expressed as mean $\pm$ SEM. When two groups were compared, the differences were assessed by the unpaired *t*-test. Comparisons among three groups were made by one-way ANOVA followed by Bonferroni *post hoc* testing for multiple comparisons. Differences were considered significant only when the $P < 0.05$.

RESULTS

Analysis of the experimental model. The body weight of the control rats at the end of the experiment was 358 ± 4 g, and the body weight of the monocrotaline-induced PH rats was 303 ± 8 g; this difference was statistically significant.

On echocardiography, the acceleration time of pulmonary arterial flow in the monocrotaline-induced PH rats was significantly lower than in the control rats (Table 1). Moreover, the RV Tei index in the monocrotaline-induced PH rats was significantly higher than in the control rats. On hemodynamic analysis, there was no difference in the systolic arterial pressures between the control rats and the monocrotaline-induced PH rats (control rats, 105 ± 4 mm Hg; monocrotaline-induced PH rats, 109 ± 9 mm Hg). However, the systolic pulmonary arterial pressure in the monocrotaline-induced PH rats was higher than in the control rats (Table 1). An index of RV hypertrophy, the ratio of the RV weight to the LV weight plus septum weight, in the monocrotaline-induced PH rats was significantly greater than in the control rats (Table 1).

Typical photographs of arterioles stained with van Gieson-staining are shown in Fig. 1*A*. Hypertrophied arterioles were observed in the monocrotaline-induced PH rats, but not in the control rats. An index of pulmonary artery remodeling, the MWT of the arterioles, was 17.8 ± 0.88 in the monocrotaline-induced PH rats and 38.5 ± 1.58 in the control rats; this difference was statistically significant (Fig. 1*B*).

ACE activity in the monocrotaline-induced PH rats was significantly lower than in the control rats (Fig. 2*A*). In contrast, chymase activity was significantly higher in the monocrotaline-induced PH rats (Fig. 2*B*). Moreover, the angiotensin II concentration was significantly higher in the monocrotaline-induced PH rats than in the control rats (Fig. 2*C*). Using angiotensin I as a substrate, the angiotensin II-forming activity in lung extracts was significantly higher in the monocrotaline-induced PH rats than in the control rats (Fig. 3*A*). ACE inhibitor treatment reduced the angiotensin II-forming activity in the control rats by 96.6%, but only by 4.7% in the monocrotaline-induced PH rats. In contrast, chymase inhibitor treatment reduced the angiotensin II-forming activity in the monocrotaline-induced PH rats by 95.1%, but only by 3.3% in the control rats (Fig. 3*B*). The RVCH mRNA level in the monocrotaline-induced PH rats was significantly higher than in the control rats, but the ACE mRNA level in the monocrotaline-induced PH rats was significantly lower (Fig. 4).

Effects of an ARB in the monocrotaline-induced PH rats. There was no significant difference between the body weights
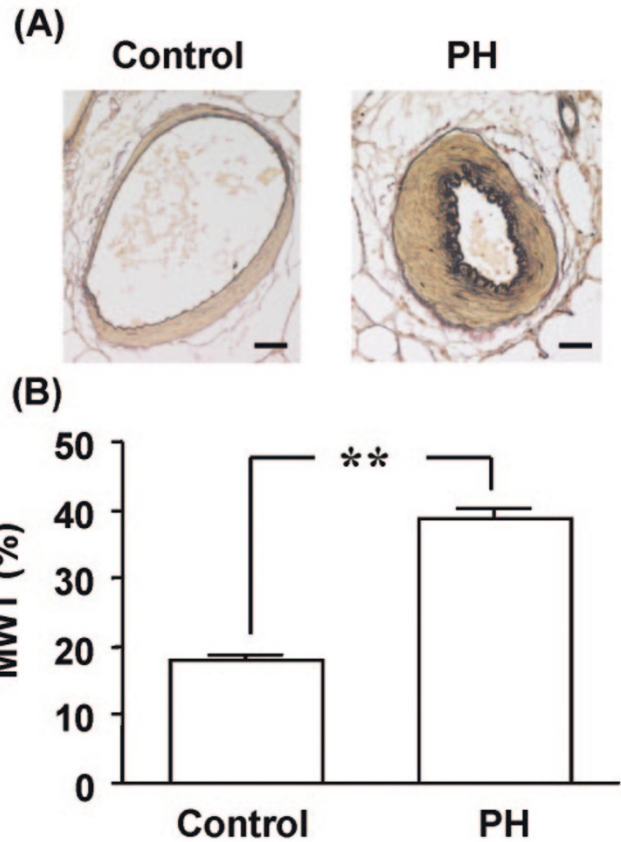
Figure 1. Typical photographs of pulmonary arterioles in control and monocrotaline-induced pulmonary hypertensive (PH) rats (*A*). MWT (%) of arterioles in control and monocrotaline-induced pulmonary hypertensive (PH) rats (*B*). Original magnification: 200×. Scale bar in A, 10 μm. ** $p < 0.01$.
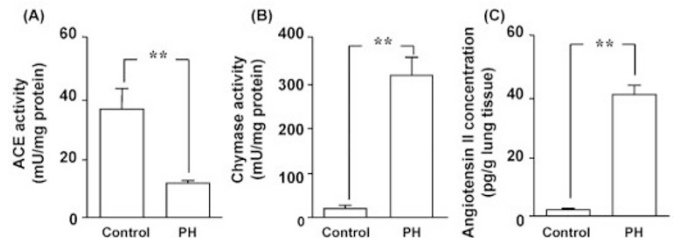
Figure 2. ACE activity (*A*), chymase activity (*B*) and angiotensin II concentration (*C*) in lungs of control and monocrotaline-induced pulmonary hypertensive (PH) rats. ** $p < 0.01$.

of the monocrotaline-induced PH rats treated with a placebo (303 ± 6 g) and those treated with an ARB (298 ± 5 g).

The acceleration time of pulmonary arterial flow in the ARB-treated group was significantly increased compared with

**Table 1.** *Characterization in control and monocrotaline-induced PH rats*

Animals	Pulmonary acceleration time (msec)	RV Tei index	RV/LV + S ratio	Pulmonary arterial pressure (mmHg)
Control rats (*n* = 6)	30 ± 1.2	0.41 ± 0.03	0.24 ± 0.01	23 ± 0.86
PH rats (*n* = 8)	20 ± 0.9**	0.73 ± 0.13*	0.40 ± 0.03**	49 ± 2.96**

PH rats, pulmonary hypertensive rats; RV Tei index, right ventricular Tei index; RV/LV + S ratio, the ratio of right ventricle (RV) to left ventricle (LV) and septum.

* $p < 0.05$ and ** $p < 0.01$ *versus* control rats.
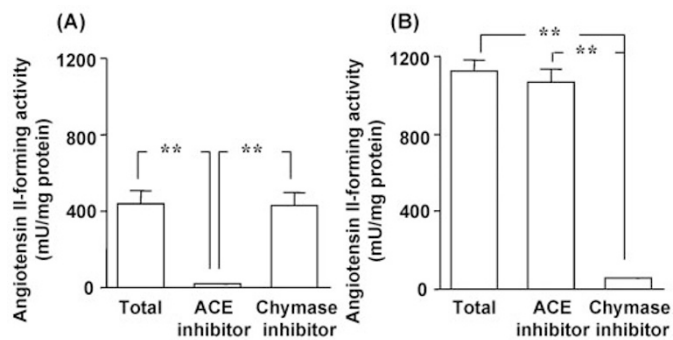
Figure 3. Lung angiotensin II-forming activity in the absence of an inhibitor (total), in the presence of an ACE inhibitor or a chymase inhibitor in control (*A*) and monocrotaline-induced pulmonary hypertensive rats (*B*). ** $p < 0.01$.
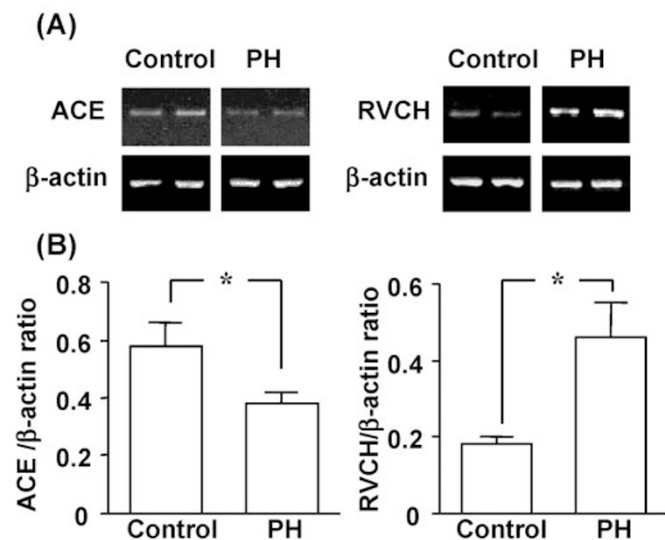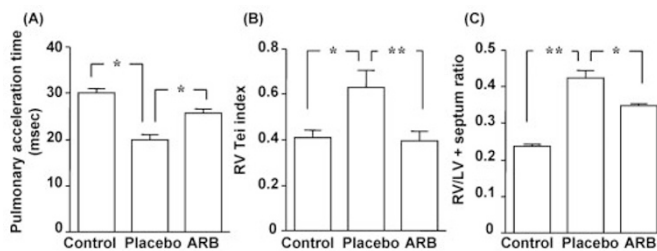

Figure 4. Typical photographs of ACE, RVCH, and β-actin expressions in lungs obtained from control and monocrotaline-induced pulmonary hypertensive (PH) rats (*A*). Ratios of ACE to β-actin expression or ratios of RVCH to β-actin expression in lungs obtained from control and monocrotaline-induced pulmonary hypertensive (PH) rats (*B*). * $p < 0.05$.
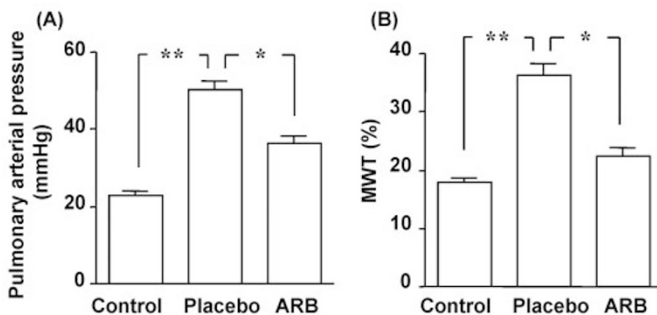

Figure 5. Effects of an ARB on the pulmonary acceleration time (*A*), RV Tei index (*B*), and RV/LV + septum ratio (*C*) in monocrotaline-induced pulmonary hypertensive rats. * $p < 0.05$ and ** $p < 0.01$.

Figure 6. Effects of an ARB on the pulmonary arterial pressure (*A*) and MWT (*B*) in monocrotaline-induced pulmonary hypertensive rats. * $p < 0.05$ and ** $p < 0.01$.

the placebo-treated group (Fig. 5*A*). The RV Tei index in the ARB-treated group was significantly lower than in the placebo-treated group (Fig. 5*B*). The ratio of the RV weight to the LV weight plus the septum weight was significantly lower in the ARB-treated group than in the placebo-treated group (Fig. 5*C*). Although systolic systemic arterial pressure tended to be lower in the ARB-treated group than in the placebo-treated group (placebo-treated group, 107 ± 8 mm Hg; ARB-treated group, 94 ± 4 mm Hg), this difference was not statistically significant ($p = 0.15$). On the other hand, a significant lowering of the systolic pulmonary arterial pressure was observed in the ARB-treated group (Fig. 6*A*). The MWT of the arterioles was also lower in the ARB-treated rats than in the placebo-treated rats (Fig. 6*B*).

DISCUSSION

Echocardiography is widely used to evaluate PH in clinical practice and in animal models (13,19). The acceleration time of pulmonary arterial flow is used to evaluate RV dysfunction.

The Tei index, which combines both systolic and diastolic parameters (13), provides a more detailed assessment of PH-induced RV dysfunction. In this study, both the acceleration time of pulmonary arterial flow and the RV Tei index had lower values in monocrotaline-induced PH rats than in control rats 4 wk after monocrotaline injection. These results confirm that RV dysfunction was present in the monocrotaline-induced PH rats. When PH is present in both human and animal models, pulmonary arterial pressure increases due to increased pulmonary artery remodeling, particularly in the MWT of the arterioles. In our model, the arteriolar MWT was significantly higher in monocrotaline-induced PH rats than in control rats. An increase in the pulmonary arterial pressure is known to induce RV hypertrophy. We also observed a significant increase of RV hypertrophy (RV/LV plus septum ratio) in monocrotaline-induced PH rats. Thus, in monocrotaline-induced PH rats, progression in arteriolar thickness might increase pulmonary arterial pressure, resulting in RV hypertrophy and RV dysfunction.

Guo *et al.* (7) found a high level of RVCH expression in the medial layers of the pulmonary artery in monocrotaline-induced PH rats, but not in saline-treated control rats. In the present study, we observed a significant increase of RVCH mRNA levels in lung. In monocrotaline-induced PH rats, chymase activity was significantly increased compared with the controls. However, as previously reported, ACE activity was significantly reduced in the monocrotaline-induced PH rats (10). Furthermore, both angiotensin II-forming activity and angiotensin II concentration were significantly increased in monocrotaline-induced PH rats. However, angiotensin II-forming activity in the controls was almost completely inhib-

ited by an ACE inhibitor, and its activity in monocrotaline-induced PH rats was almost completely inhibited by a chymase inhibitor. Such different effects of enzyme inhibitors suggest that angiotensin II-forming activity in the control rats was predominantly dependent on ACE, while, in the monocrotaline-induced PH rats, it was predominantly dependent on chymase. In other words, in the control rats, ACE carried the main function as an angiotensin II-forming enzyme, but chymase had this function in the monocrotaline-induced PH rats. In this study, the body weight tended to be lower in the ARB-treated group than in the placebo-treated group. Although there was no intermediate evaluation between the beginning and 4 wk after the monocrotaline injection, it suggests that ARB treatment may not reduce direct monocrotaline toxicity. We also found that an ARB prevented the remodeling of arterioles and the occurrence of RV hypertrophy. All these findings suggest that RVCH-dependent angiotensin II formation may play an important role in the progression of monocrotaline-induced PH.

Human chymase contributes to the formation of angiotensin II, which in turn, may be strongly correlated with pulmonary remodeling. Mitani *et al.* (20) reported that the number of chymase-containing mast cells in lung vasculature was clearly increased in PH patients compared with non-PH patients. This finding suggests that chymase is involved in the pulmonary vascular remodeling of PH. Congenital heart diseases are associated with increased pulmonary arterial pressure, resulting in PH and subsequent pulmonary vascular diseases (21). Hamada *et al.* (22) investigated whether the number of chymase-containing mast cells in the pulmonary artery might contribute to the structural changes associated with PH in patients with congestive heart diseases; they found that there was a significant correlation between the number of chymase-containing mast cells and pulmonary resistance. An increase in the number of chymase-containing mast cells may also play a possible role in the development of PH in patients with congenital heart diseases.

In general, it has been thought that, in cells other than mast cells, human chymase is expressed to only a small degree. However, some reports have shown that chymase is expressed in other cells. For example, using both immunohistochemical and *in situ* hybridization techniques, human chymase in the cytosolic granules of endothelial cells located in intramural capillaries and in some mesenchymal cells of the cardiac interstitium (23). Huang *et al.* (24) reported that the expression of human chymase in VSMCs was significantly higher in the diabetic kidney than in the normal kidney. Human chymase, like RVCH, may be expressed in human VSMCs and may contribute to angiotensin II formation in VSMCs.

In this study, lung ACE mRNA levels were significantly decreased in monocrotaline-induced PH rats. Previous investigators have reported that lung ACE activities were significantly decreased in monocrotaline-induced PH rats and have suggested that the reduction in lung ACE activity is a result rather than a cause of PH (25). However, ACE inhibitors have been shown to prevent monocrotaline-induced PH in rats (26,27), while the effects of ARBs in monocrotaline-induced PH rats have not yet been determined. Cassiss *et al.* (28)

reported that the ARB losartan failed to prevent monocrotaline-induced PH. Okada *et al.* (26) compared an ACE inhibitor, quinapril, with losartan, and though both agents were found to prevent PH, the extent of inhibition with losartan was clearly weaker than with quinapril. From these results, they speculated that the mechanism of action of ACE inhibitors in PH might depend on the increase of bradykinin rather than on the reduction of angiotensin II formation (26,28). On the other hand, the ARB irbesartan was found to significantly prevent PH (29), just as our study showed that candesartan prevented PH. The discrepancies among ARBs may be related to the different character of each ARB (18). In this study, the upregulation of angiotensin II formation in the lung after monocrotaline injection might be dependent on the increase of RVCH, but not ACE. Therefore, the combination of an ACE inhibitor and an ARB may be useful for increasing bradykinin accumulation by ACE (kininase II) inhibition and reducing angiotensin II formation by RVCH inhibition. This combination may be more effective in treating PH than monotherapy with either agent. Further studies are needed.

In the present study, our result suggests that RVCH-dependent angiotensin II formation is involved in the development of monocrotaline-induced PH in rats.

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