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Possible Involvement of Mitochondrial Energy-Producing Ability in the Development of Right Ventricular Failure in Monocrotaline-Induced Pulmonary Hypertensive Rats

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Abstract. The present study was undertaken to explore the possible involvement of alterations in the mitochondrial energy-producing ability in the development of the right ventricular failure in monocrotaline-administered rats. The rats at the 6th week after subcutaneous injection of 60 mg/kg monocrotaline revealed marked myocardial hypertrophy and fibrosis, that is, severe cardiac remodeling. The time-course study on the cardiac hemodynamics of the monocrotaline-administered rat by the cannula and echocardiographic methods showed a reduction in cardiac double product, a decrease in cardiac output index, and an increase in the right ventricular Tei index, suggesting that the right ventricular failure was induced at the 6th week after monocrotaline administration in rats. The mitochondrial oxygen consumption rate of the right ventricular muscle isolated from the monocrotaline-administered animal was decreased, which was associated with a reduction in myocardial high-energy phosphates. Furthermore, the decrease in mitochondrial oxygen consumption rate was inversely related to the increase in the right ventricular Tei index of the monocrotaline-administered rats. These results suggest that impairment of the mitochondrial energy-producing ability is involved in the development of the right ventricular failure in monocrotaline-induced pulmonary hypertensive rats.

Keywords: monocrotaline, right ventricular hypertrophy, right ventricular failure, mitochondrial dysfunction, high-energy phosphate

Introduction

Pulmonary artery hypertension is a progressive disease caused by a variety of pulmonary and/or cardiac disorders. Pulmonary artery hypertension is commonly characterized by an increase in the pulmonary vascular resistance or in the right ventricular systolic pressure. A rise in pulmonary artery hypertension induces right ventricular hypertrophy and right ventricular failure and eventually leads to premature death. It is well known that a single subcutaneous administration of the pyrrolizidine alkaloid monocrotaline to rats causes cardiovascular and pulmonary disorders similar to those seen in patients with pulmonary artery hypertension (1, 2), including severe right ventricular hypertrophy (3, 4) and right ventricular failure (5 – 8). Despite extensive studies, the fundamental mechanisms responsible for the development and progression of right ventricular failure have not been fully elucidated.

The mitochondrion is an important organelle for energy production. To maintain cardiac contractility, energy supplied by the mitochondria, which amounts to more than 90% of the energy in the heart, is essential under normoxic conditions (9). Studies on mitochondrial function in cardiac tissue from patients with heart failure showed a reduction in the capacity of the mitochondria for oxygen consumption (10 – 12). Experimental studies in rats with heart failure following left coronary artery ligation also showed decreases in myocardial high-energy phosphate levels and in mitochondrial energy-

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producing ability of the left ventricle (13). It is well known that the right ventricular remodeling with excess degree is induced in the monocrotaline-administered animals. However, changes in the right ventricular energy metabolism such as myocardial content of high-energy phosphates and mitochondrial energy-producing ability during the development of right ventricular failure remain unclear. Furthermore, no information is available about the relation between the mitochondrial energy-producing ability and the right ventricular function during the development of the right ventricular dysfunction. The present study was designed to elucidate the role of alterations in the mitochondrial energy-producing ability in the right ventricular muscle in rats with right ventricular failure following monocrotaline-induced the right ventricular hypertrophy.

Materials and Methods

Animals and treatment

Male Wistar rats (SLC, Hamamatsu) weighing 190 – 210 g were used in the present study. The animals were conditioned according to the *Guide for the Care and Use of Laboratory Animals* as published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The protocol of this study was approved by the Committee of Animal Use and Welfare of Tokyo University of Pharmacy and Life Sciences.

The rats were randomly selected to receive either a single subcutaneous injection of approximately 0.2 mL of monocrotaline solution (60 mg monocrotakine/mL) or an equal volume of saline. In a preliminary study, we examined the effects of various doses of monocrotaline ranging from 20 to 80 mg/kg, s.c. Doses of administration with less than 40 mg/kg monocrotaline did not induce the right ventricular failure in rats, whereas all animals died within 10 days after administration of 80 mg/kg monocrotaline. Therefore, we employed 60 mg/kg monocrotaline in the present study. Treated animals were subjected to the following study at different time periods after vehicle or monocrotaline administration.

Measurement of hemodynamics by cannula method

Rats at the preinjection of monocrotaline (0w-control); age-matched control rats (2w-control, 4w-control, 6w-control, and 8w-control, respectively); and rats at the 2nd (2w-MCT), 4th (4w-MCT), 6th (6w-MCT), and 8th (8w-MCT) weeks after monocrotaline administration (n = 6 for each group) were anesthetized with an intraperitoneal administration of 50 mg/kg pentobarbital sodium. A polyethylene catheter (PE50) was then introduced into the right ventricle through the right jugular vein to measure the right ventricular systolic pressure and right ventricular end-diastolic pressure. Central venous pressure was measured via a catheter that was placed in the right jugular vein and advanced to the level of the right atrium. The mean arterial blood pressure and heart rate were measured by means of a pressure transducer attached to the cannula placed into the left femoral artery and a heart rate counter, respectively (14).

Tissue weight and histological study

After measurement of hemodynamics by the cannula method, the rats (n = 6 for each group) were anesthetized with 40 m/kg, i.p. pentobarbital sodium. Then the heart was isolated and dissected free from the atria, aorta, and pulmonary artery. The free wall of the right ventricle (RV), the left ventricle (LV), and septum (Sep) were separated and then weighed. The ratio of the right ventricular weight to left ventricle + septum weight (RV weight / LV weight + Sep weight) was calculated to assess the right ventricular hypertrophy (15). The right ventricular and lung weights were measured, and their ratio to the body weight was calculated.

The other hearts (n = 2 for each group) were transversely sectioned, fixed in 4% paraformaldehyde for 24 h at 4°C, and subsequently embedded in paraffin. For examination of the time course of changes in fibrosis of the heart tissue, sections were stained with Masson's trichrome. Paraffin-embedded tissue blocks were sectioned at 3 μm. Masson's trichrome staining was performed by the standard technique.

Measurement by echocardiography

Transthoracic echocardiography was performed on rats according to the method described previously (16). In the present study, pathophysiological alterations of the rats were examined at the 2nd, 4th, 6th, and 8th weeks after monocrotaline administration (n = 6 for each group). The animals were anesthetized with 40 mg/kg, i.p. pentobarbital sodium, and then the hair on their chest was shaved off before the examination. Two-dimensional and Doppler imagings were performed by using ProSound 5500^{R} (Aloka, Tokyo) with a 10-MHz transducer. The transthoracic echocardiographic probe was placed to obtain long-axis and apical four-chamber views. To evaluate right ventricular function, we measured cardiac output at the pulmonary artery, which was confirmed to be practically adequate (16). After determination of the pulmonary arterial flow and heart rate, velocity time integral (VTI) and pulmonary arterial diameter (PAD) were measured in the long-axis view. Cardiac output and stroke volume were calculated according to the following equations (17): Cardiac

output = $(PAD/2)^2 \times \pi \times VTI \times$ heart rate, stroke volume = cardiac output / heart rate. In addition, to characterize the pulmonary outflow, we measured the pulmonary artery flow acceleration time (PAAT). PAAT was estimated as the difference between the time that the increase in the systolic blood flow started and the time at which the peak velocity of the pulmonary outflow was reached (18). Tei index in the echocardiograph is a simple and reproducible Doppler index for combined systolic and diastolic myocardial performance in patients with primary myocardial systolic dysfunction. The right ventricular Tei index was assessed from Doppler recordings of right ventricular inflow and outflow. From tricuspid inflow in the apical four-chamber view, the time interval from cessation to onset of tricuspid inflow was measured (*a*-interval). Ejection time (*b*-interval) was measured from the right ventricular outflow velocity curve recorded in the long-axis view. The right ventricular Tei index, increase of which is considered to reveal a sign of right ventricular dysfunction, was calculated as $(a - b) / b$ (19 – 21). Then, consecutive cardiac-cycle images were measured and averaged.

Measurement of right ventricular contractile force

For the measurement of right ventricular contractile force, the coronary-perfused right ventricular muscles were prepared from MCT and control rat hearts (n = 6 for each group) according to the method described by Tanaka et al. (22). The left atrium, left ventricle, and septum were dissected away from the preparation and then a cannula was placed into the aorta with the isolated right ventricular muscle. The right ventricular wall was perfused at 37°C with a constant flow rate (3.0 mL/min) of Krebs-Henseleit bicarbonate buffer of the following composition: 120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.25 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose (pH 7.4). The perfusion buffer was equilibrated with a gas mixture of 95% O_2 + 5% CO_2. The right ventricular wall apex was attached to a force displacement transducer (TB-611T; Nihon Kohden, Tokyo) in an organ bath. The resting tension on each preparation was adjusted to 0.5 g. All preparations were allowed to equilibrate for 30 min before measurement of the basal developed tension and heart rate. We estimated the cardiac double product, that is, the developed tension multiplied by the heart rate, as a measure of the right ventricular workload (23).

Determination of high-energy phosphates (HEPs)

The right ventricular HEPs, ATP and creatine phosphate, of the MCT- or vehicle-administered rats at different times after the administration (n = 6 for each group) were determined by using the coronary-perfused preparations described above. After the developed tension and heart rate had been determined, the right ventricular wall was quickly clamped with aluminum tongs pre-cooled with liquid nitrogen and quickly frozen. The frozen tissue was pulverized in a motor-driven homogenizer with a pestle, and ATP and creatine phosphate were extracted with 0.3 M perchloric acid containing 0.25 mM EDTA. The extracted ATP and creatine phosphate were determined by means of HPLC (L2000 series; Hitachi, Tokyo) according to the method of Iwai et al. (24).

Oxygen consumption rate of myocardial skinned bundle

The mitochondrial oxygen consumption rate (OCR) of cardiac tissue was determined by the method described previously (13). After perfusion of the right ventricle (n = 6 for each group), myocardial bundles (0.4 mm in diameter and 0.5 mm in length) were prepared from the right ventricular wall by use of a McIlwain tissue chopper (Mickle Lab. Engineering Co., New York, NY, USA). The bundles were incubated in buffer containing 10 mM EDTA, 3 mM $MgSO_4$, 20 mM taurine, 20 mM imidazole, 83.5 mM MOPS, 5 mM ATP, and 15 mM creatine phosphate (pH 7.0) for 20 min at 4°C in the presence of 83 μg/mL saponin, after which the saponin was washed out with the buffer lacking it. Then the OCR of the skinned bundles was determined by means of a Clark-type electrode (Central Science, Tokyo). The basal OCR was measured in the absence of ADP and creatine, and the total (maximal) oxygen consumption rate was measured in the presence of 1 mM ADP and 7.5 mM creatine. Glutamate is a typical substrate for the oxidative phosphorylation of complex I in the mitochondrial electron transport system, whereas succinate is a substrate for the oxidative phosphorylation of complex II. Since ascorbate is a donor of electrons for cytochrome *c* and the reduced cytochrome *c* transfers electrons to complex IV, ascorbate is used as a substrate for cytochrome *c* / complex IV activity. To maintain the reduced form of ascorbate, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine dihydrochrolide (TMPD) is combined with cytochrome *c*. On this basis, we used 5 mM glutamate, 5 mM succinate, and 5 mM ascorbate / 0.25 mM TMPD as the respective substrates to determine activities of complex I, II, and cytochrome *c* / complex IV in the mitochondrial electron transport system.

Statistics

The results were expressed as the means ± S.E.M. All data were normally distributed. Statistical significance of differences in echocardiographic parameters was

estimated by using 2-way ANOVA for repeated measures. Statistical significance of differences in hemodynamics, tissue weight, right ventricular contractile force, and OCR was estimated by using 2-way factorial ANOVA followed by Fisher's PLSD correction for multiple pairwise comparisons. Pearson's correlation coefficient was used to examine the relationship between the right ventricular Tei index measured by the echocardiography and the OCR of the right ventricular skinned bundles. Differences with a probability of 5% or less were considered to be significant ($P<0.05$).

Results

Time course of changes in tissue weight and related parameters

The time course of changes in body, heart, right ventricle, and lung weights of the rats and in parameters related to them are shown in Fig. 1. The body weight of the MCT rats was significantly lighter at the 4th to 8th weeks after monocrotaline administration than that of the age-matched controls at those times (Fig. 1A). The heart weight of the MCT rat was significantly heavier only at the 8th week (Fig. 1B), whereas the ratio of heart weight / body weight of the MCT rat was significantly higher at the 6th and 8th weeks after MCT administration (Fig. 1C). The right ventricular weight, the ratios right ventricular weight / body weight, and right ventricular weight / (left venricular weight + septum weight), the latter being a body weight–independent index of right ventricular hypertrophy, were significantly greater at the 4th to 8th weeks after MCT administration (Fig. 1: D, E, and F). The lung weight and the ratio of lung weight / body weight of the 4w-, 6w-, and 8w-MCT rats were significantly greater than those of the controls (Fig. 1: G and H).

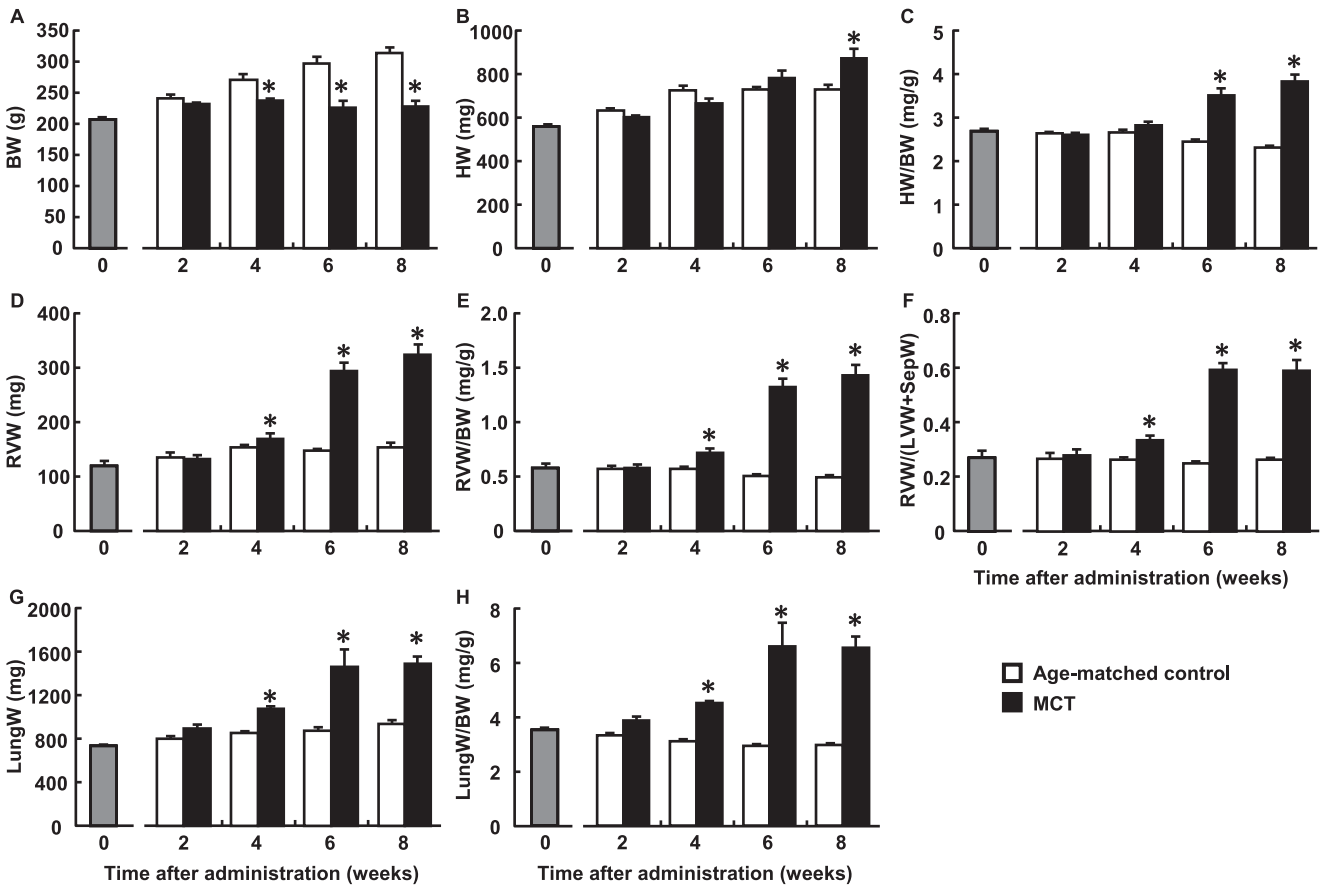

Fig. 1. Time course of changes in the tissue weight parameters of control (0w: hatched column), age-matched control (open columns), and MCT-administered (closed columns) rats at the 2nd, 4th, 6th, and 8th weeks after administration of saline and MCT, respectively. Each value represents the mean ± S.E.M. of 6 experiments. *$P<0.05$ vs. age-matched control group. Abbreviations: BW, body weight; HW, heart weight; HW/BW, heart weight / body weight; RVW, right ventricular weight; RVW/BW, right ventricular weight / body weight; RVW/(LVW + SepW), right ventricular weight / (left ventricular weight + septal weight); Lung W, lung weight; LungW/BW, lung weight / body weight; MCT, monocrotaline.

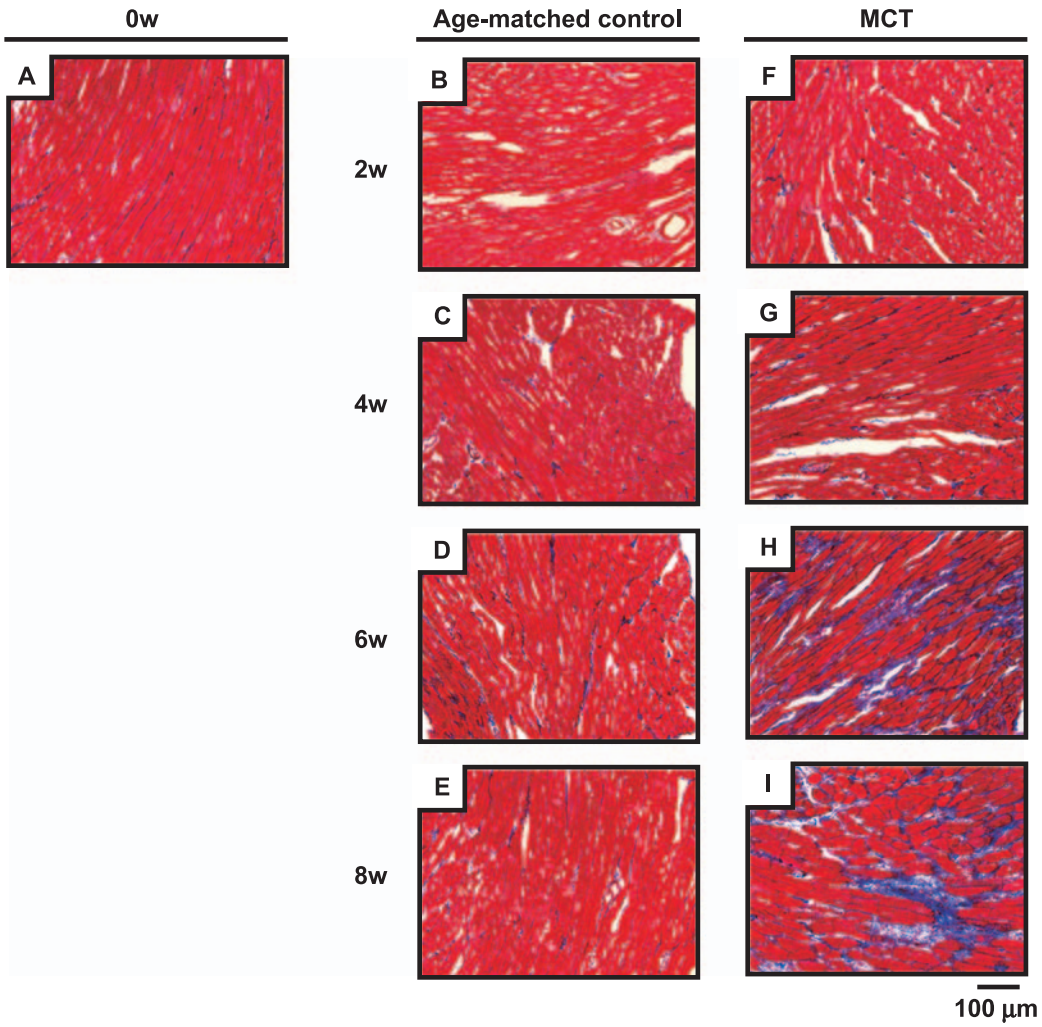

Fig. 2. Representative microphotographs of Masson's trichrome–stained right ventricle of rats before monocrotaline administration (0w: A) and in age-matched control (B – E) and MCT (F – I) rats at the 2nd (B, F), 4th (C, G), 6th (D, H), and 8th (E, I) weeks after vehicle or monocrotaline administration, respectively (n = 2 for each group). Blue-stained areas represent interstitial fibrosis. Scale bar = 100 μm.

Masson's trichrome staining

Figure 2 shows typical photos of Masson's trichrome-stained right ventricles from rats before monocrotaline administration and 2w-, 4w-, 6w-, and 8w-control and MCT rats. The microscopic observations on the 2w- to 8w-control rats were similar to those on the rats before monocrotaline administration. The microscopic observations on the 2w-MCT rats were also similar to those on the rats before monocrotaline administration, in which neither myocardial hypertrophy nor fibrosis was observed (Fig. 2: A and B). In contrast, the 4w-MCT rats showed a slight increase in the degree of fibrosis (Fig. 2C), and the 6w- and 8w-MCT ones showed apparent interstitial fibrosis and hypertrophy of the right ventricle (Fig. 2: D and E).

Hemodynamic parameters

Hemodynamic parameters of the control and MCT rats were determined by the cannula method (Fig. 3). As compared with that of the corresponding control rats, the right ventricular systolic pressure, which is equivalent to the pulmonary arterial systolic pressure, of the MCT rats increased with time after MCT administration: the right ventricular systolic pressure of the 8w-MCT rats was approximately 330% of the corresponding control value (Fig. 3A). The right ventricular end-diastolic pressure and central venous pressure were increased at the 6th and 8th weeks after MCT administration (Fig. 3: B and C). Decreases in mean arterial pressure and heart rate were observed at the 6th and 8th weeks after monocrotaline administration (Fig. 3: D and E).

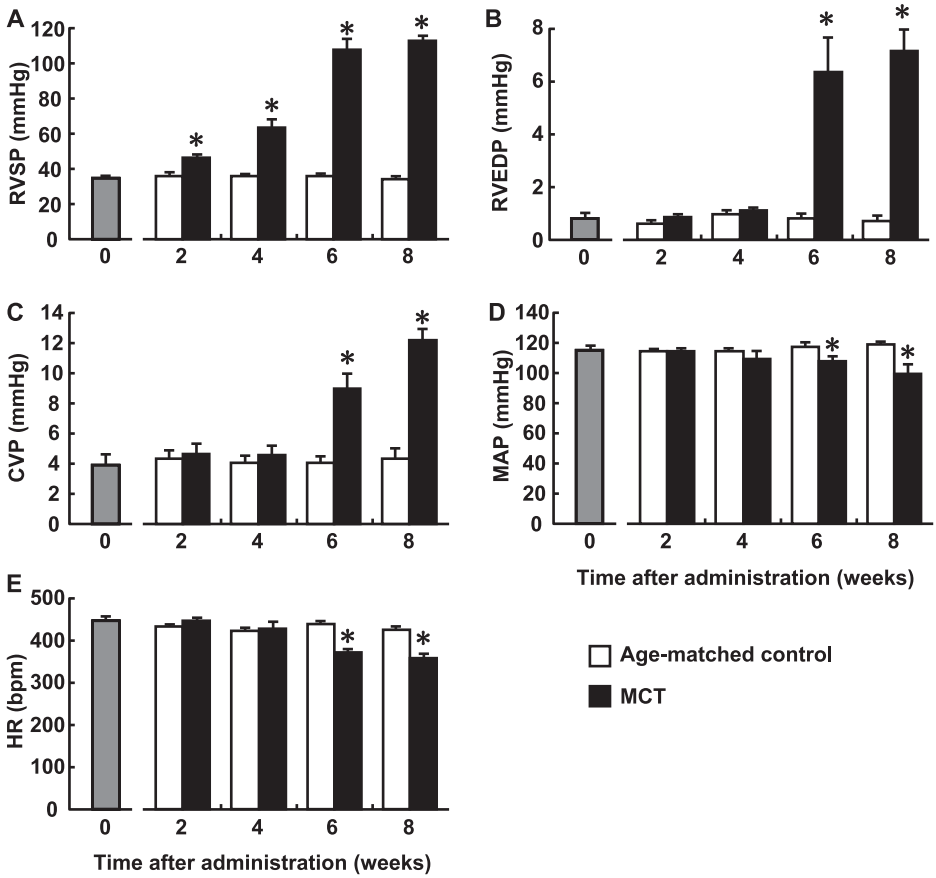

Fig. 3. Time course of changes in hemodynamic parameters of 0 week–control (0w: hatched columns), age-matched control (open columns), and MCT-administered (closed columns) rats at the 2nd, 4th, 6th, and 8th weeks after vehicle or monocrotaline administration, respectively. Each value represents the mean ± S.E.M. of 6 experiments. **P*<0.05 vs. age-matched control group. Abbreviations: RVSP, right ventricular systolic pressure; RVEDP, right ventricular end-diastolic pressure; CVP, central venous pressure; MAP, mean arterial blood pressure; HR, heart rate; MCT, monocrotaline.

Echocardiographic parameters

Figure 4 shows cardiac parameters determined by the echocardiographic system to assess the progression of pulmonary artery hypertension in rats at multiple time points. Serial echocardiographic measurements were performed on control and MCT rats at the 2nd, 4th, 6th, and 8th weeks after MCT administration. As compared with those of the control rats, the cardiac output index, stroke volume index, and heart rate of the MCT rats were decreased at the 6th week (Fig. 4: A – C). PAAT, an estimate of the pulmonary artery systolic pressure, was decreased at the 2nd to 8th weeks after monocrotaline administration (Fig. 4D). The right ventricular Tei index, an increase in which results from the development of right ventricular dysfunction and provides prognostic information on a variety of myocardial conditions, was increased at the 4th to 8th weeks after MCT administration as compared with that for the corresponding control rats (Fig. 4E).

Contractile force and heart rate of isolated right ventricle

To examine alterations in the right ventricular function of the rats, we examined the double product (developed tension × heart rate), a measure of cardiac workload, of the isolated perfused right ventricular muscle. The basal value for the double product of the control animal was 403 ± 23 g·bpm (n = 6). As shown in Fig. 5, the basal double products of the 2w- and 4w-MCT rats were similar to those of the corresponding control rats. On the other hand, the double products of both 6w- and 8w-MCT rats were significantly decreased to approximately 50% of the corresponding controls.

HEP contents of the right ventricle

To assess energy levels of the right ventricular muscle, we determined the levels of HEPs in the isolated perfused right ventricular muscles from control and MCT rats (Fig. 6). The right ventricular ATP contents of the 2w- and 4w-MCT rats were similar to those of the corresponding control rats, whereas those of the 6w-

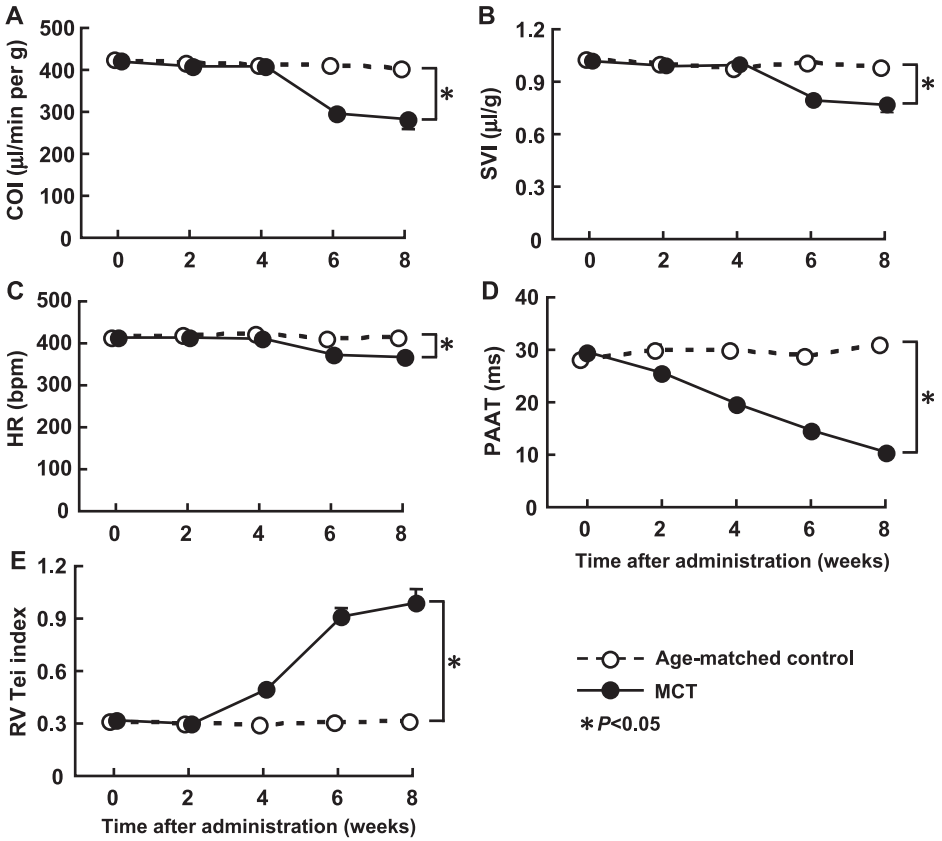

Fig. 4. Serial echocardiographic parameters of age-matched control and MCT rats at 0 to 8 weeks after vehicle or monocrotaline administration of saline and MCT, respectively. Each value represents the mean ± S.E.M. of 6 experiments. *P* values represent statistical significance among age-matched control and MCT groups. Statistical analysis was performed by two-way ANOVA for repeated measures. Abbreviations: COI, cardiac output index; SVI, stroke volume index; PAAT, pulmonary artery flow acceleration time; HR, heart rate; RV Tei index, right ventricular Tei index; MCT, monocrotaline.

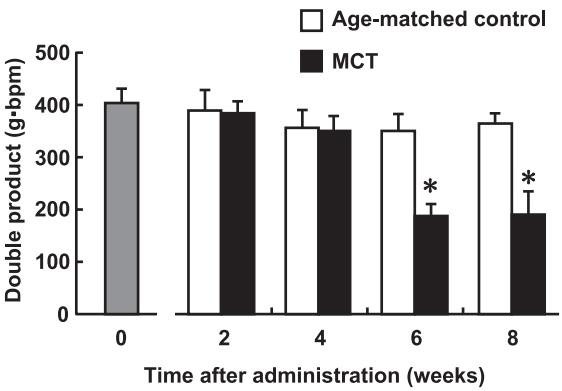

Fig. 5. Time course of changes in the basal double product of the isolated perfused right ventricular muscle of 0 week–control (0w: hatched columns), age-matched control (open columns), and MCT-administered (closed columns) rats at the 2nd, 4th, 6th, and 8th weeks after vehicle or monocrotaline administration, respectively. Each value represents the mean ± S.E.M. of 6 experiments. *P<0.05 vs. age-matched control group.

and 8w-MCT rats were decreased to approximately 75% and 70% of the respective values for the corresponding control rats. The right ventricular creatine phosphate contents of the 6w- and 8w-MCT rats were also decreased, to approximately 75% and 65% of the respective values for the corresponding control rats.

Mitochondrial oxygen consumption rate of skinned bundles

To elucidate the mitochondrial function of the MCT rats, we determined the mitochondrial OCR of the skinned bundles prepared from the right ventricle from control and MCT rats (Fig. 7). OCR of the skinned bundles of the 6w- and 8w-MCT rats in the presence of glutamate for complex I or ascorbate/TMPD for cytochrome *c* / complex IV were decreased, whereas those of the 2w- and 4w-MCT rats were not altered significantly (Fig. 7: A and C). In contrast, the OCRs of the right ventricular muscle of the MCT rats in the presence of succinate for complex II did not change throughout the experiment (Fig. 7B).

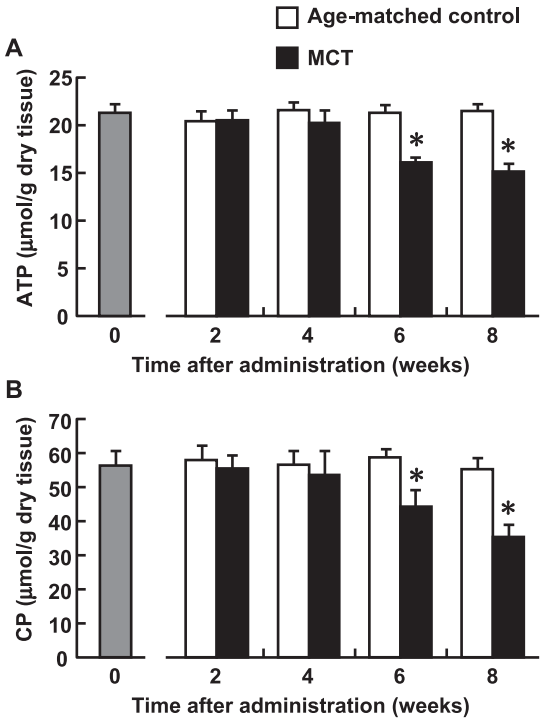

Fig. 6. Time course of changes in ATP and creatine phosphate (CP) contents in the right ventricular muscles of 0 week–control (0w: hatched columns), age-matched control (open columns), and MCT-administered (closed columns) rats at the 2nd, 4th, 6th, and 8th weeks after vehicle or monocrotaline administration, respectively. Each value represents the mean ± S.E.M. of 6 experiments. **P*<0.05 vs. age-matched control group.

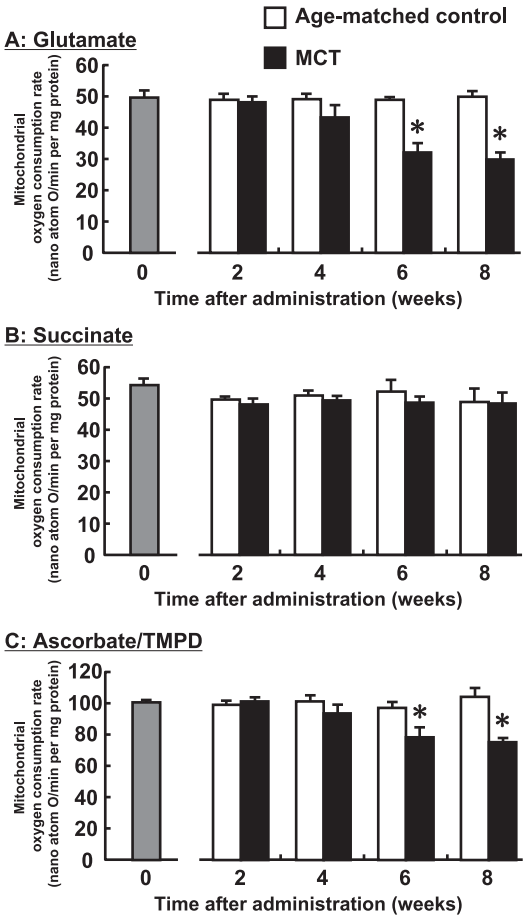

Fig. 7. Time course of changes in mitochondrial oxygen consumption rate with glutamate (A), succinate (B), or ascorbate/TMPD (C) as the substrate for the skinned bundles from the right ventricular muscles of 0 week–control (0w: hatched columns), age-matched control (open columns), and MCT-administered (closed columns) rats at the 2nd, 4th, 6th, and 8th weeks after vehicle or monocrotaline administration, respectively. Each value represents the mean ± S.E.M. of 6 experiments. **P*<0.05 vs. age-matched control group.

To elucidate the relationship between myocardial performance and the mitochondrial energy-producing ability of the right ventricular muscle, we plotted OCR of the skinned bundles from MCT rats measured in the presence of each substrate against the right ventricular Tei index (Fig. 8). OCR with glutamate or ascorbate /TMPD were inversely and highly related to the right ventricular Tei indices of the 2w-, 4w-, 6w-, and 8w-MCT rats (Fig. 8: A and C). In contrast, OCR determined with succinate did not correlate with the right ventricular Tei index (Fig. 8B).

Discussion

In the present study, we first measured the hemodynamic parameters of MCT rats by a cannula method. The right ventricular systolic pressure, that is, the systolic pressure of the pulmonary artery, of the MCT rats increased along with time after monocrotaline administration. The right ventricular end-diastolic pressure and central venous pressure also increased, at the 6th and 8th weeks, after the administration, and these increases were associated with decreases in mean arterial pressure and heart rate. Determination of tissue weights revealed greater increases in the right ventricular weight, right ventricular weight / body weight, right ventricular weight / (left ventricular weight + septum weight), lung weight, and lung weight / body weight at the 4th to 8th weeks after MCT administration, whereas a significant change in heart weight was seen at the 8th week and a significant change in heart weight / body weight was seen at the 6th and 8th weeks. The alterations in the right ventricular weight and the ratios related to them indicate that right ventricular hypertrophy preceded the increase in the whole heart weight. Since the ratio of right ventricular weight / (left ventricular weight + septum weight) is considered to be a marker of right ventricular hypertrophy, augmentation of this ratio may represent the genesis of right ventri-

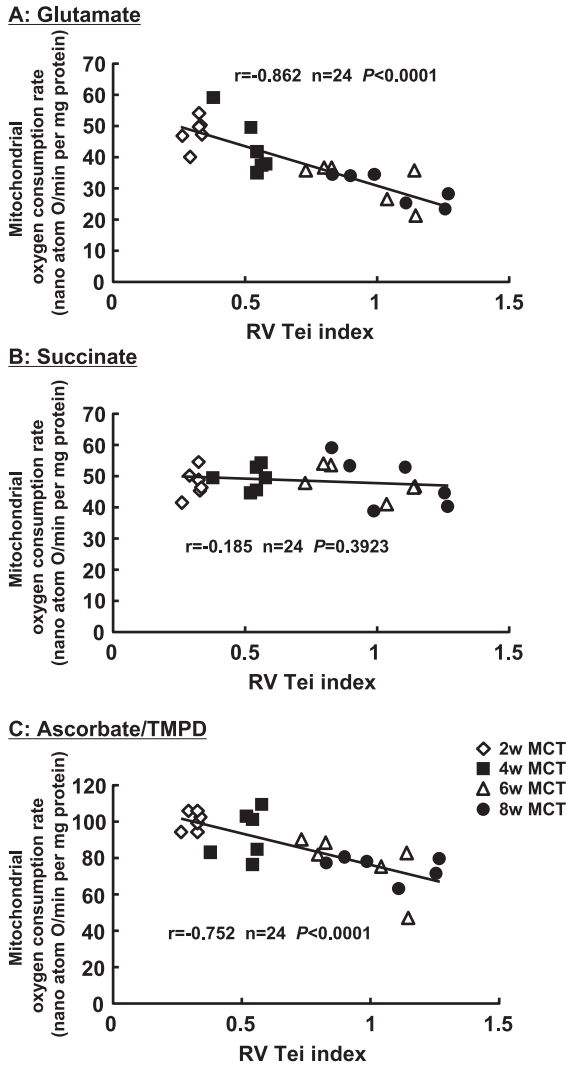

Fig. 8. Relationship between the right ventricular Tei index and mitochondrial oxygen consumption rate in the presence of glutamate (A), succinate (B), or ascorbate/TMPD (C) as the substrate for mitochondria prepared from the 2w- (open diamonds), 4w- (closed squares), 6w- (open triangles), and 8w- (closed circles) MCT rats. Significant relationships between the reduction in the right ventricular Tei index and the mitochondria oxygen consumption rate with glutamate (n = 24, $P<0.0001$) and between the decrease in the right ventricular Tei index and the reduction in the mitochondria oxygen consumption rate with ascorbate/TMPD (n = 24, $P<0.0001$) were observed.

cular hypertrophy. Accordingly, the above findings confirmed that monocrotaline was an appropriate tool for induction of pulmonary artery hypertension and right ventricular hypertrophy, as proposed by others (1 – 4). The results of Masson's trichrome staining of the 6w- and 8w-MCT rats showed apparent interstitial fibrosis and hypertrophy in the right ventricle, which were comparable to the increase in the right ventricular weight along with time after monocrotaline administration. These results suggest that monocrotaline induces pulmonary artery hypertension, followed by an increase in right ventricular pressure overload, which leads to right ventricular dysfunction.

We further examined the whole heart and right ventricular function by using echocardiography. Several studies by echocardiography have been performed to determine cardiac and right ventricular function in monocrotaline-induced pulmonary artery hypertension in rats (18, 25, 26). However, satisfactory data have not been obtained so far, probably due to the limitation of the ability of the probe of the transducer employed or technical difficulty in the application of the probe in small animals (16). We also evaluated the right ventricular function by examining the pulmonary arterial flow by using the color Doppler method. Since changes in the pulmonary arterial flow waveform and PAAT have been found to be early events in the development of pulmonary artery hypertension in rats, PAAT, a measure that is most closely correlated with increased pulmonary artery systolic pressure, can be used for estimation of the pulmonary artery systolic pressure in this model (18). The indices for cardiac output and stroke volume of the 6w- and 8w-MCT rats were decreased, suggesting the genesis of heart failure in this model. The right ventricular Tei index is used for the assessment of right ventricular dysfunction in patients with pulmonary artery hypertension (21, 27, 28). The right ventricular Tei indices of the 6w- and 8w-MCT rats were markedly increased, suggesting that the right ventricular function was maintained until the 4th week, but aggravated thereafter.

Secondly, we found that the double product, a measure of cardiac muscle workload (23), of isolated perfused right ventricular muscles of the 6w- and 8w-MCT rats, was decreased. These results suggest that the right ventricular failure occurs, at the latest, by 6 weeks after monocrotaline administration. We also obtained a very low value for the double product of the isolated perfused right ventricular muscle of the 6w-MCT rats, suggesting that the contractile machinery for the right ventricular muscle is seriously injured by this time. Such severe impairment might be compatible with the findings obtained from the Masson's trichrome staining. However, despite such a close relation between the hemodynamic parameters and histological observation, some other mechanisms responsible for alterations in the circumstances of the right ventricular muscle should be considered.

Under normal working conditions approximately 70% of the ATP in the myocardium is used for cardiac contractility and the other 30%, for generation of intracellular ion pump activity to maintain cardiac cell

membrane integrity (29). Generally, the maintenance of the cardiac workload at a high level is essential for energy provision from mitochondria, which produce more than 90% of the energy in the heart (9). As described above, studies on mitochondrial function in cardiac tissue in patients with heart failure showed that the mitochondrial capacity for oxygen consumption and oxidative phosphorylation were reduced (10 – 12). Additionally, earlier studies from our laboratory (30) have shown decreases in ATP and creatine phosphate contents and a concomitant reduction in the mitochondrial energy-producing ability in the failing heart following left coronary artery ligation. Thus, we propose the idea that the mitochondrial energy-producing ability of the right ventricular muscle after monocrotaline administration may be decreased during the development of right ventricular hypertrophy and/or right ventricular failure.

To support this idea, we examined the right ventricular ATP and creatine phosphate contents of MCT rats. These contents of the 6w- and 8w-MCT rats were decreased, whereas no significant changes in high-energy phosphates were detected in the 2w- and 4w-MCT rats, suggesting that the mitochondrial energy-producing ability declined at the 6th and 8th weeks after monocrotaline administration. The mitochondrial OCRs of the skinned bundles with glutamate as the substrate for complex I, and ascorbate/TMPD for cytochrome *c* / complex IV, in the 6w- and 8w-MCT rats were reduced, whereas those of the 2w- and 4w-MCT rats did not decrease, suggesting that a decreased enzyme activity of complex I and cytochrome *c* / complex IV in the failing heart is involved in the reduction in the mitochondrial energy-producing ability. Our findings suggest that mitochondrial complex I– and IV–dysfunction may thus play an important role in the development of right ventricular failure. Since it remains unclear whether the decrease in mitochondrial function is induced via reduction in mitochondrial oxidative phosphorylation proteins or production of inhibitory factor for mitochondrial oxidative phosphorylation, further studies are required to elucidate the mechanism underlying reduction in mitochondrial oxidative phosphorylation activity in the right ventricular failing heart.

Furthermore, we found an inverse relationship between a decrease in the right ventricular Tei index and a reduction in complex I enzyme–mediated and/or cytochrome *c* / complex IV enzyme-mediated oxidative phosphorylation. Since complex I in the mitochondrial electron transport system plays a critical role in mitochondrial oxidative phosphorylation, the reduced activity of mitochondrial complex I may cause a decrease in energy production for cardiac contractile function. This idea is comparable to the hypothesis by other investigators that a reduction in complexes I– and IV–mediated mitochondrial activities may induce a leak of electrons from the mitochondrial electron transport system, leading to generation of reactive oxygen species. In the present study, we have not yet examined the release of reactive oxygen species from the mitochondria after monocrotaline administration. In the future, further experiments should be performed to determine the contribution of reactive oxygen species to the genesis of right ventricular failure. The present study may provide evidence showing that a reduction in the mitochondrial enzyme activity in the electron transport system may be involved in the genesis of right ventricular dysfunction of MCT-administered animals.

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