Myocyte Morphological Characteristics Differ Between the Phases of Pulmonary Hypertension-Induced Ventricular Hypertrophy and Failure

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SUMMARY

Pulmonary hypertensive model rats were prepared by treating them with monochrotaline (MCT). Using these model rats, we examined myocyte remodeling in the right ventricle in response to increased right ventricular pressure.

Male Sprague-Dawley rats were divided into 2 groups. Group M received MCT and group C received physiological saline. The 2 groups were examined at weeks 2, 5, and 7 after MCT or saline injection, respectively. At week 2, a significant difference in cell form was not observed in either group. At week 5, cell volume and myocyte cross-sectional area (CSA) of the right ventricle in group M were significantly greater than those in group C. At week 7, cell volume, CSA, and cell length of the right ventricle in group M were all significantly greater than those in group C. These results suggest that pulmonary hypertension causes hypertrophy, accompanying the enlargement of CSA in the right ventricle, and that cells lengthen in the phase of right ventricular failure.

These results are similar to the changes observed in left ventricular myocytes due to overload pressure. Both right and left ventricular myocytes may share a common mechanism for myocyte remodeling as an adaptive and maladaptive response to increased ventricular pressure. (Int Heart J 2006; 47: 629-637)

Key words: Monocrotaline, Isolated myocyte, Right ventricular hypertrophy, Pressure overload

CARDIAC hypertrophy occurs under pressure overload of the ventricle. Chronic pressure overload eventually produces heart failure and then causes auxocardia (an increase in ventricular diameter). By using spontaneously hypertensive heart failure (SHHF) rats, Gerdes, et al investigated morphometric changes in left ventricular myocytes under pressure overload.1) They found that the CSA of the left ventricular myocytes increases in the hypertrophic phase and

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that the cells lengthen in the failing heart phase, suggesting the possibility that myocyte remodeling is associated with the transition from hypertrophy to failure.

On the other hand, pulmonary hypertension causes hypertrophy in the right ventricle. Chronic pressure overload leads to right ventricular failure. However, the association of myocyte remodeling with the transition from hypertrophy to failure in the right ventricle has rarely been investigated.\(^2\)\(^-\)\(^6\)

In this study, we prepared pulmonary hypertensive model rats by administering a pyrrolizine alkaloid plant extract, monochrotaline (MCT). Using this rat model, we investigated the relationship between right ventricular hypertrophy, right ventricular dysfunction, and myocyte remodeling.

**METHODS**

1. **Experimental animals:** Male Sprague-Dawley rats weighing approximately 200 g were divided into 6 groups. Group C2W, a control group, was examined 2 weeks after vehicle injection \((n = 7)\). Group M2W was examined 2 weeks after MCT injection \((n = 8)\), and group C5W or M5W was examined 5 weeks after vehicle \((n = 7)\) or MCT injection \((n = 8)\), respectively. Group C7W or M7W was examined 7 weeks after vehicle \((n = 8)\) or MCT \((n = 7)\) injection. The MCT-treated groups were given MCT crystals (Sigma Chemical Co., St Louis, USA). The crystals were dissolved in 1N HCL, neutralized with NaOH, and diluted with distilled water to create a solution containing 2% MCT. This solution was subcutaneously injected at the rate of 40 mg/kg.\(^1\) The control group received subcutaneous injections of physiological saline. All procedures conformed to the institutional guidelines of the Jikei University School of Medicine Animal Care and Use Committee.

2. **Hemodynamic measurements and echocardiographic findings:** Under anesthesia and after stabilizing body movement, blood pressure and heart rate were measured using the tail-cuff method. Ketamine hydrochloride (30 mg/kg) and xylazine (5 mg/kg) were injected intramuscularly and then echocardiography was performed.\(^7\) Standard echocardiography techniques using an echo machine with a 7.5 MHz transducer (model SONOS 100CF Agilent Technologies, Tokyo) were used to obtain 2-dimensionally targeted M-mode with short-axis views of the left ventricle at or just below the tip of the mitral valve leaflets.

3. **Obtaining isolated myocytes:** After echocardiography, 1,000 U/kg of heparin was injected into the abdominal cavity. The hearts were removed, blotted, weighed, and immediately cannulated through the aorta for retrograde perfusion with Joklik medium (Sigma) containing 0.01 mmol/L EGTA (Sigma) followed by Joklik medium plus collagenase (286 U activity/mL; Worthington Biochemical Co., Lakewood, USA). The atrium and aorta were removed from the heart and
then the ventricles were separated into 3 sections (left ventricle, ventricular septum, and right ventricle). Each tissue was minced and poured through 250 µm nylon mesh to obtain cell suspensions. The collected myocytes were fixed in a solution containing 2% glutaraldehyde.8-10)

4. Isolated myocytes: Cell volume was measured with a Coulter Channelizer (model C256, Beckman & Coulter Corp., Tokyo) by measuring the change in electrical resistance of each cell as it passed through an opening 200 µm in diameter. Each sample provided at least 10,000 measurements, from which an average cell volume was calculated. Cell length, defined as the longest length parallel to the longitudinal axis of the myocyte, was measured in at least 40 cells in order to reduce the sampling error to less than 3%.11) using a phase microscope (Nikon Corp., Tokyo), from which the average length was calculated. The cellular CSA was calculated by dividing the cell volume by the cell length.8-10)

Statistical analysis: Measurements are expressed as the mean ± SD. Student’s t test was used to determine if the differences between the control and MCT groups were significant. A 2-sided P value less than 0.05 was considered statistically significant.

RESULTS

In group M5W, neither retention of pleural effusion nor pericardial effusion was observed. Retention of pleuroperitoneal effusion was seen in group M7W, but not in group C7W.

1. Heart weight: The Table shows the changes in body and heart weights in the groups. No significant differences in body weight or heart weight, or heart/body weight ratio were seen between groups C2W and M2W. Group M5W, however,
had a significantly greater heart/body weight ratio than group C5W. No significant differences in body weight or heart weight were seen between groups C7W and M7W. Group M7W, however, had a significantly greater heart/body weight ratio than group C7W.

2. Hemodynamics: There were no significant differences in blood pressure levels between groups C2W and M2W or between groups C5W and M5W. There were also no significant differences in blood pressure levels between groups M7W and C7W.

3. Echocardiographic findings: No significant differences in left ventricular wall thickness, left ventricular diameter, fractional shortening, LV wall stress, or LV mass were found between groups M2W and C2W, between groups M5W and C5W, or between groups M7W and C7W.

4. Myocyte investigation:

   a) Left ventricular myocytes. Figure 1 shows the results for the left ventricular myocytes. There were no significant differences in cell volume, cell length, or myocyte CSA between groups M2W and C2W, or between groups M5W and C5W. There were also no significant differences in these data between groups M7W and C7W.

   b) Right ventricular myocytes. Figure 2 shows the results for the right ventricular myocytes. No significant differences were seen in the right ventricular cell forms between groups M2W and C2W. Moreover, no significant differences
in cell length were seen between groups M5W and C5W, although group M5W had greater cell volume and myocyte CSA than group C5W. Group M7W had greater cell volume, cell length, and myocyte CSA than group C7W.

**DISCUSSION**

1. **Right ventricular pressure overload model**: MCT is an alkaloid extracted from a plant. It is widely used in experiments because it causes pulmonary hypertension as a result of necrotizing pulmonary arteritis. Pulmonary hypertension increases right ventricular wall thickness under pressure overload and causes myocardial failure in the right ventricle at the end stage. In most cases, a single dose (40-60 mg/kg) of MCT is used. The death rate from failing myocardium in the right ventricle as a result of MCT administration increases in a dose-dependent manner.12-17)

Hirota, et al2) subcutaneously injected 40 mg/kg of MCT solution into rats. Two weeks later, the right ventricular systolic pressure rose to 25.4 ± 2.2 mmHg in the MCT group and to 13.3 ± 1.4 mmHg in the control group. The right/left ventricle + septal weights also increased. Furthermore, after 6 weeks, the left ventricular systolic pressure went down to 79.1 ± 3.8 mmHg in the MCT group compared to 95.7 ± 3.1 mmHg in the control group. They also reported that atrial natriuretic peptide (ANP) in the plasma and myocardial tissues rose significantly
so as to induce heart failure in the MCT group. Adopting the methods of Hirota, et al we subcutaneously injected 40 mg/kg of an MCT solution into rats and investigated their conditions 2, 5, and 7 weeks after the injections. It should be pointed out that this experiment has 2 study limitations. 1) We were unable to obtain accurate measurements of the weights of the left ventricle, ventricular septum, and right ventricle in the animals since the hearts were removed and immediately perfused with medium containing collagenase, and 2) We did not monitor pulmonary arterial pressure. However, 7 weeks after the injection, systemic edema as well as pleural and peritoneal effusion were observed, suggesting the presence of right ventricular failure. Moreover, by week 5, the cell volume of the right ventricle had increased, suggesting that pulmonary hypertension may induce right ventricular hypertrophy in this stage.

2. Analysis of isolated cardiac myocytes: In this investigation of overload pressure on the right ventricle, the cell volume and CSA of the right ventricular myocytes had increased at week 5. However, in addition to the increases in cell volume and CSA, an increase in cell length of the right ventricular myocytes at week 7 was also observed.

Various studies have been conducted concerning left ventricular hypertrophy under pressure overload and the changes in the shape of isolated cardiac myocytes in the failing heart. Gerdes, et al reported that spontaneously hypertensive heart failure (SHHF) rats, which are typical models of left ventricular heart failure under pressure overload, showed no changes in the CSA of the myocytes in the uncompensatory stage of left ventricular failure (24 months), compared to the stage of left ventricular hypertrophy (12 months), however, cell length and volume both increased. Moreover, the cell volume of the right ventricular myocytes increased remarkably, and unlike in the left ventricle, both cell length and CSA increased and the ratio of cell length and CSA remained consistent.1) Gerdes, et al suggested that the CSA of the left ventricular myocytes did not change and only the cell length increased nonuniformly, which may have caused an uncompensatory failing heart. They also remarked that the right ventricular myocytes could adapt to various conditions. Onodera, et al used SHHF rats to demonstrate that the cell volume and length both increased in the left ventricular myocytes over time, however, the CSA of the cell did not increase after 12 months of age. These unbalanced morphological changes in the cardiac myocytes may cause heart failure.10)

According to the law of Laplace, LV wall stress (left ventricular stress) can be expressed as pressure radius (inner radius) /2 (wall thickness). In other words, wall stress is proportional to the lumen and inversely proportional to wall thickness. According to this rule, increased pressure overload in the left ventricle influences wall thickening, which is the denominator of the equation, resulting in
a constant wall stress. However, when the lumen expands, the wall stress increases to an irreversible level that cannot be uncompensated, and then leads to heart failure. At the cellular level, the cardiac myocytes surrounding the ventricle, and the cell diameter, ventricular wall thickness, and cell length are proportional to the lumen diameter of the ventricle. In left ventricular myocytes under left ventricular pressure overload, the morphological changes that increase the wall thickness are compensatory remodeling, while the increased cell length in the failing heart is uncompensatory remodeling.

Zimmer, et al\(^{18}\) created a wide range of myocardial infarctions in rats by ligating the left anterior descending artery. They reported a decrease in left ventricular pressure, and an increase in end-diastolic pressure in the left ventricle. Tissue proliferation in the pulmonary arteries, followed by pulmonary hypertension was also seen. In these rats, the right ventricular weights increased remarkably, having developed right ventricular hypertrophy. Both cell length and CSA increased in the right ventricular myocytes.\(^{18}\) However, in the group of rats that had developed a wide range of myocardial infarctions, the left ventricular end-diastolic pressure increased remarkably to 32 ± 2 mmHg compared to 3.4 ± 0.8 mmHg in the control group, and blood pressure in the group with myocardial infarction was significantly lower than that in the control group, resulting in a diagnosis of severe heart failure in the left ventricle, as well as possible progression from hypertrophy to failure in the right ventricle. Morphological changes were investigated in a study using female Sprague-Dawley rats subjected to a state of intermittent hypoxia, thereby producing a pulmonary hypertension rat model. The results showed increases in cell volume and CSA in the right ventricular myocytes. The cell length, however, did not change.\(^{19}\) Therefore, in both the left and right ventricles, in the compensation stage, neither myocyte cell length nor lumen diameter increases, while in the noncompensation stage, the myocytes lengthen and the lumen expands. The cardiac hypertrophy corresponding to pressure overload and changes in cellular shapes in both ventricles of a failing heart are similar, which suggests a common physiological compensatory mechanism may be involved in pressure overload. Even in current clinical practice where treatment is advanced, partly as a result of longer life spans, patients still die of cor pulmonale caused by pulmonary disease. Suppressing this right ventricular remodeling may lead to improvements in deteriorated QOL or a decrease in the number of deaths from right ventricular failure.

**Conclusion:** Right ventricular hypertrophy was produced by injecting right ventricular dysfunction model rats with MCT. Marked increases in cell volume and CSA of the right ventricle were observed at the hypertrophied stage. Cell lengthening was observed during heart failure. These cell shape changes are similar to the changes in left ventricular cells when they respond to left ventricular pressure overload.
overload, which indicates a common physiological compensatory mechanism working on both right and left ventricles. Further studies are needed in order to obtain a better understanding of the mechanism, however, the present study results suggest the development of an effective treatment for right ventricular dysfunction in the future may be possible by targeting the mechanism.

REFERENCES