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Reverse Remodeling of Cardiac Hypertrophy in Rats with Renovascular Hypertension

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ABSTRACT

Cardiac hypertrophy is a contributory factor in heart failure, arrhythmias, and sudden death. Therefore, understanding the reverse remodeling of cardiac hypertrophy is important. Reverse remodeling of cardiac hypertrophy in rats with two-kidney, one-clip renovascular hypertension (RHT) was examined 1, 3, 7, and 14 days after removal of the clipped kidney. The cardiac geometry and the single-cell morphology of myocytes and histological changes were examined. The heart weight/body weight ratio and the left ventricular wall thickness increased markedly in the RHT rats but returned to control levels within 14 days. The average length, cross-sectional area, and volume of myocytes were significantly higher in RHT rats than in control rats. The myocyte length and volume remained increased for 14 days. On the other hand, the extent of cardiac fibrosis and collagen accumulation in the RHT rats began to decrease after 3 days and returned to control levels within 14 days. These results demonstrate that the removal of the clipped kidney in RHT rats causes immediate regression of the cardiac fibrosis and collagen accumulation before the morphological improvement of hypertrophic myocytes. These findings suggest that the regression of cardiac fibrosis in early reverse remodeling should be considered as a treatment for hypertensive cardiac hypertrophy. (Jikeikai Med J 2006 ; 53 : 101-9)

Key words : cardiac fibrosis, cardiac hypertrophy, cardiomyocyte, reverse remodeling, renovascular hypertensive rat

Introduction

Cardiac hypertrophy represents a physiological adaptation to maintain normal wall tension in the heart against pressure overload. Cardiac hypertrophy is also recognized as an independent risk factor in patients with cardiac diseases[1,2]. The pathological features of morbid cardiac hypertrophy are cellular hypertrophy of myocytes and proliferation of connective tissue. Many reports have demonstrated cellular remodeling of myocytes in cardiac hypertrophy. Increases in both the cell volume and the cross-sectional area of myocytes are involved in the development of cardiac hypertrophy. On the other hand, increase in the cell length of myocytes was observed during the progression to heart failure[3,4]. Regression of myocyte hypertrophy, including the increased cell volume, cell length, and cross-sectional area, are observed in spontaneously hypertensive rats with heart failure following the administration of an angiotensin II type 1 receptor blocker (ARB) or angiotensin converting enzyme inhibitor (ACE-I)[5]. Although these findings reflect the effects of long-term treatment with these drugs on reverse remodeling of cardiac hypertrophy, the time-course of prompt reverse remodeling has not been clarified.

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The proliferation of connective tissue in cardiac hypertrophy is mainly associated with the accumulation of collagen in the extracellular matrix. An increase in the deposition of interstitial collagen produced by fibroblasts may be involved in the pathogenesis of diastolic dysfunction, heart failure, arrhythmias, and sudden death in patients with cardiac hypertrophy[6,7]. Treatment with TCV116, an ARB, decreases the extent of cardiac fibrosis surrounding the myocytes and vessels to about one-third in rats with renovascular hypertension (RHT)[8,9]. We have previously demonstrated that treatment with an ARB or ACE-I improves myocyte hypertrophy and cardiac fibrosis in RHT rats[10]. However, the time-course of regression of the myocyte hypertrophy and cardiac fibrosis in cardiac hypertrophy has not been clarified. Therefore, we examined the morphological changes of hypertrophic myocytes and the histological changes in the extent of cardiac fibrosis and collagen accumulation in the early stage after removal of the clipped kidney in two-kidney, one-clip hypertensive (2K1C) rats.

METHODS

Experimental models

RHT was produced in 12 female Wistar rats weighing 120 to 130 g (RHT rats) by constricting the left renal artery with a silver clip, 0.4 mm in diameter, under anesthesia induced with intramuscular injection of ketamine hydrochloride (30 mg/kg) and xylazine (5 mg/kg). After 5 weeks of induction of 2K1C RHT, cardiac hypertrophy was confirmed by the increase in left ventricular (LV) wall thickness on echocardiograms[11]. Subsequently, the clipped kidney was removed for immediate reduction of the high blood pressure (BP) and LV wall stress in the RHT rats. Then, reverse remodeling of the cardiac hypertrophy was examined in the one-kidney rats 1, 3, 7, and 14 days, respectively, after uninephrectomy in the N1D group ($n=6$), N3D group ($n=6$), N7D group ($n=12$), and N14D group ($n=12$). Wistar rats matched for age with the RHT group were examined as a control group ($n=12$). The BP was measured using the tail-cuff method (BP-98A, Softron, Tokyo) starting 1 week after the clipping. The rats were housed in a temperature-controlled room (23-25°C) with a 12-hour light/dark cycle and allowed free to access to food and water. All procedures were performed in accordance with the guidelines of the institutional guidance committee of The Jikei University School of Medicine.

Echocardiography

The rats were anesthetized with ketamine (10 mg/kg) and xylazine (2 mg/kg). Five weeks after the clipping, transthoracic echocardiography was performed with the Sonos 100 CF system using a 7.5-MHz probe (Hewlett-Packard, Andover, MA, USA). After satisfactory two-dimensional images had been obtained, M-mode images of the LVs were obtained. Systolic and diastolic LV anterior wall thicknesses, systolic and diastolic LV internal diameters, and systolic and diastolic LV posterior wall thicknesses were measured. The end-systolic volume and end-diastolic volume were calculated with the Sonos 100 system.

Preparation of the heart specimens

The rats were anesthetized with an intramuscular injection of ketamine hydrochloride (30 mg/kg) and xylazine (5 mg/kg), and an intraperitoneal injection of heparin (3,000 U/kg) was administered at the end of the protocol. The hearts were excised and immediately weighed. The hearts were placed in ice-cold phosphate-buffered saline (PBS ; 136.8 mM NaCl, 2.7 mM KCl, 8.1 mM NaH_2PO_4, and 1.46 mM KH_2PO_4) and subjected to the following examinations.

Myocyte isolation

Myocytes were isolated with the method of Gerdes et al[12]. The hearts were cannulated through the aorta for retrograde perfusion (Langendorff method) and perfused for 12 minutes with Joklik's medium (Sigma-Aldrich, St. Louis, MO, USA) containing collagenase. The left ventricular wall was then minced in Joklik's medium and filtered through a nylon mesh to isolate the myocytes. The isolated myocytes were fixed in a solution containing 1.5% glutaraldehyde (Fig. 1).

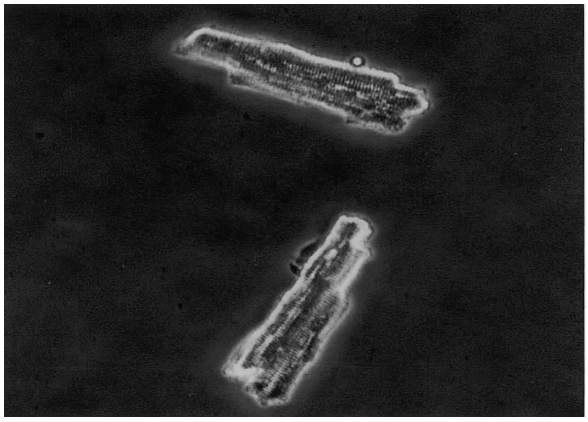

Fig. 1. A phase-contrast micrograph of isolated cardiac myocytes.

Morphological measurements of the isolated myocytes

The myocyte length, defined as the longest length parallel to the longitudinal axis, of at least 40 myocytes from each sample was measured under a phase-contrast microscope (Nikon K.K., Tokyo), and the average length was calculated. The average myocyte volume was measured with a cell counter (Z2 Coulter Counter, Beckman Coulter, Inc., Fullerton, CA., USA). The average cross-sectional area of the myocytes was calculated from the average myocyte volume divided by the average myocyte length.

Histological examinations of the heart specimens

A cross section of the internal area of the heart was cut and fixed with 10% buffered formalin. The fixed cardiac tissues were embedded in paraffin and cut into 4-μm-thick sections, which were stained with the Masson's trichrome method for detecting cardiac fibrosis and the Sirius red method for detecting collagen accumulation. To estimate the degree of cardiac fibrosis, photomicrographs were digitized with a digital imaging system (E990, Nikon K.K.). The total myocardial area and the fibrosis area were assigned numerical values with an imaging software program (NIH Image, U.S. National Institutes of Health, Bethesda, MD, USA), and the ratio of cardiac fibrosis was normalized by the cross-sectional area of the LV. These methods have been described in our previous report[13].

Reverse transcription polymerase chain reaction of atrial natriuretic peptide messenger RNA

Reverse transcription polymerase chain reaction (RT-PCR) analysis was performed to evaluate the expression levels of atrial natriuretic peptide (ANP) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) messenger (m) RNAs. Total RNA was extracted from the myocardium using the standard guanidium method, and the expression level of the ANP gene was examined with the RT-PCR method using Moloney murine leukemia virus reverse transcriptase (Gibco/Invitrogen, Carlsbad, CA,USA) and TaqDNA synthetic enzyme (Takara, Kyoto, Japan). The base sequences of the primers were as follows: ANP sense, TAC AGT GCG GTC TCC AAC ACA GAT CTG ATG GAT TTC AAG; ANP antisense, GCA ATG CGA CCA AGC TGT GTG ACA CAC CGC; GAPDH sense, TCC TGC ACC ACC TGC TTA GCC; and GAPDH antisense: TAG CCC AGG ATG CCC TTT AGT GGG. The expression level of each gene was determined by analysis of the digitized images and corrected for GAPDH expression.

Statistical analysis

Statistical analyses were performed using the Statview 5 software package (SAS Institute Inc., Cary, NC, USA). Values were expressed as the mean $\pm$ 1 standard error (SE). Differences among the groups in measurements such as the BP, echocardiographic variables, and expression of mRNA were tested by one-way analysis of variance combined with Scheffe's posthoc test. P values <0.05 were considered to indicate statistical significance.

Results

Hemodynamics and heart weight

The changes in the BP, heart rate, and heart weight/body weight ratio are shown in Table 1. The systolic and diastolic BPs in the RTH group were significantly higher than those in the control group. The systolic BPs in the N3D, N7D, and N14D groups were significantly lower than those in the RHT group. On the other hand, the systolic and diastolic BPs in the RHT group returned to the control range within 7

Table 1. Changes in the systolic and diastolic blood pressure, heart rate, body weight, heart weight, and HW/BW.
HW/BW, heart weight to body weight ratio ; N1D, N3D, N7D, and N14D : at 1 day, 3 days, 7 days and 14 days after uninephrectomy.

	Control	RHT	N1D	N3D	N7D	N14D
Systolic BP (mmHg)	125±3	208±17*	180±14*	175±4*#	137±8#	131±3#
Diastolic BP (mmHg)	98±3	160±12*	161±9*	152±5*	103±6#	100±3#
Heart Rate (bpm)	393±13	461±12*	447±15*	422±14	403±14#	391±15#
Body Weight (g)	305±16	254±11*	255±12*	257±13*	266±8*	285±8#
Heart Weight (mg)	1,028±36	1,211±52*	1,233±52*	1,216±41*	1,108±30#	1,038±32#
HW/BW	3.4±0.1	4.8±0.2*	4.5±0.4*	4.3±0.1*#	4.0±0.2*#	3.7±0.2#

*$p<0.05$ vs. control #$p<0.05$ vs. RHT

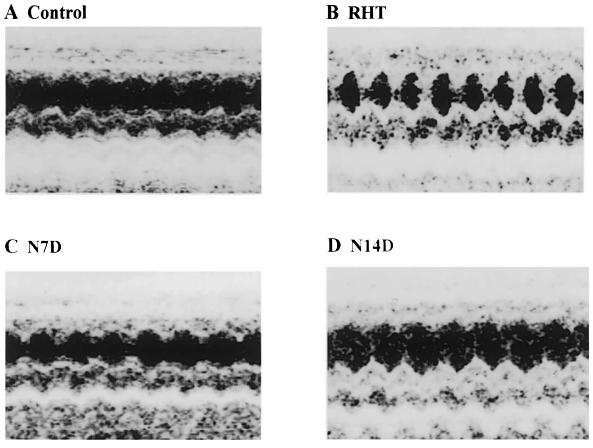

Fig. 2. Typical records of M-mode echocardiography in each group.
A : control, B : RHT, C and D : at 7 days and 14 days after uninephrectomy.

days after uninephrectomy. The body weight in the RHT group was significantly lower than that in the control group. However, after uninephrectomy, the body weight in the RHT group began to return to the baseline value. The markedly increased heart weight in the RHT group gradually reverted to the control level within 7 days after uninephrectomy. The heart weight/body weight ratio in the RHT group was significantly higher than that in the control group and reverted to the control level within 14 days after uninephrectomy.

We used only female rats to minimize the confounding influences of changes in the body weight and heart weight caused by aging, because the body weight remains more stable with aging in female rats than in male rats[12].

Table 2. Changes in the echocardiographic findings.
LVAWTs, systolic LV anterior wall thickness ; LVAWTd, diastolic LV anterior wall thickness ; LVIDs, systolic LV internal diameter ; LVIDd, diastolic LV internal diameter ; LVPWTs, systolic LV posterior wall thickness ; LVPWTd, diastolic LV internal diameter ; ESV, end-systolic volume ; EDV, end-diastolic volume.

	Control	RHT	N7D	N14D
LVAWTd	1.93±0.13	2.67±0.05*	2.41±0.16	1.91±0.05#
LVAWTs	2.62±0.08	3.42±0.18*	3.21±0.19	2.79±0.07#
LVIDd	5.46±0.13	4.44±0.24*	4.93±0.13	5.31±0.19#
LVIDs	3.14±0.09	1.73±0.13*	2.15±0.19*	2.86±0.12#
LVPWTd	2.46±0.17	3.34±0.16*	2.96±0.15	2.45±0.07#
LVPWTs	2.83±0.13	4.32±0.21*	4.12±0.21*	3.01±0.09#
EDV	0.38±0.03	0.20±0.03*	0.30±0.04	0.37±0.04#
ESV	0.070±0.009	0.024±0.002*	0.042±0.009*	0.064±0.009#

*$p<0.05$ vs. control #$p<0.05$ vs. RHT

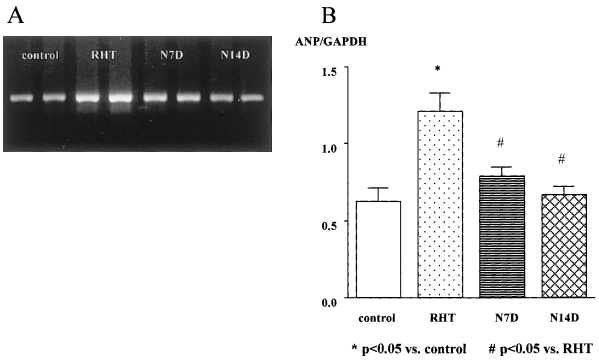

Fig. 3. The ANP mRNA expression in each group. Normalized value was expressed as ANP/GAPDH ratio. N7D and N14D, at 7 days and 14 days after uninephrectomy.

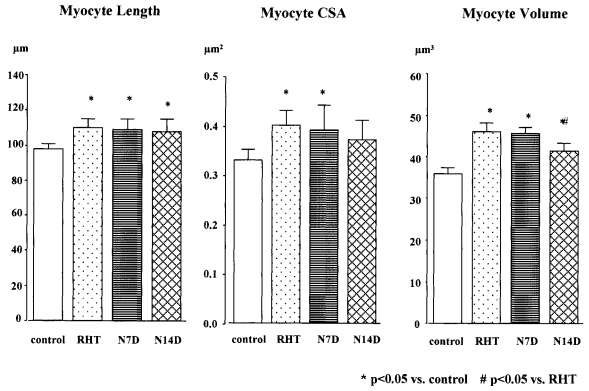

Fig. 4. Myocyte length, cross-sectional area, and volume in each group.
CSA, cross-sectional area ; N7D and N14D, at 7 days and 14 days after uninephrectomy.

Echocardiography

The changes in the echocardiographic findings are shown in Fig. 2 and summarized in Table 2. In the RHT group the LV anterior and posterior wall thicknesses were significantly greater and the LV internal lumen was significantly smaller than in the control group. The LV anterior and posterior wall thicknesses in the RHT group returned to the control values within 14 days after uninephrectomy. Also, the decreased internal lumen in the RHT group increased to the control value within 14 days after uninephrectomy.

ANP messenger RNA expression

The ANP mRNA expression in each group as an indicator of cardiac hypertrophy is shown in Fig. 3. The ANP mRNA expression in the RHT group was significantly higher than that in the control group. The high level of ANP mRNA expression in the RHT rats was rapidly decreased and returned to the control level within 14 days after uninephrectomy (control, 0.62 ± 0.03 ; RHT, 1.12 ± 0.09 ; N7D, 0.79 ± 0.06 ; and N14D, 0.67 ± 0.05).

Morphology of isolated myocytes

The morphological changes of isolated myocytes are shown in Fig. 4. The average myocyte length in the RHT group was significantly greater than that in the control group and remained significantly greater until 14 days after uninephrectomy (control, 98.0 ± 3.0 μm ; RHT, 112 ± 5 μm ; N7D, 111 ± 6 μm ; and N14D, 108 ± 5 μm). The mean myocyte cross-sectional area in the RHT group was significantly greater than that in the control group, remained higher until 7 days after uninephrectomy, and showed a slight, but not statistically significant, decrease at 14 days (control, 0.33 ± 0.03 μm^2 ; RHT, 0.40 ± 0.03 μm^2 ; N7D, 0.39 ± 0.05 μm^2 ; and N14D, 0.37 ± 0.04 μm^2). The mean myocyte volume in the RHT group was significantly greater than that in the control group and remained greater until 7 days after the uninephrectomy (control, 36.0 ± 1.4 μm^3 ; RHT, 46.0 ± 2.1 μm^3 ; N7D 45.6 ± 1.6 μm^3, N14D 41.7 ± 2.0 μm^3). The length and volume of myocytes in the N7D group and the N14D group remained higher than those in the control group.

Histological changes in the extent of cardiac fibrosis and collagen accumulation

Cardiac sections stained with Masson's trichrome staining are shown in Fig. 5. The extent of cardiac fibrosis, most marked in the perivascular areas, was evident in the RHT group. After uninephrectomy, the cardiac and perivascular fibrosis gradually regressed within 14 days after uninephrectomy. Similarly, cardiac sections stained with Sirius red are shown in Fig. 6. The extensive collagen accumulation in the perivascular and interstitial areas were noted in the RHT group and also gradually regressed within 14 days after uninephrectomy.

The rates of the cardiac fibrosis and the collagen accumulation in the heart are shown in Fig. 7. The

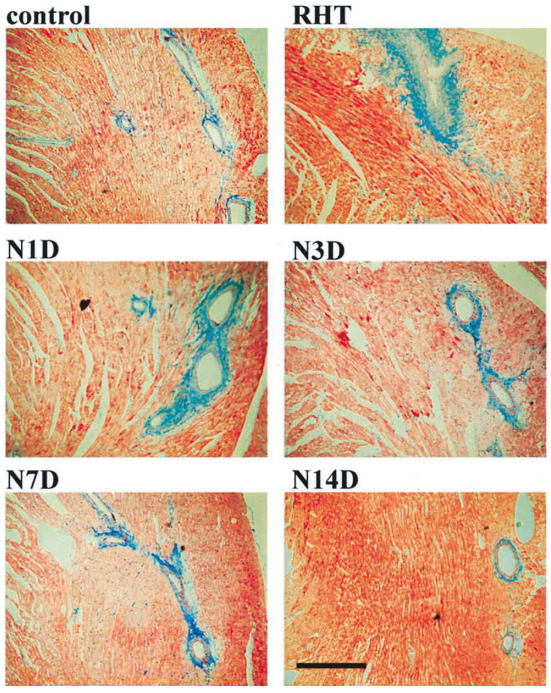

Fig. 5. Photomicrographs of cardiac sections stained with Masson's trichrome in each group.

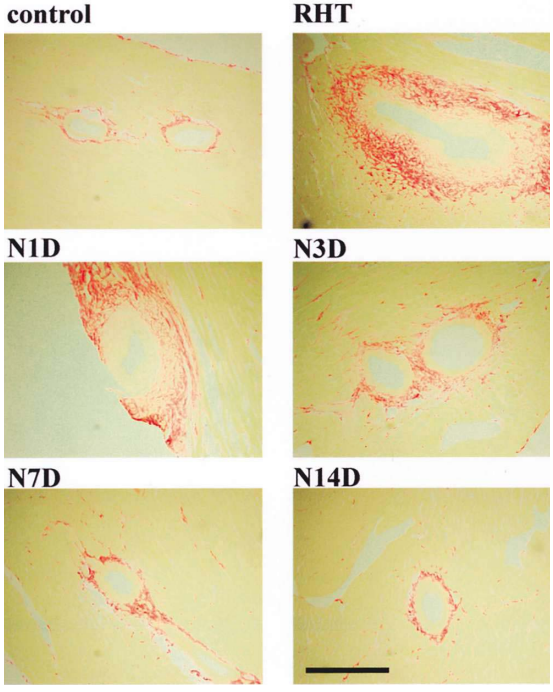

Fig. 6. Photomicrographs of cardiac sections stained with Sirius Red.
A: control; B: RHT; C, D, E, and F: at 1 day, 3 days, 7 days, and 14 days after uninerectomy. A black bar indicates 100 μm.

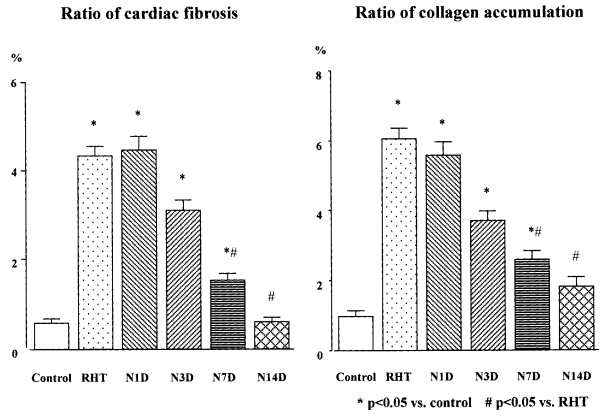

Fig. 7. The ratio of cardiac fibrosis and the ratio of collagen accumulation in each group.
N1D, N3D, N7D, and N14D: at 1 day, 3 days, 7 days, and 14 days after uninephrectomy.

rate of cardiac fibrosis in the RHT group was significantly higher than that in the control group but returned to the control level within 14 days after uninephrectomy (control, 0.6±0.1%; RHT, 4.3±0.6%; N1D, 4.4±0.6%; N3D, 3.1±0.5%; N7D, 1.5±0.3%; N14D 0.7±0.1%). Similarly, the rate of collagen accumulation in the RHT group was also significantly higher than that in the control group and decreased significantly within 7 days after uninephrectomy (control, 1.0±0.2%; RHT, 6.1±0.3%; N1D, 5.6±0.4%; N3D, 3.7±0.3%; N7D, 2.6±0.3%; N14D 1.8±0.3%).

DISCUSSION

The present study has demonstrated that the removal of the clipped kidney in RHT rats immediately reduces BP and causes regression of cardiac fibrosis and collagen accumulation before improvements in the morphology of hypertrophic myocytes. These findings suggest that the regression of cardiac fibrosis is deeply involved in the early phase of reverse remodeling of cardiac hypertrophy.

In this study, marked increases in the heart weight/body weight ratio and LV wall thickness were observed in RHT rats. The removal of the clipped kidney promptly reduced the BP and decreased the heart weight and the LV wall thickness to the control levels within 14 days after uninephrectomy. Also, the high level of ANP mRNA expression in the RHT rats

returned to the control level within 14 days after uninephrectomy. Both ANP and brain natriuretic peptide are indicators of cardiac remodeling, and the signaling from these peptides modulates myocyte growth and interstitial fibrosis in cardiac hypertrophy. Kawakami et al. have shown that the changes in ANP and brain natriuretic peptide gene expression in RHT rats are associated with the progression and regression of cardiac hypertrophy[14]. Uninephrectomy obviously improves the geometric LV hypertrophy and the humoral factor as the reverse remodeling of cardiac hypertrophy.

With regard to the remodeling of individual myocytes, Gerdes et al. have reported that pressure overload increases the cross-sectional area in concentric hypertrophy in spontaneously hypertensive rats with heart failure[3,4]. In contrast, the volume overload proportionally increases both the cell length and the cross-sectional area in the early decompensatory phase of heart failure[15,16]. In our study, the cardiac hypertrophy in the RHT group was in the transient remodeling stage between the compensatory cardiac hypertrophy phase and decompensated cardiac failure phase[8,17,18]. In this study, the morphological hypertrophy of myocytes, especially the length and volume of myocytes, in the RHT group did not return to the control level within 14 days after uninephrectomy. In spite of the improvements in the geometric cardiac hypertrophy and the ANP mRNA overexpression, the reverse remodeling of the morphological myocyte hypertrophy was delayed and may not be deeply involved in the early phase of reverse remodeling.

The proliferation of connective tissues, including fibrosis and collagen accumulation, is a characteristic finding of morbid cardiac hypertrophy. Cardiac fibrosis reduces ventricular compliance and ultimately causes diastolic heart failure. Marked perivascular fibrosis, cardiac interstitial fibrosis, and collagen accumulation were observed in the hearts of RHT rats. The cardiac fibrosis and collagen accumulation began to regress within 3 days after uninephrectomy and returned to the control level within 14 days. This prompt regression of cardiac fibrosis is closely involved in the early phase of reverse remodeling of geometric LV hypertrophy.

On the other hand, activation of the renin-angiotensin-aldosterone system in RHT rats causes marked cardiac hypertrophy and fibrosis, which are prevented by the blockade of this system[19]. Obayashi et al. have shown that treatment with an ARB reduces the length and width of myocytes in rats with hypertension generated by constriction of the abdominal aorta[20]. Nicoletti et al. have reported that treatment with an ARB reduces cardiac fibrosis in RHT rats[18]. We have previously reported that treatment with an ARB or an ACE-I prevents the progression of cardiac hypertrophy in RHT rats[10]. Recently, aldosterone has been suggested to play pivotal roles in the pathogenensis of cardiac fibrosis and fibrolysis[21,22]. The reduction in aldosterone levels after uninephrectomy may be associated with the regression of cardiac fibrosis in RHT rats. More studies are needed to clarify the role of aldosterone in the reverse remodeling of cardiac hypertrophy.

The regression of myocyte morphology and fibrosis in cardiac hypertrophy with various treatments has often been reported; however, the time-course of the reverse remodeling in removal of the clipped kidney in RHT rats has never been studied. Our experiment showed for the first time that the regression of cardiac fibrosis is a crucial factor in the early phase of reverse remodeling of cardiac hypertrophy in RHT rats. However, our results still do not explain the mechanism of regression of cardiac fibrosis in the early phase of reverse remodeling. The mechanisms are quite complex, and many factors may be involved[21,23]. Recently, transforming growth factor has been reported to increase the extracellular matrix and induce myocyte hypertrophy[24]. Moreover, the extracellular matrix is mainly degraded by matrix metalloproteinases, which are inhibited by a specific tissue inhibitor[25,26]. Elucidating the mechanism of regression of cardiac fibrosis is clinically important so that effective methods to treat cardiac hypertrophy can be developed. On the other hand, the degradation pathway of interstitial tissues and other factors in the heart may be associated with the improvement of myocyte hypertrophy. To clarify the mechanisms of reverse remodeling in cardiac hypertrophy, more studies are needed in the future.

CONCLUSION

We investigated reverse remodeling of the hypertrophic myocytes and interstitial fibrosis of cardiac hypertrophy in RHT rats after the removal of the clipped kidney. After uninephrectomy, the cardiac fibrosis and collagen accumulation immediately regressed before the morphological improvement of the hypertrophic myocytes. These findings suggest that the reverse remodeling of geometric LV hypertrophy is associated with the regression of cardiac fibrosis, which should be considered to treat hypertensive cardiac hypertrophy.

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