

Chymase is activated in the hamster heart following ventricular fibrosis during the chronic stage of hypertension

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Abstract Chronic pressure overload induces cardiac tissue remodeling. Chymase is known to regulate matrix metabolism and angiotensin II formation. In the present study, we investigated the pathophysiological functions of chymase in the pressure-overloaded hamster heart induced by a two-kidney, one-clip (2K1C) hypertension procedure. Fibrosis and apoptosis were observed in the pressure-overloaded hearts of 2K1C hamsters 32 weeks after clipping, but these histological changes were not detected at 16 weeks. Heart chymase-like activity of 2K1C hamsters at 32 weeks increased 5.2-fold compared with that at 16 weeks, while angiotensin-converting enzyme was not activated. Chymase might be involved in cardiac tissue remodeling during the chronic stage of hypertension.

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Key words: Angiotensin-converting enzyme; Chymase; Fibrosis; Apoptosis; Pressure overload

1. Introduction

Chymase is a chymotrypsin-like serine protease and is able to hydrolyze a variety of substrates including extracellular matrix [1–3], metalloproteases [4,5], vasoactive substances [6–8], cytokines [9], and lipoproteins [10]. However, its precise *in vivo* pathophysiological role as well as the actual substrate for chymase have not been determined. Chymases of human, monkey, dog, and hamster are highly efficient in converting angiotensin (ANG) I to ANG II [11–17], and the chymase-dependent ANG II-forming pathway is recognized as a major pathway for ANG II formation in cardiovascular tissues. ANG II is a potent bioactive peptide with diverse actions, and is involved in the paracrine regulation of cardiovascular tissue remodeling [18]. Our recent studies demonstrated that chymase was upregulated in the process of myocardial hypertrophy after balloon catheterization [16]. This result suggests that chymase is involved in the mechanisms of vascular tissue remodeling through the production of ANG II. Chymase has also been identified in the human heart [13], but the possible roles of chymase in the pathogenesis of cardiac diseases have not been determined.

Hypertension is a major risk factor of heart failure. Chronic pressure overload has been shown to induce dysfunction of myocardial contractile tissue with loss of myocytes and ventricular fibrosis after the compensated stage of hypertrophy [19,20]. Myocyte loss and fibrosis are also recognized in the human heart in patients with chronic hypertension [21]. Many studies have been performed to investigate the factors promoting myocyte growth and the synthesis of extracellular ma-

trix. However, the precise mechanisms for the transition from compensated hypertrophy to heart failure have not been established. In the process of tissue remodeling, growth factors and tissue proteases regulate the formation of extracellular matrix and cellular components. In the present study, we investigated the pathophysiological functions of chymase in hamster heart during the chronic stage of hypertension.

2. Materials and methods

2.1. Animal treatments

Six-week-old male Syrian hamsters weighing 90–110 g were purchased from SLC Japan (Shizuoka, Japan) and divided into two groups: the two-kidney, one-clip (2K1C) renal hypertensive group and the sham-operated control group. The 2K1C hypertension was induced as reported previously [22]. Sixteen weeks and 32 weeks after the procedure, blood pressure was measured, and the animals were killed by decapitation under pentobarbital anesthesia and the hearts were collected. The experimental procedures for animals were in accordance with our institutional guidelines.

2.2. Measurement of angiotensin-converting enzyme (ACE) and chymase-like activities

Heart ACE activity was determined using the synthetic substrate hippuryl-histidyl-leucine (HHL, Peptide Institute, Inc., Minoh, Japan) specifically designed for ACE [22]. Heart chymase-like activity was measured using the procedure of Okunishi et al. with modifications [12,17].

2.3. *In situ* detection of DNA fragmentation

Apoptotic cells were identified using the terminal deoxynucleotidyl transferase UTP nick end labeling assay [23]. Hearts were collected from the 2K1C and sham-operated control hamsters 16 and 32 weeks after operation, and fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin sections (5 μ m) were mounted on slides, and *in situ* detection of apoptosis was performed with an ApopTag kit (Oncor, Gaithersburg, MD, USA). To assess the myocardial fibrosis and calcification, paraffin sections from each heart were stained with azan and subjected to von Kossa's method for calcium.

2.4. *In vitro* detection of DNA fragmentation

Genomic DNA was extracted from the hearts of 2K1C hamsters 16 and 32 weeks after clipping and from the sham-operated control hamsters 32 weeks after the operation using the TurboGen genomic DNA purification kit (Invitrogen, San Diego, CA, USA). Three micrograms of genomic DNA were electrophoretically separated on 2% agarose gels and stained with ethidium bromide.

2.5. Statistical analysis

All numerical data shown in the text are expressed as mean \pm S.E.M. Significant differences between the means of different groups were evaluated by Student's *t*-test for unpaired data.

3. Results

The mean blood pressure of 2K1C hamsters 16 weeks after clipping was higher (162 ± 7 mm Hg, $n = 5$) than that of age-

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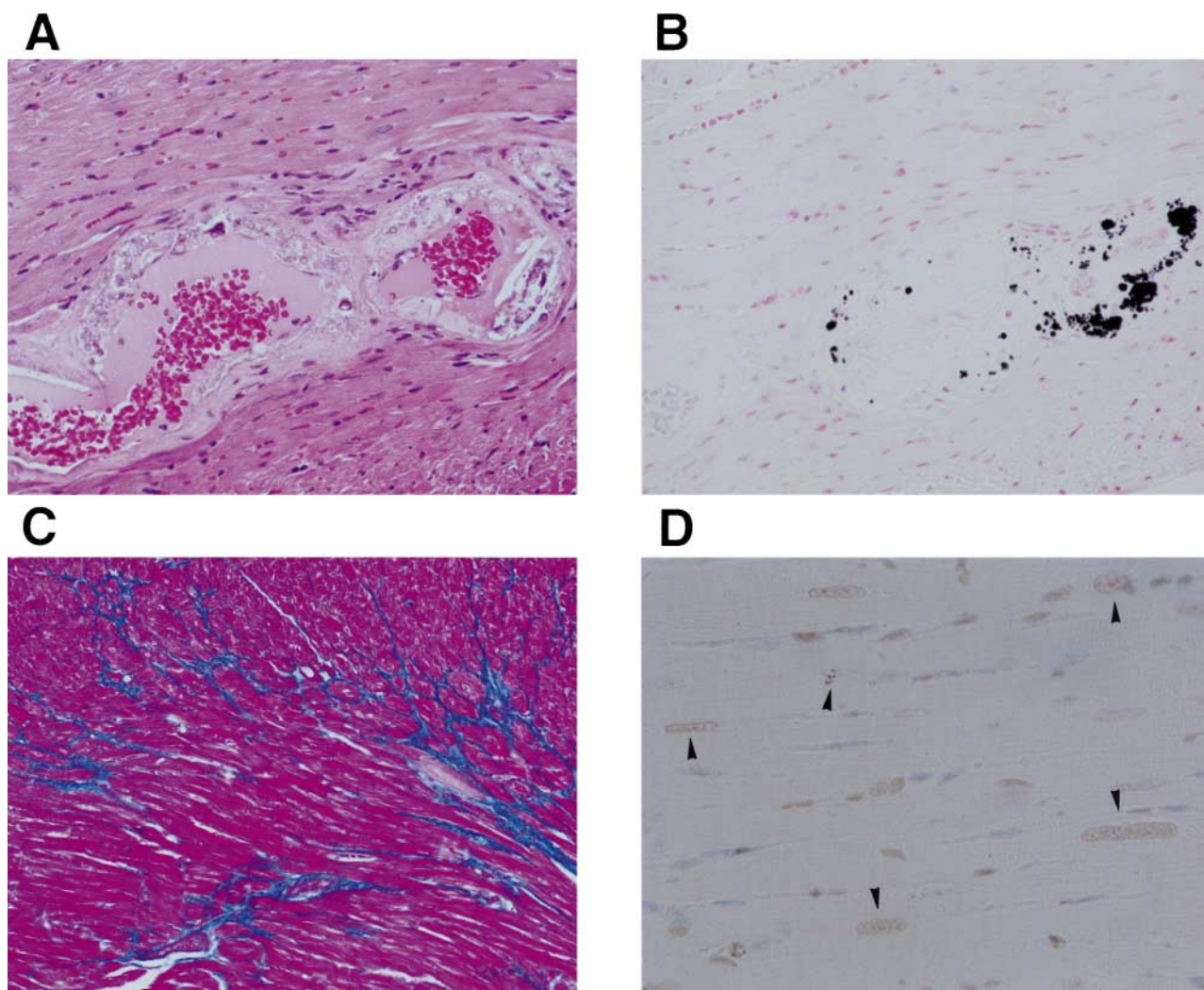


Fig. 1. Histological sections of hearts of 2K1C hypertensive hamsters 32 weeks after clipping. A: Atherosclerotic degeneration of an intramural arteriole in the left ventricle (hematoxylin-eosin stain, original magnification $\times 200$). B: Calcification of an intramural arteriole in the left ventricle (von Kossa's method for calcium, original magnification $\times 200$). C: Left ventricle showing extensive myocardial fibrosis (azan stain, original magnification $\times 100$). D: In situ detection of DNA fragmentation in hamster heart employing an Apoptag kit. Arrowheads indicate positive staining of nuclei in cardiomyocytes (original magnification $\times 400$).

matched sham-operated control hamsters (112 ± 5 mm Hg, $n=5$, $P<0.01$) and remained high 32 weeks after clipping (146 ± 2 mm Hg, $n=6$, versus 107 ± 3 mm Hg, $n=6$, $P<0.01$). The heart weight of 2K1C hamsters was significantly higher than that of age-matched controls, at 16 weeks (436 ± 12 versus 321 ± 11 mg, $n=5$, $P<0.01$) and 32 weeks (440 ± 18 versus 348 ± 7 mg, $n=5$, $P<0.01$). Widespread myocardial fibrosis and atherosclerotic degeneration of small branches of the coronary artery were observed in the hearts of 2K1C hypertensive hamsters 32 weeks after clipping (Fig. 1A–C). The results of in situ DNA end labeling with the terminal deoxynucleotidyl transferase assay showed that apoptosis occurred in the cardiomyocytes of 2K1C hypertensive hamsters 32 weeks after clipping (Fig. 1D). These histological abnormalities were not detected in the hearts of 2K1C hamsters 16 weeks after clipping and in sham-operated control hamsters 16 weeks and 32 weeks after operation. The oligonucleosomal laddering in DNA was detected clearly in the genomic DNA extracted from 2K1C hamsters 32 weeks after clipping, but not in age-matched sham-operated controls and 2K1C ham-

sters 16 weeks after clipping (Fig. 2). The heart chymase-like activity of 2K1C hamsters 32 weeks after clipping exhibited a 5.2-fold increase when compared with that 16 weeks after clipping (Fig. 3A), while the heart ACE was not activated throughout the experimental period (Fig. 3B). The heart ACE and chymase-like activities of the sham-operated controls were not changed between 16 weeks and 32 weeks after the sham operation (Fig. 3).

4. Discussion

The present study demonstrated for the first time that chymase was markedly activated during the chronic stage (32 weeks after clipping) of the 2K1C hypertensive heart concurrently with the development of cardiac fibrosis. Hamster chymase, like human chymase, is a highly efficient ANG II-forming enzyme [17]. ANG II is known to promote fibroblast proliferation and collagen synthesis in order to induce myocardial fibrosis [18]. Recently, we also demonstrated that the cardiac chymase-like activity in BiO 14.6 cardiomyopathic

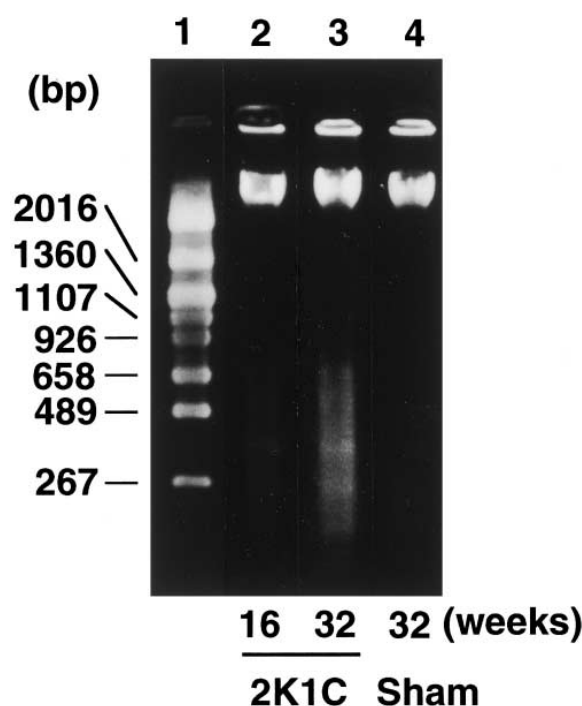


Fig. 2. Nucleosome-sized fragmentation of genomic DNA extracted from hearts. Lane 1, DNA marker; lane 2, 2K1C hamster 16 weeks after clipping; lane 3, 2K1C hamster 32 weeks after clipping; lane 4, sham-operated control hamster 32 weeks after operation.

hamsters increased significantly with the development of cardiac fibrosis, while ACE was not upregulated in the cardiomyopathic heart throughout the experimental period [24]. After chronic therapy with the ANG II receptor antagonist, the area of cardiac fibrosis in BiO 14.6 cardiomyopathic hamsters was markedly reduced by 53% [24]. These results suggested that augmented ANG II formation through a chymase-dependent pathway might be involved in cardiac tissue remodeling. However, recent clinical observations demonstrated that chymase expression is not upregulated in the terminal-stage failing heart [25]. In the present study, hypertensive hamsters did not yet show any clinical symptoms of heart failure such as increase of body weight and liver mass, or the appearance

of pleural and peritoneal fluids 32 weeks after clipping. Chymase might be activated in the initial step of the transition state from compensated hypertrophy to heart failure, where the process of tissue remodeling including myocyte loss and fibrosis are most activated.

Chymase is also known to be involved in the regulation of the proteolysis of extracellular matrix [1–3]. Extracellular matrix has the ability to bind growth factors such as basic fibroblast growth factor, TGF- β 1, and GM-CSF. Human chymase was shown to activate procollagenase directly [5], and to liberate latent TGF- β 1 from the extracellular matrix [26]. In addition, chymase was shown to digest fibronectin and vitronectin, and alter cellular functions by preventing cell-matrix interaction [2,3]. Over-expressed heart chymase was predicted to exaggerate the destruction of the cardiac interstitium, and the excessive amount of growth factors would be liberated from the matrix. These growth factors, cooperating with ANG II, could then promote the excessive wound-healing response resulting in cardiac fibrosis. Chymase may have a prevalent role in the complex network formed between matrix-producing factors and proteinases.

Recent observations indicated the possibility that apoptosis might account for the cardiomyocyte loss in the process of heart remodeling [20,27–29]. It was suggested that hypoxia induces apoptosis in cultured cardiomyocytes [30]. In the present study, the small ventricular arteries of 2K1C hamsters showed atherosclerotic degeneration at 32 weeks. This histological change might induce a reduction in coronary blood flow and the hypoxic condition of the myocardium. Chymase was shown to stimulate cholesterol accumulation in macrophages and in vascular smooth muscle cells, and to be involved in the pathogenesis of atherosclerosis [10]. Our previous report demonstrated that chymase was activated in hypertrophied vessels in response to balloon catheterization, and promoted the mechanisms of vascular remodeling through the production of ANG II [16]. Furthermore, ANG II induced oncogenes such as *c-fos* and *c-myc* in myocytes [31]. These factors have also been reported to regulate the process of apoptosis [32,33]. Chymase might be involved in the apoptosis of cardiomyocytes through the production of ANG II or the modulation of cell-matrix interaction.

In conclusion, the present study demonstrated for the first

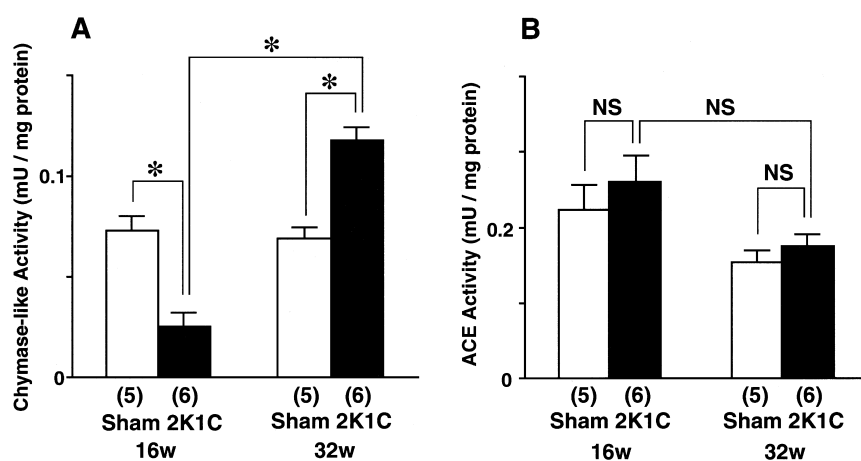


Fig. 3. Heart chymase-like activity (A) and ACE activity (B) of 2K1C hypertensive hamsters and sham-operated control hamsters 16 weeks and 32 weeks after operation. The number of hamsters is shown in parentheses. The vertical bars represent S.E.M. * $P < 0.05$. NS, not significant.

time that heart chymase was activated in the chronic pressure-overloaded heart, and might be involved in heart remodeling in the transition state to heart failure. A full understanding of the pathogenetic role of heart chymase requires further experiments using specific inhibitors for chymase.

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