Effects of Angiotensin-Converting Enzyme Inhibitor and Aldosterone Antagonist on Myocardial Collagen in Cardiomyopathic Hamsters

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To examine the effects of angiotensin-converting enzyme (ACE) inhibitor and aldosterone antagonist on myocardial collagen in the cardiomyopathic hamster, the collagen concentration was measured by determining the hydroxyproline concentration, and the ratio of type I to type III collagen (type I/III ratio) was measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Five-week-old Bio14.6 cardiomyopathic hamsters were treated with the ACE inhibitor captopril (20 mg/kg per day) or the aldosterone antagonist K-canrenoate (20 mg/kg per day) in drinking water for 20 weeks, and the collagen concentration and type I/III ratio at 25 weeks were compared with those in 25-week-old untreated Bio14.6 and normal F1b hamsters. The collagen concentration markedly increased and the type I/III ratio significantly decreased (ie, type III collagen dominant) in untreated Bio14.6 compared with F1b at 25 weeks. Captopril and K-canrenoate treatment significantly reduced the collagen concentration and reversed the changes in the type I/III ratio in cardiomyopathic hamster. These results suggest that ACE inhibitor and aldosterone antagonist improve myocardial collagen in the cardiomyopathic hamster, not only quantitatively but also qualitatively, and that the mechanism of this improvement may be related to the cardiac renin-angiotensin-aldosterone system.

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Collagen is the major component of the cardiac interstitium, and its quantitative and qualitative changes are thought to exert some influence on cardiac structure and function. Recently, Weber1 emphasized the significance of the cardiac interstitium, especially collagen, and proposed "Interstitial heart disease". Moreover, Weber and Brilla2 studied the association between fibrosis and the cardiac renin-angiotensin-aldosterone system in hypertrophied hearts, and reported that angiotensin II or aldosterone was responsible for cardiac fibroblast proliferation and enhanced collagen synthesis, and that either the ACE inhibitor captopril or the aldosterone antagonist spironolactone prevented fibrosis.

The cardiomyopathic hamster is frequently used as an animal model of human cardiomyopathy. In this model, interstitial fibrosis commonly increases with aging.3 However, the details of myocardial collagen are still unclear.

In the present study, to examine the effects of angiotensin-converting enzyme (ACE) inhibitor and aldosterone antagonist on myocardial collagen in the cardiomyo-
All hamsters were weighed, and their hearts were then removed immediately after anesthesia with diethylether and washed in ice-cold 0.05 mol/L Tris-HCl buffer (pH 7.4) containing 5 mmol/L ethylenediaminetetra acetic acid (EDTA) and 1 mol/L NaCl. The atria and great vessels were separated from the heart and the remaining ventricular wet weight was measured as the heart weight. A small part of the left ventricular free wall was resected for determination of the hydroxyproline concentration.

Bio14.6 and F1b hamsters were obtained from Biobreeders (Fitchburg, USA). They were taken care of according to our institution's guide for the care and use of laboratory animals.

*Extraction of Collagen*

The tissue was cut into small pieces and homogenized in ice-cold 0.05 mol/L Tris-HCl buffer (pH 7.4) containing 5 mmol/L EDTA and 1 mol/L NaCl with a homogenizer. The homogenates were washed in the same buffer for 24 h and centrifuged at 3,000 × g for 15 min at 4 °C. The precipitates were suspended and washed in 0.5 mol/L acetic acid for 24 h, and centrifuged at 3,000 × g for 15 min at 4 °C. The precipitates were digested with pepsin (10 mg pepsin/g wet weight of tissue, Boeringer, Ingelheim, Germany) in 0.5 mol/L acetic acid (10 ml acetic acid/g wet weight of tissue) for 24 h and centrifuged at 10,000 × g for 60 min at 4 °C, and the supernatants were obtained. The relative percentage of pepsin-extracted collagen from the myocardium of 5-week-old Bio14.6 hamster was 60% of the total collagen. The pepsin in the supernatants was inactivated with 5 mol/L NaOH for 24 h at room temperature, and the gelatinous precipitates, predominantly consisting of collagen types I and III, were separated by centrifugation at 10,000 × g for 60 min at 4 °C. The separated collagen were resolubilized in 5 mmol/L acetic acid and analyzed by SDS-PAGE.

*SDS-PAGE*

The phenotypes of pepsin-extracted collagen were analyzed by SDS-PAGE in the presence of 3 mol/L urea according to the method described by Hayashi and Nagai with some modifications. Briefly, SDS-polyacrylamide gel consisted of 3% stacking

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**MATERIALS AND METHODS**

*Experimental Animals*

Five-week-old male Bio14.6 cardiomyopathic hamsters were treated with the ACE inhibitor captopril (20 mg/kg per day, Sankyo, Tokyo, Japan) or the aldosterone antagonist K-canrenoate (20 mg/kg per day, Searle, Osaka, Japan) in drinking water for 20 weeks. The heart weight-to-body weight ratio, collagen concentration and type I/III ratio at 25 weeks (hypertrophy, dilatation and scar phase) were measured and compared with those in 25-week-old untreated Bio14.6 and normal F1b hamsters. Each group consisted of 6 hamsters.

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Fig. 1. SDS-PAGE of pepsin-extracted collagen from hamster myocardium. Lanes 1 and 2 show the electrophoretic patterns of standard type I and type III collagen (reduced) from porcine skin as a control. Lanes 3 and 4 show pepsin-extracted collagen from hamster myocardium which was reduced (lane 3) or not reduced (lane 4).
acetic acid and methanol. Under electrophoresis in the presence of urea, clear separation of α1 and α2 chains of type I collagen and α1 chains of type III collagen was achieved (Fig. 1), although α1 chains of collagen types I and III move at the same rate in the absence of urea. The stained gels were scanned using a densitometer (Konishiroku PDS-15, Tokyo), and the areas under each peak were determined using a planimeter (Tamaya PLANIX, Tokyo). The areas representing α1 chains were used to calculate the relative percentages of collagen types I and III.

**Collagen Concentration**

Myocardial tissues used to determine the hydroxyproline concentration were heated with 6 mol/L HCl at 110 °C for 24 h to hydrolyze collagen into its component amino acids. The hydroxyproline concentration was measured spectrophotometrically (Hitachi 200–20, Tokyo) by its reaction with Ehrlich's reagent according to the method described by Inayama et al. The collagen concentration was calculated by multiplying the hydroxyproline concentration by 7.46, and was expressed as milligrams of collagen per gram ventricular wet weight.

**Histologic Examination**

Myocardial tissues for light microscopy were immediately submerged in 20% neutral buffered formalin (pH 7.4), embedded in paraffin, cut into sections 4 μm thick, and stained with Mallory-Azan stain to assess the degree of fibrosis.

**Statistical Analysis**

All results were expressed as mean ± SD, and intergroup differences were evaluated by ANOVA followed by Scheffe’s multiple comparison test. Statistical significance was defined as p < 0.05.

**RESULTS**

**Heart Weight-to-Body Weight Ratio**

The heart weight-to-body weight ratios of the normal hamsters, and untreated, captopril-treated and K-carnrenoate-treated cardiomyopathic hamsters at 5 and 25 weeks are shown in Fig. 2. There was a marked increase in the heart weight-to-body weight.
ratio with aging in untreated Bio 14.6 (5 wk; 3.64±0.23 mg/g, 25 wk; 4.42±0.11 mg/g) compared with F1b (5 wk; 3.04±0.30 mg/g, 25 wk; 3.17±0.17 mg/g). Treatment with either captopril or K-canrenoate for 20 weeks significantly decreased this ratio in Bio 14.6 from 4.42±0.11 to 3.91±0.27 mg/g (p<0.05) or 3.43±0.23 mg/g (p<0.01), respectively.

**Collagen Concentration**

The collagen concentrations in each group are shown in Fig. 3. There was a marked increase in the collagen concentration with aging in untreated Bio 14.6 (5 wk; 3.80±0.52 mg/g, 25 wk; 6.14±0.48 mg/g) compared with F1b (5 wk; 3.11±0.31 mg/g, 25 wk; 3.46±0.31 mg/g). Treatment with either captopril or K-canrenoate for 20 weeks significantly reduced the collagen concentration in Bio 14.6 from 6.14±0.48 to 4.53±0.48 mg/g (p<0.01) or 2.74±0.33 mg/g (p<0.01), respectively.

**Type I/III Ratio**

The type I/III ratios in each group are shown in Fig. 4. The type I/III ratio significantly decreased (ie, type III collagen dominant) in untreated Bio 14.6 (5 wk; 0.93±0.11, 25 wk; 0.81±0.21) compared with F1b (5 wk; 1.62±0.32, 25 wk; 2.07±0.40). Treatment with K-canrenoate for 20 weeks significantly increased the type I/III ratio in Bio 14.6 from 0.81±0.21 to 1.85±0.17 (p<0.05). Treatment with captopril also tended to increase the type I/III ratio (1.32±0.22).

**Histologic Findings**

Histologic findings in the left ventricles of 25-week-old hamsters in each group are shown in Fig. 5. There was a marked increase in interstitial fibrosis and calcification in untreated Bio 14.6 compared with F1b. Treatment with either captopril or K-canrenoate decreased interstitial fibrosis and calcification.

**DISCUSSION**

**Collagen and Cardiomyopathy**

Human dilated cardiomyopathy is characterized by chamber dilatation and decreased myocardial contractility. Histologically, a marked increase in interstitial fibrosis is observed, with the hypertrophy and degeneration of myocytes. Recently, Weber et al. reported an increase in the proportion of type III collagen, which is thinner and has less tensile strength than type I collagen, and suggested an association between this increase and chamber dilatation and decreased myocardial contractility in human dilated cardiomyopathy. The cardiomyopathic ham-
Myocardial Collagen in Cardiomyopathic Hamsters

Fig. 5. Histologic findings in the myocardium in 25-week-old hamsters. (A) normal F1b; (B) untreated Bio 14.6; (C) captopril-treated Bio 14.6; (D) K-canrenoate-treated Bio 14.6. (Mallory-Azan stain, original magnification ×25.)

Weber and Brilla\textsuperscript{2} studied the association between fibrosis and the cardiac renin-angiotensin-aldosterone system in hypertrophied hearts, and reported that angiotensin II or aldosterone was responsible for cardiac fibroblast proliferation and enhanced collagen synthesis, and that either the ACE inhibitor captopril or the aldosterone antagonist spironolactone prevented fibrosis. Mukherjee and Sen\textsuperscript{7} examined the effects of captopril on collagen content and type I/III ratio in SHRs, and reported that captopril treatment not only reduced the collagen content but also reversed the altered type I/III ratio. Makino et al\textsuperscript{8} also reported that enalapril treatment reduced the collagen content and the expression of type III collagen mRNA in aortic banding rat hearts. Brilla et al\textsuperscript{9} examined the effects of spironolactone on myocardial fibrosis in several models of arterial hypertension in rats, and reported that treatment with spironolactone prevented myocardial fibrosis in renovascular hypertension and continuous aldosterone infusion models.

Although Kato et al\textsuperscript{10} reported the effects of captopril and enalapril on the onset and progress of cardiomyopathy in the cardiomyo-

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opathic hamster J-2-N, and suggested the significance of increases in tissue bradykinins and vasodilatory prostaglandins, there have been no reports on the effects of ACE inhibitor and aldosterone antagonist on myocardial collagen, especially regarding changes in collagen phenotypes, in the cardiomyopathic hamster. In this paper, the increases in the collagen concentration and proportion of type III collagen observed in untreated cardiomyopathic hamster were suppressed by treatment with either captopril or K-canrenoate. These results suggest that ACE inhibitor and aldosterone antagonist improve myocardial collagen in the cardiomyopathic hamster, not only quantitatively but also qualitatively, and that the mechanism of this improvement may be related to the cardiac renin-angiotensin-aldosterone system in both cardiomyopathic hearts and hypertrophied hearts. Since Kato et al.\cite{11} reported that angiotensin II stimulated collagen synthesis in cultured smooth muscle cells, we speculate that angiotensin II stimulates collagen synthesis in cardiac fibroblasts in cardiomyopathic hearts and that ACE inhibitor suppresses such synthesis by blocking angiotensin II. Moreover, since Campbell et al.\cite{12} reported that chronic elevations in plasma aldosterone were associated with a reactive interstitial fibrosis, we speculate that aldosterone stimulates collagen synthesis directly, as well as angiotensin II, and that aldosterone antagonist suppresses this synthesis by blocking aldosterone.

In summary, we clarified the quantitative and qualitative changes in myocardial collagen in the cardiomyopathic hamster, and the improvement of these disorders by ACE inhibitor or aldosterone antagonist. It is unclear whether this improvement depends on direct effects of these drugs on the cardiac renin-angiotensin-aldosterone system or on the indirect effect of hemodynamic improvement, since we did not evaluate humoral or hemodynamic parameters. Further studies are needed to examine the effects of these drugs on collagen synthesis in cultured cardiac fibroblasts.

REFERENCES