Experimental Studies

Collagen Synthesis by Cultured Cardiac Fibroblasts Obtained from Cardiomyopathic Hamsters

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SUMMARY

The aim of the present study was to clarify myocardial collagen metabolism in cardiomyopathic hamsters and the effects of the angiotensin converting enzyme inhibitor, captopril, on collagen synthesis.

Cardiac fibroblasts from Bio14.6 cardiomyopathic hamsters and from non-cardiomyopathic F1b hamsters were cultured and used in the 4th passage. The synthetic activity of collagenous protein from the two types of hamsters was determined by measuring ^3H -proline uptake, and the collagen type was subsequently analyzed by SDS-PAGE in cultured cardiac fibroblasts. Also studied were the effects of the angiotensin converting enzyme inhibitor captopril ($1\text{ }\mu\text{M}$) on collagen synthesis by cardiac fibroblasts from cardiomyopathic hamsters.

Twenty five-week-old Bio14.6 hamsters had significantly higher synthetic activity of collagenous protein and rate of collagen synthesis compared with 13-week-old Bio14.6 hamsters or 25-week-old F1b hamsters [Bio14.6(25-week); 12.4 ± 1.6 , Bio14.6(13-week); 4.8 ± 0.4 , F1b (25-week); 8.7 ± 0.9 cpm/cell, Bio14.6(25-week); 11.0 ± 0.9 , Bio14.6(13-week); 3.9 ± 0.4 , F1b (25-week); $4.8 \pm 0.4\%$, $p < 0.05$]. Qualitatively, 25-week-old Bio14.6 hamsters had significantly higher synthetic activity of type I, III, IV and V collagens compared with 25-week-old F1b hamsters. The synthetic activity of type III collagen was increased the most in the cardiomyopathic group. Captopril ($1\text{ }\mu\text{M}$) caused a significant decrease in synthetic activity of collagenous protein in 25-week-old Bio14.6 hamsters (12.4 ± 1.6 cpm/cell $\rightarrow$ 10.9 ± 1.1 cpm/cell, $p < 0.05$). Qualitatively, the synthetic activity of type III collagen was decreased to about half.

Our study revealed enhanced collagen synthetic activity in cardiac fibroblasts from Bio14.6 hamsters. Captopril improved collagen metabolism in cultured cardiac fibroblasts from Bio14.6 hamsters not only quantitatively but also qualitatively. The mechanism of this improvement may be related to the cardiac renin angiotensin system. (Jpn Heart J 1998; 39: 97-108)

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HYPERTROPHIC cardiomyopathy is a disease characterized by an increase of fibrosis in the cardiac interstitium with hypertrophy and disarray of cardiac myocytes histopathologically. Sugihara *et al.*¹⁾ reported that ventricular dysfunction in hypertrophic cardiomyopathy, especially diastolic dysfunction, is influenced by thickening of the ventricular wall with an increase of fibrosis in the cardiac interstitium. Brilla *et al.*²⁾ studied the relationship between a structural remodeling of the myocardium produced by pressure overload and diastolic cardiac function in spontaneously hypertensive rats (SHRs). In SHRs a structural remodeling of the myocardium has been found that includes myocyte hypertrophy and fibrosis in the cardiac interstitium (replacement fibrosis that occurs in response to cell loss and reactive fibrosis that occurs in the absence of myocyte necrosis).³⁻⁵⁾ They revealed a negative correlation between the grade of fibrosis in the cardiac interstitium and peak cardiac output and concluded that a structural remodeling of the cardiac interstitium plays an important role in cardiac function.

The myocardium is comprised of many different cells. Cardiac myocytes constitute only one third of the cell population. All remaining cells, by definition, are found in the cardiac interstitium and include cardiac fibroblasts, endothelial cells, vascular smooth muscle cells, macrophages and mast cells.⁶⁾ Cardiac fibroblasts have the responsibility to produce the extracellular matrix proteins, collagen and fibronectin. The connective tissue, mostly composed of collagen, tethers parenchymal cells to stromal cells in the myocardium to maintain the structure and several functions of the myocardium. Therefore, collagen metabolism in cardiac fibroblasts is thought to exert some influence on cardiac structure and function.

These findings suggest that, besides abnormality in parenchymal cells in the myocardium, abnormality in the collagen metabolism of cardiac fibroblasts is present in hypertrophic cardiomyopathy. In the present study, we investigated the collagen metabolism in cultured cardiac fibroblasts from the cardiomyopathic Syrian hamster (Bio14.6).⁷⁾ In addition, the effects of angiotensin-converting enzyme (ACE) inhibitor on collagen metabolism in the cultured cardiac fibroblasts were studied, since the tissue renin angiotensin system may play an important role in cardiac hypertrophy in not only various experimental animal models and humans,^{8,9)} but also in cardiomyopathic Syrian hamsters.¹⁰⁾

METHODS

This study was approved by the University of Kanazawa Animal Care and Use Committee and conforms to the *Guide to the Care and Use of Laboratory Animals* published by the US National Institutes of Health.

Animal: Thirteen-week-old male Bio14.6 cardiomyopathic hamsters⁷⁾ (Bio Breeders, Boston, MA, USA) weighing 104.2 (SD 2.5) g and 25-week-old male Bio14.6 cardiomyopathic hamsters⁷⁾ weighing 120.4 (SD 1.9) g were used. Thirteen- and 25-week-old normal F1b hamsters weighing 129.3 (SD 2.8) g and 134.6 (SD 2.2) g on average, respectively, were used as control groups.

Cell culture: Cardiac fibroblasts were obtained through the method of Ross.¹¹⁾ All hamsters were weighed and then the hearts immediately removed after anesthesia with diethyl ether. The atria and great vessels were dissected from the heart and the remaining ventricles minced finely to manufacture explants. These explants were plated on 100-mm culture dishes on Dulbecco's modified Eagle medium (DMEM) supplemented with antibiotics (100 μ g/ml kanamycin, 5 μ g/ml amphotericin B) plus 10% fetal bovine serum (FBS) (Gibco, Grand Island, NB, USA) and incubated in a humidified environment of 5% CO₂/95% air at 37°C. Every two days the medium was exchanged with fresh medium, and the cells were allowed to grow until confluence and subsequently passaged. Cell passage was performed with 2% ethylene diamine tetraacetate-0.25% trypsin. All cells used in these experiments were from passage 4.

Immunocytochemistry: The fibroblastic nature of confluent cells as well as the purity of the cultured cell population was determined by immunocytochemical staining of the cell layer with anti-human factor VIII antibody for endothelial cells, anti-desmin antibody for smooth muscle cells, and anti-vimentin antibody for fibroblasts (Dako Corp, Kyoto, Japan). An avidin-biotinylated peroxidase complex (ABC) method was used for immunocytochemical staining of our cell culture preparations. Immunocytochemical staining was conducted on all cell cultures prior to the collagen assays. The cell population on the slide glass was fixed in acetone and embedded in paraffin. Deparaffinized cell sections were treated with methanolic hydrogen peroxide followed by 3% normal mouse serum to block the endogenous peroxidase activity and to reduce the nonspecific background staining. Primary antibodies were subsequently added and incubated at room temperature for 30 min. The sections were then treated with biotin-labeled mouse antirabbit IgG antibodies for 30 min at room temperature followed by ABC. The final reaction was achieved by incubating the sections with 0.01% hydrogen peroxide and 0.05% diaminobenzidine tetrahydrochloride in 0.05M Tris buffer, pH 7.2, for 5 min. Counterstaining was done with hematoxylin and eosin.

Analysis of synthetic activity of collagenous protein: Cultured fibroblasts of passage 4 were plated onto 35-mm culture dishes in 1 ml DMEM plus 10% FBS at an approximate density of 100 000 cells/cm². Then, every two days the medium was exchanged with fresh medium. On the tenth day [³H] proline (50 μ Ci/ml) with a specific activity of 45 Ci/mmol (Amersham, Amersham, Buckinghamshire, England), 0.1 mM ascorbic acid sodium salt and 0.5 mM aminopropionitrile fumarate were added to the new medium. After labelling the cells for 24 hours, they were harvested with the medium and sonicated to be samples for quantitative and qualitative analysis of collagenous protein.

In the group of 25-week-old male Bio14.6 cardiomyopathic hamsters we made an ACE inhibitor-supplemented subgroup to examine the effects of ACEI. Since the serum is rich in ACE,¹²⁾ the medium was changed to fresh serum-free medium before supplementation with captopril (Sankyo Company Ltd., Tokyo, Japan) to exclude the effect of circulating ACE. Peak plasma levels of 0.8 to 1.8 μ g/ml were measured after oral therapeutic administration of captopril clinically, that is, 5 to 10 μ M captopril.¹³⁾ In the present study, we used 1 μ M as one captopril concentration which was considered to be in the therapeutic range. On the ninth day after the cells were seeded in 35-mm culture dishes, the medium was changed to serum-free medium containing 1 μ M captopril. On the tenth day [³H] proline (50 μ Ci/ml), 0.1 mM ascorbic acid sodium salt and 0.5 mM aminopropionitrile fumarate were added to the medium. Samples were then prepared according to the same procedure as in the other groups.

Quantitative analysis of collagenous protein: Samples were boiled for 5 minutes to inactivate endogenous enzymatic activity. Quantitative analysis of collagenous protein was performed by the collagenase digestion method of Peterkofsky *et al.*¹⁴⁾ After sufficient dialysis against 0.05 M acetic acid, the samples were lyophilized. The dried samples were redissolved with 0.04 N NaOH, neutralized with Tris-HCl buffer (pH 7.4) and digested with collagenase (*Clostridium histolyticum* collagenase, Form III) (Advance Biofacture Co., Lynbrook, NY, USA) at 37°C for 2 hours. After digestion the samples were supplemented with 10% (w/v) trichloroacetic acid containing 0.5% tannic acid and centrifuged at 2,200 rpm for 5 minutes to obtain a fraction of high molecular weight in the precipitate (non-collagenous protein) and of low molecular weight in the supernate (collagenous protein). The radioactivity of each fraction was determined by a liquid scintillation counter LSC-700 (Aloka, Tokyo). The radioactivity of non-collagenous protein and collagenous protein per cell was then calculated, and the rate of collagen/total protein synthesis calculated using the following formula of Peterkofsky *et al.*¹⁴⁾:

$$R = [CP / (CP + 5.4 \times NCP)] \times 100$$

where R is the rate of collagen/total protein synthesis in %, CP is the radioactivity of collagenous protein per cell in cpm, and NCP is the radioactivity of non-collagenous protein per cell in cpm. Six experiments were performed from different cell isolations, each of them in triplicate.

Qualitative analysis of collagenous protein: Neutral protease inhibitor (0.25 M ethylene diamine tetraacetic acid 2Na, 10 mM phenylmethyl sulfonyl fluoride, 0.1M N-ethylmaleimide and 1.8M Tris-HCl) was added to the samples. Qualitative analysis of collagenous protein was performed by the method of Hata *et al.*¹⁵⁾ The samples were supplemented with 176 mg/ml ammonium sulfate and centrifuged at 12,000 rpm for 30 minutes to obtain a fraction of precipitation. The precipitated samples were redissolved with 0.5 M acetic acid and digested with pepsin (Sigma Chemical Co., St. Louis, MO, USA) at 4°C for 6 hours. The samples were supplemented with 2M Tris-HCl buffer and 5 N NaOH, sufficiently dialyzed against 0.05M acetic acid, and lyophilized to obtain samples for electrophoresis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure described by Weber *et al.*¹⁶⁾ Briefly, the SDS-polyacrylamide gel consisted of a 3% (pH 6.8) stacking gel and a 5% (pH 8.8) separating gel in a vertical flat-bed electrophoresis apparatus. Stacking was allowed to occur at a constant current of 20 mA. Once the sample entered the separating gel, the current was increased to 30 mA, and electrophoresis continued until the dye marker reached the bottom of the gel. For reduction, 0.1M

Figure 1. SDS-PAGE (fluorogram) of ³H-proline labeled collagenous protein synthesized by cultured cardiac fibroblasts. Lanes 1 and 2, non-reduction; lanes 3 and 4, reduction by DTT; lanes 1 and 3, Bio14.6 (25 weeks); lanes 2 and 4, captopril supplemented Bio14.6 (25 weeks); $\alpha_1(I)$ and $\alpha_2(I)$, type I collagen; III, type III collagen; IV, type IV collagen; $\alpha_1(V)$, type V collagen.

dithiothreitol (Wako Pure Chemical Industries, Ltd, Osaka, Japan) was used.

After electrophoresis, the gel was soaked with 1.1M sodium salicylate solution for 1 hour and dried. Then fluorography^{17,18)} was performed. Radiographic film XAR-5 (Eastman Kodak Company, Rochester, NY, USA) was exposed to the dried gel at -80°C for 5–10 days, developed and fixed. The densities of the bands corresponding to each collagen chain ($\alpha_1(\text{I}) + \alpha_2(\text{I})$: type I collagen, $\alpha(\text{III})$: type III collagen, $\alpha(\text{IV})$: type IV collagen, $\alpha_1(\text{V})$: type V collagen) on the exposed film (Figure 1) were measured with a densitometer, and the recordings of the areas under each peak were determined using a planimeter. The areas representing each chain were used to calculate the relative percentages of each type of collagen.

Statistical analysis: Variables measured are expressed as mean (SD). The statistical significance of differences in mean values was assessed by one-way analysis of variance followed by Scheffe's test. Probability values less than 0.05 were considered to be significant.

RESULTS

Immunocytochemistry: Almost all cells ($94.5 \pm 2.4\%$) were positively stained with anti-vimentin antibody, but negatively with anti-desmin and anti-human factor VIII antibody; almost all cells used in our experiments proved to be cardiac fibroblasts.

Quantitative analysis of collagenous protein:

Synthesis of collagenous protein by cultured fibroblasts from Bio14.6. At 13 weeks of age, there was no significant difference in the synthetic activity of collagenous protein and rate of collagen synthesis in F1b hamsters and Bio14.6 hamsters (Bio14.6;

Figure 2. Synthesis of collagenous protein of cultured cardiac fibroblasts from Bio14.6 (13 weeks, 25 weeks) and F1b (13 weeks, 25 weeks). * $p < 0.05$.

Figure 3. Rate of collagen synthesis of cultured cardiac fibroblasts from Bio14.6 (13 weeks, 25 weeks) and F1b (13 weeks, 25 weeks). * $p < 0.05$.

Figure 4. Effects of captopril on the synthesis of collagenous protein of cultured cardiac fibroblasts from Bio14.6 (25 weeks). * $p < 0.05$.

4.8 ± 0.4, F1b; 6.5 ± 0.9 cpm/cell, Bio14.6; 3.9 ± 0.4, F1b; 3.8 ± 0.4%, NS). At 25 weeks of age, both Bio14.6 hamsters and F1b hamsters had significantly higher synthetic activity of collagenous protein and rate of collagen synthesis compared with those at 13 weeks of age, being significantly higher in the former than in the latter (Bio14.6; 12.4 ± 1.6, F1b; 8.7 ± 0.9 cpm/cell, Bio14.6; 11.0 ± 0.9, F1b; 4.8 ± 0.4%, $p < 0.05$) (Figure 2 and 3).

Effect of captopril on synthesis of collagenous protein of a cultured fibroblast from Bio14.6.

Captopril supplementation caused a significant decrease in the synthetic activity of collagenous protein of a cultured fibroblast from 25-week-old Bio14.6 hamsters (12.4 ± 1.6 cpm/cell → 10.9 ± 1.1 cpm/cell, $p < 0.05$) (Figure 4).

Qualitative analysis of collagenous protein:

Synthetic activity of type I, III, IV and V collagens of a cultured fibroblast from Bio14.6. At 13 weeks of age, Bio14.6 hamsters had significantly lower synthetic activity of type I and IV collagens compared with F1b hamsters (Table I). At 25 weeks of age, Bio14.6 hamsters had significantly higher synthetic activity of type I, III, IV and V collagens compared with F1b hamsters (Table II). The synthetic activity of type III collagen showed the greatest increase.

Effect of captopril on synthetic activity of type I, III, IV and V collagens of cultured fibroblasts from Bio14.6. Captopril supplementation caused a significant decrease in the synthetic activity of type III and IV collagens of fibroblasts cultured from 25-week-old Bio14.6 hamsters and a significant increase in the synthetic activity of type V collagen (Table III). The synthetic activity of type III collagen was de-

Table I. Synthesis of Type I, III, IV and V Collagens of a Cultured Fibroblast from Bio14.6 (13 weeks) and F1b (13 weeks).

	Synthesis of collagenous protein (cpm $\times 10^{-1}$ /cell)			
	I	III	IV	V
F1b (13 weeks)	50.2(8.4)	2.0(0.2)	10.7(1.5)	2.2(0.3)
Bio14.6 (13 weeks)	39.4(3.3)*	2.3(0.2)	4.3(0.7)*	2.4(0.2)

Values are means (SD), $n = 6$. I = type I collagen; III = type III collagen; IV = type IV collagen; V = type V collagen. * $p < 0.05$ vs F1b (13 weeks).

Table II. Synthesis of Type I, III, IV and V Collagens of a Cultured Fibroblast from Bio14.6 (25 weeks) and F1b (25 weeks).

	Synthesis of collagenous protein (cpm $\times 10^{-1}$ /cell)			
	I	III	IV	V
F1b (25 weeks)	74.2 (10.0)	1.1(0.1)	8.7 (1.1)	2.6 (0.3)
Bio14.6 (25 weeks)	88.8 (9.5)*	11.7(1.4)*	12.1 (1.3)*	11.9 (1.1)*

Values are means (SD), $n = 6$. I = type I collagen; III = type III collagen; IV = type IV collagen; V = type V collagen. * $p < 0.05$ vs F1b (25 weeks).

Table III. Synthesis of Type I, III, IV and V Collagens of a Cultured Fibroblast from Bio14.6 (25 weeks) and Captopril supplemented Bio14.6 (25 weeks).

	Synthesis of collagenous protein (cpm $\times 10^{-1}$ /cell)			
	I	III	IV	V
Bio14.6 (25 weeks)	88.8(9.5)	11.7(1.4)	12.1(1.3)	11.9(1.1)
Captopril-supplemented Bio14.6 (25 weeks)	85.7(8.9)	5.2(0.5)*	0.4(0.1)*	17.7(1.5)*

Values are means (SD), $n = 6$. I = type I collagen; III = type III collagen; IV = type IV collagen; V = type V collagen. * $p < 0.05$ vs Bio14.6 (25 weeks).

creased by about half.

DISCUSSION

Human hypertrophic cardiomyopathy is a disease characterized by an increase in fibrosis in the cardiac interstitium with hypertrophy and disarray of cardiac myocytes histopathologically. A marked increase in interstitial fibrosis, mostly composed of collagen, and deteriorated cardiac function are observed with progression of the stage of the disease. In hypertrophic cardiomyopathy, therefore, besides an abnormality in cardiac myocytes, an abnormality in collagen metabolism of cardiac fibroblasts may be present.

Bajusz⁷⁾ stated that the cardiac disease that occurs in the Bio14.6 strain of cardiomyopathic Syrian hamsters is transmitted by an autosomal recessive gene. He pointed out that in its clinical course progressive, chronic congestive cardiac failure appears to be the ultimate cause of death in more than 90 per cent of the cardiomyopathic hamsters. The chronic cardiac condition of the hamster resembles human idiopathic cardiomyopathy. There are some reports on the histopathology of the heart muscle lesions of Bio14.6 hamsters. Jasmin *et al.*¹⁹⁾ reported that in the heart of Bio14.6 hamsters, the disease develops through four stages: 1) necrotic stage (30–100 days); 2) fibrotic and healing stage (100–160 days); 3) hypertrophy, dilatation and scar (160–200 days); and 4) continuing dilatation and terminal heart failure (200–300 days). Five major lesions of degeneration of cardiac myocytes, degeneration of mitochondria, degeneration of myofibrils, fibrosis in cardiac interstitium and cardiac hypertrophy are also observed in human cardiomyopathy.

We used Bio14.6 cardiomyopathic hamsters, the cardiac condition of which resembles human idiopathic cardiomyopathy closely in clinical course and pathologic aspects, to study collagen metabolism in cultured cardiac fibroblasts. At 25 weeks of age, the synthetic activity of collagenous protein in Bio14.6 hamsters had increased to about triple that in the 13-week-old hamsters, and was significantly higher than that in F1b hamsters. Qualitatively, Bio14.6 hamsters had significantly higher synthetic activity of type I, III, IV and V collagens compared with F1b hamsters. The synthetic activity of type III collagen was increased the most. Araki *et al.*²⁰⁾ observed that 25-week-old Bio14.6 hamsters have significantly more collagen content in the myocardium compared with 25-week-old F1b hamsters and show an increase in the proportion of type III collagens compared with 5-week-old Bio14.6 hamsters *in vivo*, but their observation was influenced by the hemodynamics of the hamsters. Our study on cultured cardiac fibroblasts *in vitro* supports their study to reveal abnormal collagen metabolism in cardiac fibroblasts of Bio14.6 hamsters.

Recently, the renin, angiotensinogen and ACE genes were found to be expressed in cardiomyocytes and vascular endothelial cells through molecular biological methods,^{21,22)} and much attention has been drawn to the tissue renin angiotensin system. It is suspected that the tissue renin angiotensin system plays an important role in cardiac hypertrophy of various experimental animal models and humans^{8,9)} and angiotensin II may be responsible for enhanced collagen synthesis in cardiac fibroblasts and fibrosis in cardiac interstitium.²³⁻²⁷⁾ Hirakata *et al.*¹⁰⁾ reported that the receptor for angiotensin II in the left ventricle of 300-day-old Bio14.6 cardiomyopathic hamsters has 35 percent higher affinity compared with that of normal hamsters. The tissue renin angiotensin system may be responsible for the etiology in Bio14.6 cardiomyopathic hamsters; therefore, we studied the effects of the ACE inhibitor, captopril, on collagen synthesis in cultured cardiac fibroblasts of Bio14.6 hamsters. One μ M captopril, considered to be in the therapeutic plasma level range clinically, caused a significant decrease in synthetic activity of type III collagen in 25-week-old Bio14.6 hamsters. Araki *et al.*²⁰⁾ treated 5-week-old Bio14.6 cardiomyopathic hamsters with captopril (20 mg/kg/day) in their drinking water for 20 weeks and observed that captopril treatment tends to retard the increase in the proportion of type III collagen in the cardiomyopathic hamster *in vivo*. Also, Masutomo *et al.*²⁸⁾ treated 10-week-old Bio14.6 cardiomyopathic hamsters with enalapril (10 mg/kg/day) in their drinking water for 10 weeks and observed that enalapril treatment tends to decrease the collagen type III:I ratio and type III collagen mRNA expression. But, these *in vivo* observations were influenced by the hemodynamics of the hamsters. Our study on cultured cardiac fibroblasts *in vitro* reveals that the effect of captopril may be related, in part, to inhibition of the effect of cardiac ACE on enhanced collagen synthesis.

Collagen is the major component of the extracellular matrix of the myocardium. In the heart, six different collagen types (I, III, IV, V, VI, VIII) have been detected.²⁹⁾ The extracellular collagen matrix in the myocardium consists primarily of fibrillar type I and III collagens, and 85% of total collagen is represented by type I collagen while type III collagen represents 11% in the normal condition.³⁰⁾ Type I collagen normally aggregates into predominantly thick fibers and has a substantial tensile strength, whereas type III collagen typically forms thin fibers and possesses a resilience that is ideal for maintaining structural integrity and distensibility of the myocardium.³¹⁻³³⁾ Weber *et al.*³⁾ reported that in the established phase of hypertrophy in the experimentally pressure overloaded rat, when the collagen volume fraction has risen significantly from normal (3% to 5%) to occupy 8% to 12% of the myocardium, systolic stiffness and diastolic stiffness at larger strains are each increased and that for greater increments in the collagen volume fraction, representing up to 20% or more of the hypertrophied myocar-

dium, systolic stiffness declines. They also analyzed the collagen remodeling qualitatively and reported that when type III collagen has risen to 20% of total collagen protein, diastolic stiffness declines while systolic stiffness remains normal.³⁴⁾ In postmortem human hearts with idiopathic cardiomyopathy, an excess proportion of thin collagen fibers, perhaps representing type III collagen is observed.³⁾

Judging from the above, quantitative and qualitative changes in collagen in the cardiac interstitium are thought to exert a great influence on cardiac structure and function. Since type III collagen is thinner and has less tensile strength than type I collagen, it is susceptible to rupture.^{35,36)} The increase in the proportion of type III collagen may permit chamber dilatation and deteriorated cardiac function with wall thinning. Our experiments suggest that captopril improves collagen metabolism in cultured cardiac fibroblasts from Bio14.6 cardiomyopathic hamsters, not only quantitatively, but also qualitatively. The effects of captopril on the remodeling of the cardiomyopathic heart in the Bio14.6 hamsters have the potential to lead to improvement of cardiac function. The mechanism of this improvement may be related to the cardiac renin angiotensin system.

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