Experimental Studies

Effects of Enalapril on the Collagen Matrix in Cardiomyopathic Syrian Hamsters (BIO 14.6 and 53.58)

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The hereditary cardiomyopathic strain of Syrian hamster has been extensively studied as a model of cardiomyopathy and heart failure. We attempted to determine whether an angiotensin converting enzyme (ACE) inhibitor, enalapril, prevents the increase in extracellular collagen matrix which connects the myocytes in cardiomyopathy. Enalapril was administered at an average dosage of 10 mg/kg per day to 10- to 20-week-old hamsters with hypertrophic (Bio 14.6) and dilated (Bio 53.58) cardiomyopathy, as well as to control Syrian hamsters (F1β). Collagen concentration estimated by hydroxyproline concentration and the collagen type III:I ratio significantly increased in the hearts of the Bio 14.6 and Bio 53.58 strains at 20 and 40 weeks of age as, compared with those in age-matched F1β hamsters. When Bio 14.6 hamsters were given enalapril for 10 weeks from 10 to 20 weeks of age, the collagen concentration, the collagen type III:I ratio and type III collagen mRNA expression were significantly decreased, compared with those in untreated animals of the same strain. After the administration of enalapril, scanning electron microscopic examination also revealed a decrease in fibrillar collagen accumulation in the interstitium and the network surrounding the cardiac myocytes. These prophylactic effects were not observed in the Bio 53.58 strain. These results indicate that the administration of ACE inhibitor prevents type III collagen production in the Bio 14.6 strain but not in the Bio 53.58 strain of Syrian hamster.

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The Bio strain of hereditary cardiomyopathic Syrian hamster has proved to be a useful model of genetically-determined cardiomyopathy and heart failure. This model has been used to study fundamental cellular abnormalities in heart failure. While the pathogenesis of the disease state is not fully understood, focal necrosis is apparent in the Bio 14.6 strain as early as 30 days after birth and persists until the age of 4 months, resulting in extensive replacement of viable myocardial fibrotic connective tissue! Thus, distinctive patterns of reparative myocardial fibrosis alter diastolic and systolic stiffness and may lead to pathologic hypertrophy.4 Alternatively, a loss of collagen tethers or a decline in matrix tensile strength may be responsible for the global transformations in myocardial architecture and function seen in dilated and congestive cardiomyopathy.5 In the Bio 14.6 strain, perivascular fibrosis and

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calcified lesions would be expected to decrease coronary perfusion as well as impair ventricular stiffness and function. It is now accepted that myocardium contains a collagen matrix composed primarily of type I and type III collagen, which is a major determinant of myocardial architecture, structural integrity, and mechanical properties. Cardiomyopathic Syrian hamsters have been used to study the effects of various pharmacological agents, including digitalis, calcium channel blockers, and α₁-adrenergic antagonists, on the progression of left ventricular failure. The effects of long-term therapy with angiotensin converting enzyme (ACE) inhibitors were also recently studied in this model, and a significant cardioprotective effect and increased survival were reported. The inhibition of angiotensin II production by the administration of ACE inhibitor results not only in vasodilation, but also leads to an increase in coronary blood flow. Furthermore, treatment with an ACE inhibitor decreases calcium overload within myocytes despite reduced systemic pressure. In spontaneously hypertensive rats (SHR) with established left ventricular hypertrophy, Brilla et al have shown that treatment with the ACE inhibitor lisinopril reduces collagen accumulation in myocardium. However, the precise mechanism by which chronic ACE inhibition attenuates collagen formation in the myocardium of cardiomyopathic Syrian hamsters remains to be determined. Therefore, we investigated age-related changes in fibrillar collagen metabolism in cardiomyopathic Syrian hamsters (strains Bio 14.6 and Bio 53.58). In addition, we examined the effects of chronic therapy with the ACE inhibitor enalapril on fibrillar collagen matrix in these hamsters, since the influence of this drug class on fibrillar collagen matrix has not yet been documented in animal models of cardiomyopathy.

MATERIALS AND METHODS

Animals

All of the experiments were conducted with male Syrian hamsters obtained from Bio Breeders, Inc., Fitchburg, MA. All of the animals were treated according to the Guiding Principles for the Care & Use of Animals approved by the Council of the Physiological Society of Japan. The Bio 14.6 strain was used as a model of hypertrophic cardiomyopathy and the Bio 53.58 strain was used as a model of dilated cardiomyopathy. The hamsters were 5, 10, 20 and 40 weeks old. Healthy age-matched inbred golden Syrian hamsters (F₁β) were used as controls. Each age group consisted of 6 animals. All of the hamsters were obtained at 4 weeks of age and housed under controlled conditions (20±2°C, 55±20% humidity, 12:12 h light/dark cycle) with free access to laboratory food and water. The cardiomyopathic Syrian hamster is a widely used model of cardiomyopathy. The Bio 14.6 strain displays the following characteristic pathological changes: cardiac myolysis at 4–5 weeks of age, cardiac hypertrophy at approximately 20 weeks of age, cardiac dilatation at approximately 30–40 weeks of age, and congestive heart failure at approximately 1 year of age. In contrast to the Bio 14.6 strain, Bio 53.58 hamsters do not display either myolysis or hypertrophy before dilatation. Bio 53.58 hamsters gradually develop cardiac dilatation accompanied by diffuse cell death at approximately 4–20 weeks of age. They also have a short life span and demonstrate reduced cardiac function at an early age. Therefore, the Bio 53.58 strain is a model of cardiac dilatation that contrasts with the hypertrophic Bio 14.6 strain.

Experimental Design

This study was designed to determine the effect of preventive treatment with an ACE inhibitor, enalapril, on the fibrillar collagen matrix in cardiomyopathic hamsters. The Bio 14.6, Bio 53.58 and F₁β strains were randomly separated into 2 groups of 28 animals each. Each strain was divided into 2 groups which were given either enalapril (n=14) or tap water (n=14) as the control for 10 weeks from 10 to 20 weeks of age. The drug was dissolved in drinking water and the concentration was adjusted to achieve a daily intake of 10 mg/kg body weight for enalapril. The hamsters were weighed weekly during the treatment phase. Hamsters given enalapril or vehicle were housed under identical conditions. The experimental design of the study was approved...
by our institution. The dose of enalapril (10 mg/kg, od) in this study was based on a pilot study in which plasma ACE inhibition was measured by the method of Cushman and Cheung\textsuperscript{19} in 20-week-old golden Syrian hamsters 6 h after they were given enalapril at 5, 10 or 20 mg/kg od (n = 5 for each dose). Compared with the placebo, 5, 10 and 20 mg/kg of enalapril inhibited plasma ACE activity by 68\%, 83\% and 86\%, respectively. Therefore, we chose the 10 mg/kg dose for the present study, since it was the lowest dose required for efficient plasma ACE inhibition in Syrian hamsters. Two Bio 14.6 hamsters that died accidentally were excluded. The final number of hamsters in each treatment group was as follows: 12 in the Bio 14.6 vehicle group, 12 in the Bio 53.58 vehicle group, 14 in the F1\(\beta\) vehicle group, 14 in the Bio 14.6 enalapril group, 14 in the Bio 53.58 enalapril group and 14 in the F1\(\beta\) enalapril group.

Collection of Tissue

The hamsters were weighed, heparinized (1000 units ip) and anesthetized with pentobarbital (50 mg/kg ip). Their hearts were then quickly removed and rinsed with phosphate-buffered saline (PBS). After dissecting the pericardium, the great vessels and the atria, the ventricles were weighed and quick-frozen in liquid nitrogen. The tissue samples were stored at -70\(^\circ\)C before the extraction of either RNA or collagen. Other samples from each group were used for collagen measurement and morphological examination.

RNA Isolation and Northern Blot Analysis

Total RNA was extracted from the cardiac tissue using the procedure of Chirgwin et al\textsuperscript{20} with minor modifications. Briefly, the hearts were rapidly dissected, the atria were removed, and the ventricular myocardium was homogenized in 4 mol/L guanidine thiocyanate solution on ice. The homogenate was cleared of cell debris by centrifugation at 12,000 g for 10 min. RNA was pelleted on a cesium chloride cushion and then redissolved in Tris-EDTA (pH 7.4) and purified by 3 ethanol precipitations. Total RNA was quantified by absorbance at 260 nm, assuming 40 \(\mu\)g/ml for each unit of absorbance. Steady-state levels of mRNA were determined by Northern hybridization analysis. Total RNA (20 mg) was denatured in 50\% formamide, 17.5\% formaldehyde, and 1\(\times\) MOPS (20 mmol/L 3-[N-morpholino] propanesulfonic acid at pH 7.0, 5 mmol/L sodium acetate, and 1 mmol/L Na\(\textsubscript{2}\)-EDTA at pH 8.0), electrophoresed in 1\% agarose gel, transferred to a Gene-Screen Filter (New England Nuclear, Boston, MA), and baked for 2 h at 80\(^\circ\)C in a vacuum oven. The blots were prehybridized in 1 mol/L sodium chloride, 1\% sodium dodecyl sulfate (SDS), 10\% dextran sulfate, 50\% deionized formamide and 100 \(\mu\)g/ml salmon sperm DNA for 4–8 h at 42\(^\circ\)C with the same buffer containing the appropriate radioactive probes to obtain 0.5–1.0\(\times\)10\(^6\) counts/min per ml of hybridization medium. The cDNAs were radioactively labeled by random primer extension as described by Feinberg and Vogelstein\textsuperscript{21} through the use of the Amersham Multi-prime DNA labeling system according to the manufacturer’s instructions (Amersham, UK). [\(\textsuperscript{32}\)P]-dATP (specific activity, 11.1\(\times\) 10\(^6\) GBq/mM: Amersham) was included in the reaction mixture to obtain a specific activity of 4–10\(\times\)10\(^8\) counts/min per \(\mu\)g DNA. The blots were subsequently washed once in 0.2\(\times\)standard saline citrate buffer (SSC) (1\(\times\)SSC in 0.15 mol/L NaCl, 0.015 mol/L sodium citrate) at room temperature for 10 min and once in 0.1\% SDS and 0.1\(\times\)SSC at 55\(^\circ\)C for 15 min, and autoradiography (X-Omat AR) was carried out for 1–5 days at -70\(^\circ\)C. Densitometry (Videodensitometer, Model 620, Bio-Rad) was used to quantitate the relative signal intensity of the obtained bands.

Collagen Composition of the Myocardium

The myocardial collagen concentration was measured by determining the hydroxyproline concentration in the left ventricle (100–200 mg)\textsuperscript{22} After drying the heart for 24 h, the specimens were hydrolyzed in 6 mol/L hydrogen chloride solution at 100\(^\circ\)C. After resolution in a buffer at pH 7.0, p-dimethylamino-benzaldehyde (Ehrlich’s reagent) was added to form a complex with hydroxyproline. The concentration of hydroxyproline was measured by spectrophotometric analysis at a wavelength of 558 nm. The collagen concentration was estimated by multiplying the hydroxyproline content by a factor
TABLE I COLLAGEN EXTRACTION FROM THE LEFT VENTRICLE OF THE SYRIAN HAMSTERS AT 20 WEEKS OF AGE

<table>
<thead>
<tr>
<th>Extractant</th>
<th>F1β</th>
<th>Bio 14.6</th>
<th>Bio 53.58</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 M NaCl</td>
<td>0.013</td>
<td>0.007</td>
<td>0.014</td>
</tr>
<tr>
<td>0.5 M Acetic acid</td>
<td>0.004</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Pepsin (1 mg/ml)</td>
<td>0.656</td>
<td>0.544</td>
<td>1.066</td>
</tr>
<tr>
<td>Residue</td>
<td>0.164</td>
<td>0.258</td>
<td>0.398</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0.837</td>
<td>0.818</td>
<td>1.484</td>
</tr>
</tbody>
</table>

Each value is the mean amount of collagen (mg) [%/Total] of 4-6 experiments.

of 8.2. The concentration of collagen was expressed as milligrams of collagen per gram dry weight. The myocardium sent for collagen typing was prepared as follows: the sections were cut into small pieces and lyophilized. The lyophilized material was pulverized, and an aliquot was used for solubilization and subsequent determination of collagen types as reported by Weber et al. Salt-soluble and acid-soluble collagen was first obtained in brief, samples were initially extracted with 1 mol/L NaCl in 0.05 mol/L Tris buffer, pH 7.4, containing a protease inhibitor for 24 h at 4°C. The supernatants were separated by centrifugation and the heart residue was extracted in 0.5 mol/L acetic acid at 4°C for 24 h. The samples were then centrifuged to remove the supernatants. The precipitates were subjected to pepsin digestion (1 mg pepsin/100 mg wet weight of tissue) at 4°C in 0.5 mol/L acetic acid for a minimum of 24 h. Table I indicates the relative effectiveness of the extraction procedure. The initial extractions using sodium chloride and acetic acid yielded only 0.4%–0.6% of total collagen, while over 66% of the collagen was solubilized by 3 pepsin extractions. No differences were seen in collagen solubility between the F1β and cardiomyopathic hearts. Three pepsin extracts were pooled and collagen was precipitated by adding sodium chloride to a final concentration of 0.8 mol/L at 4°C and collected by centrifugation. The collagen precipitate was resolubilized in 0.5 mol/L acetic acid and dialyzed against 0.02 mol/L Na2PO4 in 0.05 mol/L Tris, pH 7.4. The collagen types in these samples were determined using the interrupted sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis procedure of Hayashi and Nagai. Standard purified collagen types I and III were also included in the SDS gel electrophoresis. After electrophoresis, the gels were stained with Coomassie blue; they were subsequently destained in a solution of acetic acid and methanol. Under these conditions for electrophoresis, a clear separation of type I and III chains was obtained. The gels were scanned using a densitometric Scan (Bio-Rad) at 580 nm. The cardiac collagen showed an electrophoretic pattern with a similar degree of background staining. Quantitation of the relative amount of type III:I collagen was accomplished by determining the relationship between the amount of collagen applied to the gel and that found in the peak, which is distinctive for each type of collagen.

Scanning Electron Microscopic (SEM) Examination

SEM was performed to assess the morphological features of the collagen matrix in cardiomyopathic hearts after treatment with enalapril. Tissue preparation and staining were carried out as previously described. Briefly, samples were fixed in Karnovsky’s fixative for 3 h at 4°C. After rinsing in 0.1 mol/L cacodylate buffer (pH 7.4), tissue samples were immersed in a 2 mol/L NaOH solution for 5–7 days at room temperature. They were then put in a 40% dimethylsulfoxide solution and freeze-cracked with a razor blade in liquid nitrogen. The tissue pieces were thoroughly washed in a physiological saline solution containing 5% Tween 20 for 2 h at 40°C, placed in a 1.0% aqueous solution of tannic acid for 2 h and post-fixed with cacodylate-buffered 2% osmium tetroxide

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for 2 h. After dehydration and drying, the specimens were spatter-coated with gold and viewed in a H-S800 SEM.

cDNA Probes
The cDNA probes used in the present study were pAZ 1002 for chick \( \alpha_2 \) type I collagen and pDT 1505 for mouse \( \alpha_1 \) type III collagen; both were a gift from Benoit de Crombrugge of the M.D. Anderson Cancer Center, Houston, TX. The cDNA chick \( \beta \)-actin probe was obtained from Dr. Miyoshi.

Statistical Analysis
Results are expressed as the mean±SD. One-way analysis of variance (ANOVA) revealed significant differences between the groups. The results among groups were then compared using Bonferroni’s multiple comparison test. Statistical significance was set at \( p<0.05 \).

RESULTS
Heart and Body Weights in Cardiomyopathic Hamsters
The heart weight in Bio 53.58 hamsters was significantly less (\( p<0.05 \)) than that in age-matched F1\( \beta \) hamsters at 5, 10, 20 and 40 weeks of age. The heart weight in Bio 14.6 hamsters showed no difference from that in age-matched F1\( \beta \) hamsters at 5, 10 and 40 weeks of age, but was significantly less (\( p<0.05 \)) at 20 weeks (Table II). The body weight in Bio 53.58 hamsters was also significantly less (\( p<0.05 \)) than that in age-matched F1\( \beta \) hamsters from 5 to 40 weeks, while that in Bio 14.6 hamsters was significantly less (\( p<0.05 \)) than that in age-matched F1\( \beta \) hamsters at 10 and 20 weeks of age. The heart weight to body weight ratio was higher in Bio 14.6 hamsters than in age-matched F1\( \beta \) hamsters from 5 to 40 weeks of age. In contrast, there was no significant difference in this ratio between Bio 53.58 and F1\( \beta \) hamsters from 5 to 40 weeks of age (Table II).

Myocardial Collagen Concentration
Collagen concentration, defined as the milligrams of collagen per gram of heart tissue estimated from the quantification of hydroxyproline, was significantly greater in cardiomyopathic hamsters (Bio 14.6, 2.38±0.25 mg/g heart weight, \( n=6 \), \( p<0.05 \); Bio 53.58, 3.16±0.49 mg/g heart weight, \( n=6 \)) as than in F1\( \beta \) hamsters (1.50±0.12 mg/g heart weight, \( n=6 \)) at 20 weeks of age (Fig 1). At 40 weeks of age, the myocardial collagen concentration in both strains was significantly greater than that in the F1\( \beta \) strain. The observed value was higher in Bio 53.58 hamsters than in Bio 14.6 hamsters (5.50±0.33 vs 4.02±0.21 mg/mg heart wet weight, \( n=6 \), \( p<0.01 \)). There were no significant differences in collagen concentration between cardiomyopathic and F1\( \beta \) hamsters at 5 or 10 weeks of age.

Collagen Phenotypes
Gel electrophoresis gave excellent separation of the individual type I and III collagen
Effects of Enalapril on Collagen Matrix in Cardiomyopathy

Fig 1. Age-related changes in the myocardial collagen concentration in F1β (○), Bio 14.6 (●) and Bio 53.58 (□) hamsters. Each value is the mean ± SD of 6 experiments. *p<0.05, **p<0.01 compared with age-matched F1β hamsters. +p<0.05, #p<0.01 compared with age-matched Bio 14.6 hamsters.

Fig 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of pepsin-digested protein obtained from heart tissue for the separation of individual type α1(I) and type α1(III) collagen chains. Each lane contained 40 mg of protein digested with pepsin from the hearts of 26-week old F1β (lane 1), Bio 14.6 (lane 2) and Bio 53.58 (lane 3) hamsters. Lanes 4 and 5 show standard type I collagen from bovine achilles tendon and type III collagen from calf skin, respectively.

Fig 3. The relative ratio of type III to type I collagen obtained from densitometric scanning in F1β (●), Bio 14.6 (□) and Bio 53.58 (▵) hamsters aged 5 to 40 weeks, calculated from data in Fig 2. Each bar represents the mean ± SD of 6 experiments. *p<0.05, **p<0.01 vs age-matched F1β hamsters. +p<0.05 vs age-matched Bio 14.6 hamsters.

However, the type III:I ratio at 20 weeks of age significantly increased by 50% and 85% in the Bio 14.6 and Bio 53.58 strains, respectively, relative to that in the F1β strain. At 40 weeks of age, the type III:I ratio in the Bio 53.38 hamsters was significantly increased by 2.6- and 3.6-fold compared with that in Bio 14.6 and F1β hamsters, respectively. However, no difference in this ratio was observed between the Bio 14.6 and Bio 53.58 hamsters at 5, 10 and 20 weeks of age.

Effects of Enalapril on Cardiomyopathic Hamsters

Enalapril was administered to cardiomyopathic Bio and F1β hamsters at an average dosage of 10.1±0.3 mg/kg per day from 10 to 20 weeks of age. Table III shows the effects of enalapril on the heart weight, body weight, heart weight to body ratio, collagen concentration and the collagen type III:I ratio at the end of the study (20 weeks of age). In Bio 14.6 hamsters treated with enalapril, the heart weight and body weight were significantly increased and the heart weight to body weight ratio was significantly decreased as compared with that in untreated hamsters of the same strain. The ratio did not change in Bio 53.58 or F1β hamsters, regardless of treatment. The collagen III:I ratio and collagen concentration were also significantly decreased in
TABLE III  HEART WEIGHT, BODY WEIGHT, HEART WEIGHT: BODY WEIGHT RATIO, COLLAGEN CONCENTRATION AND COLLAGEN III/I RATIO IN TREATED AND UNTREATED BIO CARDIOMYOPATHIC HAMSTERS

<table>
<thead>
<tr>
<th></th>
<th>Heart Weight (mg)</th>
<th>Body Weight (g)</th>
<th>HW/BW ratio</th>
<th>Collagen Concentration (μg/mgHW)</th>
<th>Collagen III/I ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1-β</td>
<td>untreated</td>
<td>434.6±16.2</td>
<td>142.8±6.1</td>
<td>3.05±0.22</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>419.3±30.5</td>
<td>147.2±7.4</td>
<td>2.85±0.09</td>
<td>1.1±0.1*</td>
</tr>
<tr>
<td>Bio 14.6</td>
<td>untreated</td>
<td>350.2±9.0</td>
<td>88.0±4.3</td>
<td>3.98±0.15</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>425.2±32.1*</td>
<td>114.5±0.5*</td>
<td>3.71±0.12*</td>
<td>0.9±0.1*</td>
</tr>
<tr>
<td>Bio 53.58</td>
<td>untreated</td>
<td>328.9±14.8</td>
<td>109.3±7.2</td>
<td>3.01±0.09</td>
<td>3.2±0.9</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>334.4±24.8</td>
<td>102.8±5.4</td>
<td>3.25±0.20</td>
<td>3.9±0.9</td>
</tr>
</tbody>
</table>

Treated values are the data in hamsters after enalapril administration (10 mg/kg per day) from 10 to 20 weeks of age. Each value is the mean±SD of 6 experiments. HW; heart weight, BW; body weight. *p<0.05 compared with untreated hamsters.

Fig 4. Northern blot analysis of cardiac RNA for α2 (I), α1 (III) collagen and β-actin. RNA was extracted from the hearts of Bio 14.6, Bio 53.58 and F1β hamsters which either were or were not treated enalapril. Each autoradiogram represents 3 individual experiments.

Fig 5. The relative ratio of mRNA expression of collagen type III to β-actin obtained from densitometric scanning in Bio 14.6, Bio 53.58 and F1β hamsters either treated (○) or not treated (□) with enalapril. Each bar represents the mean±SD of 6 experiments. *p<0.05, **p<0.01 vs treated or untreated F1β hamsters. #p<0.01 vs untreated Bio 14.6 hamsters.

Bio hamster. In contrast, α1 (III) mRNA collagen expression corrected by β-actin was significantly depressed in the hearts of enalapril-treated Bio 14.6 hamsters compared with those of untreated Bio 14.6 hamsters (Fig 5). In the Bio 53.58 strain, α1 (III) mRNA collagen expression was not altered by treatment with enalapril.

Morphological Examination
To clarify the mechanism of the beneficial effect of enalapril on the arrangement of myocardial collagen in Bio 14.6 hamsters,
SEM examination was performed at 20 weeks of age in control F1β (Fig 6A), and untreated Bio 14.6 (Fig 6B) and Bio 53.58 (Fig 6C) hamsters. There was a difference between the F1β and untreated Bio 14.6 hamsters with respect to fibrillar collagen.
sheaths that formed a woven network around the myocytes. It was apparent that the fibrillar collagen sheaths were a densely woven network in the hearts of the Bio 14.6 hamsters. These SEM findings in Bio 14.6 hamsters were similar to the findings in Bio 53.58 hamsters. The fibrillar collagen sheaths formed a looser network in the hearts of Bio 14.6 hamsters treated with enalapril (Fig 6D) than in those of untreated Bio 14.6 hamsters. This observation in the treated Bio 14.6 hamsters was similar to that in F1/β hamsters. However, treatment with enalapril did not affect the SEM findings in Bio 53.58 hamsters.

DISCUSSION

In the present study, we showed that the collagen concentration was increased in the myocardium of cardiomyopathic Syrian hamsters, and that this elevation was associated with an increase in type III collagen. We also showed that treatment with enalapril can prevent the increase in the collagen concentration, the collagen type III:I ratio, and mRNA expression of type III collagen seen in cardiomyopathic Bio 14.6 hamsters. Since the collagen matrix is a major determinant of myocardial architecture, structural integrity, and mechanical properties, the increased deposition of type III collagen described here may be partly responsible for changing the compliance of the myocardium, resulting in dilatation of the heart and possibly leading to heart failure.

Although we did not quantitate the amount of each type of collagen, the amount of type I collagen was similar during the progression of cardiomyopathy in both types of hamster because mRNA expression in the type I collagen to β-actin ratio did not vary significantly. Therefore, we can assume that an increase in type III collagen is associated with the changes in the mechanical properties of cardiomyopathic heart. In fact, type III collagen fiber is known to have less tensile strength than the thicker type I fiber, and may be responsible for the dilatation of the myocardium that results in the spherical formation of the left ventricular chamber. In the normal state, the proportion of type I to type III collagen present in the myocardium offers an optimal balance between resistance and elasticity. Shekhonin et al.29 have shown that in postinfarction cardiosclerosis, type III collagen is diffusely distributed, while type I collagen is restricted to the deeper regions of fibrous tissue. Mukherjee and Sen reported30 that the concentration of type III collagen was significantly increased in patients with ischemic cardiomyopathy. On the other hand, Bishop et al.31 have shown that excessive collagen production, with a preponderance of type I collagen, occurs in human cardiomyopathic hearts, and they demonstrated remodeling of the collagen matrix in diseased hearts. Weber et al.23 have noted that the proportion of type III collagen was increased in left ventricular hypertrophy in the absence of myocyte necrosis, and the proportion of collagen type I to type III returned to the control level in the presence of myocyte necrosis. Furthermore, they indicated that a loss of collagen tethers or a decline in tensile strength in the matrix can be responsible for both the regional and global transformation in myocardial architecture and function seen in stunned myocardium and dilated cardiomyopathy. Thus, it appears that the remodeling of collagen matrix varies with the progression of left ventricular hypertrophy. Our data revealed an increase in the tissue collagen concentration and the type III:I ratio in both strains of myopathic hamster at 20 weeks of age. At 40 weeks of age, the collagen type III:I ratio in the Bio 14.6 strain was unchanged, and was not significantly different from the results in age-matched F1/β hamsters. These results in Bio 14.6 hamsters were consistent with those in a nonhuman primate model of pressure overloaded hypertrophy described by Weber et al.23 On the other hand, in the Bio 53.58 strain as, which is a model of dilated cardiomyopathy, an increased deposition of type III collagen may be partly responsible for the impairment of left ventricular function, resulting in dilatation of the heart. Since the ratio of type I collagen to β-actin did not vary during the progression of cardiomyopathy, type III collagen is believed to be the dominant component of fibrillar connective tissue in myocardium in the Bio 53.58 hamster.

When Bio 14.6 hamsters were given enalapril for 10 weeks from the prehypertrophic
(10-week-old) to the hypertrophic (20-week-old) stage, there was a considerable decrease in the heart weight to body weight ratio, the collagen concentration and the collagen type III:I ratio as well as in the mRNA expression of type III collagen compared with the findings in untreated Bio 14.6 hamsters. In contrast, enalapril did not prevent the increase in collagen concentration in Bio 53.58 hamsters. Thus, the effects of enalapril may be quite different between the Bio 14.6 and Bio 53.58 strains of cardiomyopathic hamster. These observations may be explained as follows: intracellular calcium handling is seriously disturbed from a young age in the Bio 53.58 hamster; since the cytosolic Ca^{2+} concentration was high in 40-week-old Bio 53.58 hamsters but well-preserved in the early stages of cardiomyopathy in Bio 14.6 hamsters. This also differs from calcium handling in another model of hypertrophic cardiomyopathy, UM-X7.132 In addition, it has been reported that diminished β-adrenergic receptor responsiveness and cardiac dilatation in the hearts of Bio 53.58 hamsters are associated with a functional abnormality of the guanine nucleotide-binding regulatory protein that stimulates adenyl cyclase (Gs).33 In the present study, enalapril did not affect collagen metabolism in the Bio 53.58 hamster, presumably because collagen accumulation is more extensive in the Bio 53.58 strain than in the Bio 14.6 strain according to histological examination.34 Chemla et al.35 reported that perindopril (1 mg/kg per day), an ACE inhibitor, preserved both myocardial contractility and myothelial economy in 1-month-old Bio 53.58 hamsters. If we had administered enalapril to Bio 53.58 hamsters from a very young age, as described by Chemla et al, the drug may have prevented type III collagen production. Haleen et al.36 recently demonstrated that chronic therapy with the ACE inhibitor quinapril (112 mg/kg per day) had a significant cardioprotective effect in the CHF 146 CM hamster, which is a similar to the Bio 14.6 strain. Thus, ACE inhibitors appear to have prophylactic effects when administered to cardiomyopathic hamsters from the early stages of the disease. Furthermore, the ACE inhibitor captopril also normalized the collagen type III:I ratio in SHR36. In the present study, we administered enalapril at a dosage of 10 mg/kg per day, which was the same as that in an experiment in the SHR model by Nagano et al.37 who found that 10 mg/kg enalapril reduced systolic blood pressure, left ventricular weight, and left ventricular angiotensin II content.37

The mechanism by which collagen increases in the cardiomyopathic hamster may involve hypoxia due to microvascular spasms.34 The initial increase in collagen deposition could produce further hypoxia, thus setting off a cycle leading to severe myocardial dysfunction. Although it is not yet known how ACE inhibitors reduce fibrosis, some or all of the following factors may be involved: [1] nonspecific action through a reduction in afterload, [2] specific and exclusive inhibition of the effects of angiotensin II, [3] a direct action through factors involved in collagen regulation, and [4] inactivation of collagenase inhibitors. These factors may explain in part why ACE inhibitors have beneficial effects in the treatment of cardiovascular disease. Although the exact mechanism by which enalapril alters collagen metabolism remains to be elucidated, the present study demonstrates that the drug affects collagen content and phenotypic distribution, and reduces cardiac hypertrophy in Bio 14.6, as seen in the SHR model.36,37

The results presented here also illustrate that alterations in the proportion of type I to type III collagen in cardiomyopathy are at least partly regulated at the transcriptional level. The transcriptional factors at this level are unknown. However, several studies have examined the effects of various chemical factors, including transforming growth factor-β, glucocorticoids, and ascorbic acid, on collagen gene expression. Cell density, growth rate, and ECM environment have also been shown to influence collagen synthesis in vitro. Our data revealed a specific increase in type III collagen in Bio 14.6 and Bio 53.58 Syrian hamsters. At present, it is not clear how fibroblasts increase collagen expression in the cardiomyopathic hamster. Further studies are needed to identify the mechanism by which collagen synthesis is regulated and how changes in cardiomyopathy are produced by ACE inhibitors.
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