Cellular Mechanisms of Captopril-Induced Matrix Remodeling in Syrian Hamster Cardiomyopathy

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Background Although angiotensin-converting enzyme (ACE) inhibitors have become a mainstay of treatment for chronic congestive heart failure (CHF), it is not known whether the cardiac remodeling effects are a secondary phenomenon, resulting from ACE inhibitors' hemodynamic actions of afterload reduction, or occur through an independent mechanism.

Methods and Results We used ultrasonic tissue characterization to define potentially salutary effects of treatment with ACE inhibitors on the material properties of the heart and its potential influence on cardiac remodeling at the cellular level. Ten 1-month-old, cardiomyopathic (CM) Syrian hamsters and 6 normal (NL) hamsters were treated with captopril (2 g/L water ad libitum), and 10 CM hamsters and 10 NL hamsters were maintained untreated for 3 months. Hearts were excised, and backscattered radiofrequency data were acquired from 1200 independent sites from each specimen with a high-resolution 50-MHz acoustic microscope for calculation of integrated backscatter (IB). Treatment with captopril reduced left ventricular mass, calcium concentration, and IB in CM hearts without affecting myofiber size or collagen concentration. The IB from grossly normal regions of myocardium in NL hamsters, treated CM hamsters, and untreated CM hamsters was not significantly different. The IB from the microscopic regions of scar tissue in treated CM hamsters was significantly less ($P=.0004$) than that from scar tissue in untreated CM hamsters.

Conclusions The reduced IB from treated scar tissue components reflects specific alterations in the material properties (elastic stiffness, density) of fibrous regions in CM hearts induced by captopril. This is the first report that defines specific cellular effects of ACE inhibitors on the material properties of isolated components of cardiac tissue in experimental cardiomyopathy. These alterations in material properties of scar tissue components represent a potential mechanism for the salutary actions of ACE inhibitors in heart failure.

(Circulation. 1994;90:1334-1342.)

Key Words • captopril • cardiac remodeling • backscatter • cardiomyopathy

Angiotensin-converting enzyme (ACE) inhibitors have become a mainstay of treatment for chronic congestive heart failure (CHF) based on compelling evidence that they improve exercise tolerance\textsuperscript{1,2} and hemodynamic status\textsuperscript{3} and decrease heart size and mortality.\textsuperscript{4,5} ACE inhibitors also induce a regression of left ventricular hypertrophy (LVH) in patients with hypertensive cardiomyopathy.\textsuperscript{6} Despite these salutary effects, the cellular events responsible for these actions remain incompletely defined. Whether the cardiac remodeling effects of ACE inhibitors represent a secondary phenomenon that results from their hemodynamic actions of afterload reduction or whether remodeling occurs through a primary mechanism, such as direct inhibition of the local tissue renin-angiotensin axis,\textsuperscript{7-9} has not been resolved. Furthermore, determination of the specific mechanisms responsible for improved systolic and diastolic functions are confounded by inherently imprecise measurements of global ventricular function that are highly dependent on preload and afterload.

A principal objective of this research was to define potentially beneficial effects of treatment with ACE inhibitors on the material properties of heart tissue and to elucidate cellular mechanisms of improved cardiac remodeling. Our laboratory has previously reported that quantitative ultrasonic characterization of myocardial tissue based on analysis of frequency-dependent backscatter and attenuation can delineate fundamental physical properties and structural constituents of the human left ventricle\textsuperscript{10} under conditions of ischemia,\textsuperscript{11} infarction,\textsuperscript{12} and cardiomyopathy.\textsuperscript{13} We have shown that ultrasonic tissue characterization can sensitively depict pathological changes in the content\textsuperscript{14} and organization\textsuperscript{15} of the myocardial connective tissue matrix. Recent work from our laboratory has demonstrated the validity of ultrasonic analysis for quantification of the elastic properties of heart tissue.\textsuperscript{16} Ultrasonic methods can be used to determine values for Young's modulus for heart tissue, which closely approximate those obtained by conventional methods based on deformation stress-strain relations. Because the interaction of ultrasound with the heart depends on the material properties of cardiac tissue\textsuperscript{17,18} such as density and elastic stiffness, ultrasonic tissue characterization provides a unique approach for detection of the effects of ACE inhibitors on cardiac remodeling. The use of high-resolution, high-frequency acoustic microscopy permits delineation of these material properties at the microscopic level.\textsuperscript{19}

We hypothesized that ultrasonic tissue characterization could detect the potentially beneficial effects of ACE inhibitors on microscopic cardiac structure and material properties in experimental cardiomyopathy.
Selected strains of Syrian hamsters exhibit a heritable hypertrophic cardiomyopathy that progresses to a dilated stage according to a predictable time course.\textsuperscript{20,21} The cardiomyopathy is characterized by focal myocardial myolysis and necrosis beginning at approximately 30 days of age and subsequent replacement of myocytes with fibrotic connective tissue. As the fibrosis increases, there is a compensatory hypertrophy followed by chamber dilation and finally death occurring from CHF at approximately 280 days of age.\textsuperscript{20,21}

The focality of the myocardial lesions seen in this cardiomyopathy has led many investigators to postulate that microvascular spasm may be the primary etiology of the cardiomyopathy. Factor et al\textsuperscript{22} demonstrated that verapamil treatment caused histological resolution of the myocardial lesions of cardiomyopathic (CM) Syrian hamsters. Other investigators have also shown that therapy with verapamil\textsuperscript{23,24} or diltiazem\textsuperscript{25} can significantly improve or prevent the pathological myocardial changes of CM Syrian hamsters. We propose that the presence of multiple discrete areas of myocardial fibrosis and hypertrophy in these hearts would afford an invaluable model for studying cellular effects of ACE inhibitors on cardiac scar tissue remodeling. Because ACE inhibitors have been shown to reduce global myocardial chamber stiffness\textsuperscript{26} without a concomitant improvement in the passive stiffness of the noninfarcted myocardium,\textsuperscript{27} we hypothesized that captopril treatment would elicit specific changes in material properties of isolated scar tissue components. Accordingly, we report the use of quantitative ultrasound to characterize the specific effects of ACE inhibitor therapy on the material properties of isolated scar tissue components in cardiomyopathy.

Methods

Animal Preparations

A total of 46 1-month-old, male Syrian hamsters were used. The hearts of 5 normal (NL) Syrian hamsters (Bio F1-B strain, Biobreeders Inc) and 5 CM Syrian hamsters (Bio TO-2 strain, Biobreeders Inc) were examined at 1 month of age to obtain baseline morphological and ultrasound heart measurements before the institution of ACE inhibitor therapy. All hamsters were housed 3 to a cage with free access to laboratory rodent chow (Purina) and were maintained in Hepa-filtered “stay clean” units with a 12-hour light/dark cycle. Ten CM and 6 NL hamsters were treated with captopril (2 g/L water ad libitum). Ten CM and 10 NL hamsters were maintained untreated for 3 months. Body mass was measured every 2 weeks. At the end of the study, the 4-month-old hamsters were first anesthetized with methoxyflurane, then killed by pentobarbital overdose (>120 mg/kg IP). The study protocol was approved by the Animal Care Committee of the Jewish Hospital at Washington University Medical Center.

Morphometric Analysis

After fixation in formalin, the hearts were sectioned. The right ventricle was removed, the left atrium and great vessels were trimmed away, and the left ventricle was weighed. The left ventricular (LV) apex was used for analysis of collagen content by the method of Woessner.\textsuperscript{28} A 500-\textmu m section from the midmyocardium was used for determination of calcium content by quantitative photometric absorption.\textsuperscript{29} A section of the LV posterior wall was stained with hematoxylin-eosin, Masson's trichrome stain, and Von Kossa's stain for calcium. Collagen and calcium content also were calculated with computer-assisted planimetry of stained sections according to methods previously described.\textsuperscript{13} Calcium content was determined by planimetry in all scar tissue regions of both CM groups and expressed as percentage of total scar. Myofiber diameter was determined to the nearest hundredth of a micron with computer-assisted planimetry by measuring approximately 140 fibers from each specimen within 25 high-power fields.

Acquisition and Analysis of Ultrasonic Data

A vibratome (model Pelco 101, Ted Pella, Inc) was used to cut a 500-\textmu m-thick section from the midmyocardium of each heart. These sections were then mounted in a holding device with the endocardial side facing the transducer. The tissue holder and the mounted specimen were then immersed in degassed distilled water at room temperature for ultrasonic insonification. A custom-designed acoustic microscope was used to collect ultrasonic data as described previously by our laboratory.\textsuperscript{13} Briefly, a 50-MHz, broadband, focused, piezoelectric delay-line transducer (1/4-in. diameter, 1/8-in. focal length, model V390, Panametrics Co) was operated in the pulse-echo mode. A Tektronix DSA 601 digitizing oscilloscope was used to digitize backscattered radiofrequency data at 500 megasamples per second with eight-bit resolution. Radiofrequency data were acquired from approximately 1200 independent sites from each specimen.

The radiofrequency data were stored in a low-resolution, raster scan format and analyzed with custom software. Segments of the radiofrequency lines 500 nanoseconds in duration and 100 nanoseconds below the cut surface of the section were gated for analysis to avoid the front and back wall specular echoes from the specimen. The gated data were multiplied by a Hamming window, and their power spectra were determined by fast-Fourier transformation. The power spectra from the myocardial specimens were referenced to the power spectrum returned from a near-perfect steel planar reflector, according to methods previously described.\textsuperscript{10,12} Integrated backscatter (IB), a measure of the relative efficiency of ultrasonic scattering from tissue, was computed from the average of the frequency-dependent backscatter transfer function across the useful bandwidth of the transducer (30 to 50 MHz). IB was expressed in decibels relative to scattering from the steel plate. The IB data at every independent site within each tissue slice were converted into a gray scale map to allow visual inspection and quantitative analysis of backscatter from regions of grossly normal or fibrotic tissue (Fig 1). The gray scale map allowed us to use the grossly normal regions as control data.

Statistical Analysis

Ultrasonic, morphological, and biochemical measurements were compared with the use of commercially available statistical software (STATVIEW 4.0, Abacus Concepts). Two groups of 1-month-old hamsters (NL and CM) were used to determine the baseline comparability of IB, LV mass %, and collagen content (based on hydroxyproline analysis), with the use of an unpaired t test. Three groups of 4-month-old hamsters (NL, treated, and untreated CM) were used to determine the effects of 3 months of captopril therapy on IB, LV mass %, collagen content (based on hydroxyproline analysis), weekly weight gain, and body mass, with the use of ANOVA methods to test the significance of differences, which was assigned at the P<.05 level. Testing of significance differences among these three groups was performed with the Fisher's protected least squares difference t test. All data are expressed as mean±SE unless otherwise indicated.

Results

Baseline Measurements for Untreated 1-Month-Old NL and CM Hamsters

Because all hamsters were approximately 1 month of age at the start of the study, we sought to establish
baseline measurements for both NL and CM hamster hearts. There were no statistically significant differences in LV mass % (normalized for body mass) or collagen concentration (mg/g LV dry mass) between the 1-month-old NL and CM hamsters (Fig 2). Fig 2 also shows that 1-month-old NL hamsters exhibited slightly greater myocardial scattering than did the CM hamsters ($P=.005$).

**Effects of Captopril Therapy**

**Body Weight**

Fig 3 shows the growth curves for NL, untreated, and treated CM hamsters over the 3-month study period. Baseline weights among the three groups were not statistically different at the beginning of the study. After 3 months, however, the NL hamster group exhibited a significantly higher mean weight than did either of the two CM hamster groups, which were equivalent ($P<.0001$). These data indicate that captopril treatment did not affect the body weights for the CM hamsters. Both treated and untreated CM hamsters gained less weight than did the NL hamsters despite starting at equivalent weights at 1 month of age (see above).

**LV Mass and Myofiber Diameter**

Fig 4 shows the LV mass % (normalized for body mass) for 4-month-old NL, untreated CM, and treated CM hamsters. The hearts of the NL and treated CM hamsters exhibited a similar LV mass %, which was significantly less than that of the untreated CM hamsters ($P<.0001$). Grossly, both groups of CM hamsters manifested whitish bands of fibrous tissue running throughout the myocardium, whereas hearts of NL hamsters had none. Microscopic analysis of the grossly
normal tissue in each heart revealed that myofiber diameters differed minimally among the three groups: 9.8±0.1 μm for untreated NL hamsters, 9.3±0.1 μm for treated NL hamsters, 8.9±0.1 μm for untreated CM hamsters, and 9.7±0.1 μm for treated CM hamsters. Thus, treatment with captopril elicited a 23% decrease in LV mass in treated as compared with untreated CM hamsters, with minimal changes in the size of grossly normal myofibers.

Normal hamsters were treated to determine the general effect that ACE inhibitors have on LV mass. After 3 months of captopril therapy, the treated NL hamsters manifested an 18% decrease in LV mass % as compared with the untreated NL hamsters (0.197±0.007 versus 0.240±0.005, P=.0004). These data show that captopril therapy starting at 1 month of age can inhibit heart growth in both NL and CM hamsters.

**Collagen Concentration**

Fig 5 shows the LV collagen concentration (mg/g left ventricle) for 4-month-old NL, untreated CM, and treated CM hamsters. After 3 months, both treated and untreated CM hamsters exhibited increases in LV collagen to levels significantly greater than that for the NL hamsters (P=.0002). Computer-assisted planimetry of histological sections stained with Masson’s trichrome also corroborated the increased LV collagen concentration of the CM hamsters compared with NL hamster hearts. Both the hydroxyproline assay and the computer-assisted planimetry yielded CM collagen concentrations approximately 1.5 to 2 times control levels in NL animals. The collagen concentrations measured by the two methods were highly correlated (r=.86). Thus, 3 months of captopril treatment does not appear to significantly reduce the average concentration of collagen (P=NS), both of which were significantly greater than the normal hamsters (NL) (P=.0002).

**Calcium Concentration**

Fig 6 shows the LV calcium concentration (mg/g left ventricle) for 4-month-old NL, untreated CM, and treated CM hamsters. After 3 months, the whole hearts of both treated and untreated CM hamsters exhibited LV calcium concentrations significantly greater than that for the NL hamsters (P=.0001). Treatment with captopril resulted in a 37% decrease in calcium concentration for the treated as compared with the untreated CM hearts (P=.001). Examination of histological sections stained with Von Kossa’s demonstrated little gross calcification in the normal myocardium. Computer-assisted planimetry of scar tissue regions corroborated the increased calcification of scar tissue in the untreated CM hearts as compared with the captopril-treated CM hearts (55±1% versus 43±2%, P<.0001). Thus, 3 months of captopril treatment significantly reduced the average concentration of calcium in the scar tissue regions of CM hamsters. There was also a strong correlation between LV mass % and planimetry scar calcium content (r=.85, P=.0005).

**Quantitative Ultrasonic Indexes**

Fig 7 illustrates the effect of 3 months of captopril therapy on IB. Both treated and untreated CM hamster hearts demonstrated substantially greater IB as compared with the NL hearts. Captopril therapy, however, resulted in a 2.1-dB (1.6-fold) decrease in IB for the treated as compared with the untreated CM hearts (P<.02). The hearts of the 4-month-old untreated CM hamsters exhibited a 6.5±0.7-dB (4.5-fold) increase in IB as compared with the 1-month-old untreated CM hamsters (P<.0001). The hearts of the 4-month-old
treated CM hamsters exhibited a 4.4±0.8-dB (2.7-fold) increase in IB as compared with the 1-month-old untreated CM hamsters (P=.0006). Thus, the effect of treatment with captopril produces a 2.1-dB (1.6-fold) decrease in IB in CM hamsters. The values of IB from the 1-month-old (see Fig 2) and 4-month-old NL hearts were not significantly different. Thus, aging did not alter the ultrasonic scattering from NL hearts but significantly augmented IB from CM hearts. This progressive increase in IB from CM hearts was mitigated by captopril.

The IB data reported above were acquired from unselected regions of myocardial tissue, comprising admixed grossly normal and fibrotic tissue in the case of CM hamsters. To address the question of which component of the myocardial tissue in CM hearts might be most affected by captopril therapy, we reanalyzed the data by partitioning the heart into zones of high and low scattering according to the IB gray scale map (see Fig 1). By comparing the histological sections from the same regions on which backscatter analysis was performed, we determined that the areas of high scattering corresponded precisely to the whitish bands of fibrous tissue observed in the gross specimens (Fig 8). Therefore, for each CM specimen, all radiofrequency lines lying within a high scattering zone were counted as fibrous scar, and all radiofrequency lines lying within low scattering zones were counted as grossly normal myocardium. For any given sample of CM myocardium, the scarred areas made up no more than 10% to 20% of the total radiofrequency data (see Fig 1).

Fig 9 illustrates that the grossly normal (low scattering) zones exhibited no significant difference in IB among the NL, treated, and untreated CM hamster hearts (P=NS). Fig 10 shows that the magnitude of IB from the fibrous (high scattering) zones in CM animals was significantly less in the treated than in the untreated CM hamsters (P=.0004). The IB from these high scattering fibrous zones correlated linearly with scar tissue calcium content measured by planimetry (r=.60, P=.01). These data indicate that over the 3 months of the study, IB increased in both treated and untreated CM hamster hearts due primarily to the presence of bandlike areas of fibrosis. The magnitude of IB in the fibrous scar zones reflected in part calcium content of scar tissue. Captopril therapy resulted in a specific reduction of IB from fibrous scar tissue in treated animals but had no effect on IB from grossly normal areas in any hearts.

Additional evidence of this specific effect of captopril on scar tissue is provided by comparing grossly normal and fibrotic regions from individual animals. This analysis uses each animal as its own control. The difference between IB from normal and fibrotic regions was 13.0±0.4 dB in the treated CM hamsters (P<.0001) and 15.4±0.8 dB in the untreated CM hamsters, a 2.4-dB (1.7-fold) increase (P<.0001). These data verify that IB was significantly greater in fibrotic as compared with grossly normal regions in CM hearts. Therefore, therapy with captopril resulted in reduced backscatter in scar tissue components.

Discussion

In this study, we used ultrasonic tissue characterization methods to elucidate the cellular effects of captopril on remodeling in a heritable cardiomyopathy. These
data indicate that captopril exerts a prominent remodeling effect on the material properties of the scar tissue component of cardiomyopathic hearts, whereas the material properties of the interspersed grossly normal myocardium remain unchanged. The use of cardiomyopathic Syrian hamsters permitted accurate control of experimental conditions necessary to elucidate specific features of ACE inhibitors in cardiac remodeling. The use of high-frequency, high-resolution acoustic microscopy was crucial for defining the material properties of intact cardiac tissue with microscopic precision.

Four predictable morphological/histological and clinical stages evolve in this heritable CM disease process: prenecrotic, necrotic or myolytic, hypertrophic, and terminal.20,30,31 Grossly visible white streaks are caused by fibrosis and calcification of the degenerating muscle and follow the direction of the myocardial fibers at all levels of the hypertrophied left ventricle.31 The CM hamster proved to be an ideal model for studying the microscopic process of cardiac remodeling by providing multiple discrete areas of fibrosis for examination. IB increased in both treated and untreated CM hamsters, but captopril therapy significantly limited the increase in IB in the treated CM hamsters. These differences in ultrasonic scattering were due to the effects of captopril on the fibrous scar regions because the IB from the grossly normal (nonscarred) myocardium was not different among the NL, treated, and untreated CM animals. Therefore, captopril induced a specific remodeling effect primarily on the material properties of the scar tissue component, resulting in a 3.8-dB (37.2-dB versus 33.4-dB for untreated versus treated CM fibrous areas) or 2.4-fold decrease in IB from these regions. The material properties of the grossly normal, non-scarred myocardium, however, were unaffected by captopril treatment.

Other investigators have demonstrated salutary effects of ACE inhibitors in CM Syrian hamsters. Haleen et al.32 showed that administration of quinapril to CM hamsters from 180 to 300 days of age prevented the decline of LV function in vitro and coronary flow in vitro, reduced the age-dependent increases in LV volume, and increased median survival time 33%, as compared with vehicle-treated CM Syrian hamsters. Kato et al.33 observed that administration of captopril to 5-week-old CM Syrian hamsters ameliorated the expected elevations of creatine kinase, aldolase, V, myosin, and malondialdehyde (MDA) typically observed in untreated CM hamsters. They attributed the rise of MDA in untreated CM Syrian hamsters to an increase in active oxygen species as a consequence of damage to the active oxygen scavenger systems after cell membrane damage.33 Nasmussen et al.34 recently showed that enalapril treatment restores intracellular energy reserve systolic performance in CM Syrian hamsters.

Our data indicate that captopril elicited an antihypertrophic effect, resulting in CM cardiac remodeling in IB (relative IB from the CM hamster group). Treatment with captopril caused a decrease in calcium concentration in the scar tissue regions but did not affect myofiber diameter or relative collagen concentration (normalized for LV mass). Only a small difference in myofiber diameter of approximately 0.7 μm (7%) was noted in the untreated CM hamster. A generalized downregulation of heart growth was observed in both NL and CM animals after treatment with captopril. Thus, ACE inhibitors appeared to induce a proportionate reduction in the amount of both grossly normal myofibrils and fibrous tissues for NL and CM animals. Therefore, this effect appears generalized in contrast to the specific effect on the material properties of scar tissue components.

The hypothesis that remodeling of scar tissue elements alters the material properties of the fibrous tissue is supported by previous data from our laboratory. We and others have shown that one of the primary determinants of scattering from myocardial tissue is collagen.33 Hoyt et al.35 and Wong et al.36 also demonstrated a close correspondence between the magnitude of IB and collagen concentration. In the normal myocardium, IB is greater in the right ventricle than in the left ventricle, a difference corresponding to a higher collagen content in the right ventricle.36 However, the physical determinants of backscatter are more complex. O’Donnell et al.37 showed that the organization of collagen represents an important parameter of scattering behavior in infarct scar tissue. They demonstrated the magnitude of IB increases dramatically as scar tissue ages and contracts. Mimb’s et al.38 further defined the role of collagen organization in scattering from rabbit myocardium. They showed that perfusion of infarct regions with collagenase resulted in a substantial decrease in IB, although the concentration of collagen fragments measured in the infarct region remained unchanged. These data demonstrate that the organization of the complex structure of intact collagen is a more important determinant of scattering than merely the amount of its substrate present in the tissue.

Wickline et al.39 recently showed that the three-dimensional organization of scar tissue elements profoundly influences scattering behavior in human myocardial infarct scars. Furthermore, the angle dependence of insonification or ultrasonic anisotropy of grossly normal and infarcted tissue also influences scattering behavior.39,10,12,16 as IB is maximal in a direction perpendicular to the fibers and minimal in a direction parallel to or along the fiber axis. These anisotropic effects are even more pronounced in tissue composed primarily of collagen. Hoffmeister et al.40 showed a 39-dB difference in IB when insonifying either parallel or perpendicular to human Achilles tendon (80% to 90% type I collagen by dry weight), whereas normal myocardium displayed only 15 dB of anisotropy. Because we insonified all tissue sections perpendicular to the ultrasonic beam, the effect of anisotropy on the magnitude of IB was minimized for these data. These observations stress the importance of the organization and tertiary structure of collagen as additional critical determinants of the magnitude of IB.

Our laboratory has demonstrated that backscatter depends on the intrinsic material properties of the tissue.41 These material properties, elastic stiffness (E) and density (ρ), determine tissue material imaging parameter, Zof the tissue, where Z= (Eρ)0.5. Local (microscopic) variations in acoustic impedance (or elastic stiffness and density) elicit marked changes in scattering behavior. The differences observed in IB between the scar tissue and grossly normal myocardial regions therefore confirm that scar tissue is either stiffer or denser than normal tissue. These alterations in material properties might represent increased cross-linking of colla-
Potential Mechanisms Responsible for Captopril-Induced Remodeling

The mechanisms responsible for the downregulation of LV mass and the reorganization of scar tissue elements remain unknown, although several hypotheses seem plausible. ACE inhibitors have long been recognized to cause regression of LVH in animal models and patients.50-52 Previously, this antihypertrophic effect had mostly been considered a secondary phenomenon attributed to hemodynamic changes brought about by ACE inhibitors. However, angiotensin II is now known to be a potent cardiac myocyte growth factor in vitro,46 which can be blocked by ACE inhibitors.48 Evidence of a dissociation between antihypertrophic and systemic hemodynamic effects was provided by studies from Linz et al and Baker et al,49 which demonstrated that treatment with “subtherapeutic” doses of ACE inhibitors did not affect blood pressure48 or afterload49 but resulted in prevention and regression of LVH in aortic-banded rats.

Our data are compatible with these in vitro observations. Captopril caused a reduction in LV mass % (normalized for body mass) in treated NL hamsters as compared with untreated NL controls. Thus, the lower LV mass observed in the captopril-treated CM hamster group may have been due to inhibition of the growth-promoting effects of angiotensin II. However, this effect may be unrelated to scar tissue formation, since it was observed in both NL and CM myocardium. Despite reducing LV mass %, captopril treatment did not result in any significant changes in myofiber diameter in both NL and CM groups, which suggests an antihyperplastic effect. Thus, treatment with captopril would seem to elicit a nonspecific downregulation of growth in all tissue components.

Angiotensin II also may induce fibroblast proliferation,50 in vitro collagen synthesis,51 and in vivo myocardial fibrosis.52-55 Tan et al53 showed that chronic infusion of supraserous doses of angiotensin II resulted in a fibrotic response in rat hearts. ACE inhibitors could function to decrease or prevent fibrogenic propensity of angiotensin II. Captopril has been shown to augment intracellular collagen degradation.54 Angiotensin II stimulates collagen synthesis from cardiac fibroblasts in vitro, whereas prostaglandin E2 inhibits this process.51 ACE inhibition could decrease levels of both circulating and tissue angiotensin II and increase myocardial prostaglandin E2, which together may ameliorate myocardial fibrosis. Furthermore, ACE inhibition may concomitantly increase collagenase activity, which could further decrease the extent of myocardial fibrosis. Previous observations from our laboratory have demonstrated the profound impact of treatment of myocardial scar tissue with clostridial collagenase to reduce the magnitude of IB.55 We observed a similar increase in collagen concentration in both CM groups. Enhanced collagen degradation could have played a role in both the decrease in IB as well as the decreased LV mass % in treated CM hamsters.

Other studies have shown that CM Syrian hamsters demonstrate a significant increase in mitochondrial free radicals,24,55 antioxidant activity,56 and myocardial lipid peroxides24 compared with normal age-matched control hamsters. The upregulation of antioxidant activity is apparently compensatory although inadequate, because treatment with α-tocopherol, a free radical scavenger, elicits a significant decrease in myocardial damage and scarification as compared with untreated CM control hearts.55

It has been demonstrated that those ACE inhibitors that contain a sulfhydryl group (captopril and zofenopril) possess free radical scavenger properties,56 whereas others do not.57 Many studies have shown a cardioprotective effect with captopril treatment in both stunned myocardium and injury, whereas enalapril effects were somewhat more variable.58 Other theoretical mechanisms warrant additional consideration, however, because both quinapril32 and enalapril,34 ACE inhibitors without a sulfhydryl group, exhibit significant salutary effects in Syrian hamster CM.

Because captopril appears to induce a proportional downregulation of growth in all tissue components, it may have delayed the natural history of the cardiomyopathy, resulting in reduced LV mass and essentially “younger” scar tissue in the treated CM hamsters at 4 months of age. This hypothesis is further supported by the strong correlation between LV mass % and scar tissue calcium content: The larger the left ventricle, the further the progression of the natural history, and the older the scar, the more extensive the calcification. Our laboratory has previously shown that the magnitude of IB increases dramatically as scar tissue ages and contracts.14 If the scarred regions of the captopril-treated CM hamster hearts were less mature or less well organized (either less cross-linked and/or calcified), scars would be expected to produce less backscatter as compared with the more mature fibrotic regions of the untreated CM hamsters. Thus, the effect of captopril to delay scar tissue calcification may be due to molecular mechanisms that may inhibit the deposition of a tissue matrix that would favor calcification.

Clinical Implications

In our study, captopril induced potentially beneficial changes in the material properties of scarred myocardium. The ability to detect these changes with ultrasonic tissue characterization may have many clinical ramifications. Although ACE inhibitors have been shown to cause regression of LVH and reduce mortality in chronic CHF,54 and after myocardial infarction,55 it is unclear whether these results are a product of a hemodynamic effect or operate directly on tissue through an independent mechanism. Our study demonstrated one of the potential reasons why ACE inhibitors may play a salutary role in various forms of heart disease. As elucidated with ultrasonic tissue characterization, the scarred myocardium from captopril-treated
CM hamsters either manifested a delayed natural history or a direct reorganization of scar tissue components, either of which could result in a more compliant scar with reduced cross-linking and calcification. Hypothetically, more compliant scars would result in lower LV filling pressures, thereby improving diastolic function and ameliorating the need for a compensatory LVH or LV dilation to maintain systolic function.

Our data indicate that treatment of CM hamsters with captopril for 3 months produced an antihypertrophic effect, evidenced by a 23% decrease in LV mass compared with the untreated CM group. We demonstrated that ultrasonic tissue characterization can sensitively detect captopril-induced changes in material properties of scar tissue components of the heart. These are the first data to show that captopril elicits specific changes in scar tissue material properties, which may reflect retarded maturation and calcification of scar. To date, there are no reliable methods for monitoring the remodeling effects of ACE inhibitors at the cellular level. Quantitative ultrasonic tissue characterization may ultimately play a role in initiating and guiding the therapy with ACE inhibitors in patients with cardiomyopathy and other forms of CHF.

Acknowledgments

This study was supported in part by grants HL-42950, HL-40302, and an Established Investigator Award from the American Heart Association (Dr. Wickline).

References


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_Circulation_. 1994;90:1334-1342
doi: 10.1161/01.CIR.90.3.1334
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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