Background—This study was designed to determine the effects of pretreatment with an angiotensin receptor blocker on left ventricular (LV) function and remodeling during acute myocardial infarction (MI).

Methods and Results—Sprague-Dawley rats were pretreated with candesartan (10 mg · kg⁻¹ · d⁻¹) for 2 weeks and studied at 1, 3, and 6 minutes after MI. Compared with untreated rats, pretreatment with candesartan lowered (P<0.05) LV systolic pressure and the first derivative of LV pressure with respect to time but did not change LV end-diastolic pressure or improve LV regional function. With candesartan pretreatment, LV fractional shortening and ejection fraction increased (P<0.05) by 37% and 28%, and LV chamber dilation was attenuated (P<0.05). At 6 minutes after MI, LV endothelial nitric oxide synthase decreased in the infarcted and noninfarcted wall 47% (P=0.04) and 70% (P=0.002), and constitutive microtubulin increased 260% (P=0.0005) and 111% (P=0.003). Candesartan had no effect on LV tissue endothelial nitric oxide synthase levels but attenuated the increase in constitutive microtubulin by 77% (P=0.004) and 37% (P<0.05).

Conclusions—Pretreatment with candesartan before an acute MI improves global LV function, prevents LV dilation, and blunts the increase in constitutive microtubulin, with minimal effects on LV hemodynamics, regional function, or tissue endothelial nitric oxide synthase. Thus, candesartan given before an MI attenuates LV remodeling and alters the cytoskeleton matrix of the left ventricle. (Circulation. 2006;114:1933-1939.)

Key Words: angiotensin | heart failure | myocardial infarction | nitric oxide synthase

Although blocking activation of the renin-angiotensin system (RAS) after a myocardial infarction (MI) is standard treatment to reverse maladaptive left ventricular (LV) remodeling, the efficacy of this treatment as a preventive modality is unclear. Recent clinical trials have proposed a preventive strategy with blockade of the RAS in patients at risk for atherosclerotic heart disease to prevent ischemic events and to improve cardiovascular morbidity/mortality.1–3 However, many patients treated with an angiotensin-converting enzyme inhibitor (ACEI) and/or angiotensin receptor blocker (ARB) will still go on to have a MI. The hypothesis is that pretreating patients with neurohormonal blockade will attenuate maladaptive LV remodeling at the time of the acute infarct. This assumption would be difficult to study in clinical trials. Therefore, in the present study, we examined this hypothesis in the rat coronary artery ligation model of acute MI in which we are able to collect data during the acute MI. To explore selected signaling components that may play a role in myocardial remodeling, we examined possible changes in 2 different molecules in acute MI and heart failure: endothelial nitric oxide synthase (eNOS) and constitutive microtubulin (CM). We chose eNOS because, although tissue eNOS activity is downregulated in chronic heart failure and is associated with decreased LV function,4–6 the effects of acute MI on eNOS are unknown.4,7 Similarly, we selected CM because it has previously been implicated in LV remodeling. Additionally, CM is upregulated in both chronic ischemic and hypertensive heart failure, but there is little information about changes to CM in an acute infarct.8–10

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Methods

Experimental Design

Normal adult male Sprague-Dawley rats (8 to 10 weeks old; Harlan, Indianapolis, Ind) were randomized to placebo or pretreatment with candesartan (candesartan cilexetil, AstraZeneca, Wilmington, Del) given orally for 14 days in the drinking water at a dose of 10 mg · kg⁻¹ · d⁻¹. The experiments were performed in an American Association for Accreditation of Laboratory Animal Care-accredited facility with approval from the animal use committees of the

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Southern Arizona Veterans Affairs Health Care System and the University of Arizona.

MI Model
Adult male Sprague-Dawley rats underwent experimental MI by standard techniques developed in our laboratory.11–13 In brief, rats were anesthetized with inactin; a left thoracotomy was performed; the heart was expressed from the thorax; and a ligature was placed around the proximal left coronary artery. The heart was returned to the chest, and the thorax was closed.

Hemodynamics
Before the coronary artery ligation, a 1-mm micromanometer-tipped catheter (Millar Instruments, Houston, Tex) was inserted into the right carotid artery. The catheter was advanced into the aorta and then into the left ventricle under constant pressure monitoring. A zero-pressure baseline was obtained by placing the pressure sensor in 37°C saline before measurements. After a stabilization period of 15 minutes, LV pressures were recorded and digitized at 1000 Hz with a computer equipped with an analog-to-digital converter and customized software. We measured heart rate, LV end-diastolic pressure (LVEDP), the first derivative of LV pressure with respect to time (dP/dt), and the time constant of LV relaxation (τ).12–15 This approach allowed us to obtain data at baseline and 1, 3, and 6 minutes after coronary artery ligation.

Echocardiography
We obtained open-chest intrathoracic echocardiography at baseline and 1, 3, and 6 minutes after coronary artery ligation. We used a Vingmed, Vivid 7 System (GE Ultrasound, Waukeasha, Wis) echo machine with EchoPac programming software and a 10-MHz transducer with views obtained in the parasternal short-axis view. These images were digitized in sequential frames for 2-dimensional and M-mode measurements of LV dimensions throughout the cardiac cycle. The anterior wall was noted as the infarcted segment; the posterior wall was noted as the noninfarcted segment. Data from these regional measurements were used to calculate systolic displacement representing the contraction and relaxation patterns of the myocardium.

LV Ejection Fraction and Fractional Shortening
Linear measurements to determine LV performance such as ejection fraction and fractional shortening were obtained by M-mode in the parasternal short-axis view. Cursor placement was performed in diastole and systole through the interventricular septum, the internal dimensions of the left ventricle, and the posterior wall in its minor axis. Fractional shortening was obtained by the following formula

\[ FS = \frac{LVIDd - LVIDs}{LVIDd}, \]

where LVIDd is the LV internal dimension in diastole and LVIDs is the systolic internal LV dimension.

Regional Systolic Displacement
Echocardiography images of the left ventricle were obtained in the parasternal short-axis view and both 2-dimensional and M-mode views of the infarcted (anterior) region and noninfarcted (posterior) region. Systolic displacement was calculated by measuring the differences in LV wall thickness between peak systole and diastole of both the anterior and posterior regions. Five cardiac cycles were used to obtain several data sets.

Pressure-Diameter Changes During Filling
We evaluated changes in LV filling by recording the pressure-diameter relationship from the peak systolic pressure to the lowest LVEDP continuously throughout the cardiac cycle. We also evaluated LV filling by measuring τ as noted above. To measure the LV pressure-diameter relationship, we obtained echocardiograms in the parasternal short axis at the same time we had the 1-mm microm-

LV Tissue eNOS and CM
The left ventricle was removed immediately after completion of the echocardiography studies. Left ventricles were dissected into anterior, posterior, lateral, and septal portions. The tissue was immediately incubated in microtubule stabilization buffer16 on ice for 1 hour. The stabilization buffer stock was made with 50 mL glycerol and 5 mL dimethyl sulfoxide and contained (in final concentration) 10 mmol/L sodium phosphate, 0.5 mmol/L MgCl₂, 0.5 mmol/L GTP, 0.5 mmol/L EDTA, pH 8, and 100 U/mL aprotinin. The buffer was brought to a 100-ML volume with water; pH was then brought to 6.95, and the buffer was stored at −4°C. Just before use, protease inhibitors (10 mmol/L benzamidine, 1 mmol/L phenylmethylsulfonyl fluoride, 10 mmol/L aprotinin, 10 mmol/L leupeptin, and 10 mmol/L pepstatin A) were freshly added to the stabilizing buffer to prevent artificial depolymerization of microtubules. The tissue was homogenized on ice for 5 minutes, and the homogenate was centrifuged at 100 000g for 15 minutes at room temperature. The supernatant was removed and considered to hold the free tubulin fraction of the cardiomyocytes.

Protein analysis was carried out with Lowry reagent and folin ciocalteu’s reagent and analyzed on a Packard Spectra machine (wavelength, 620 nm). The supernatant (25 μg for tubulin, 50 μg for eNOS) was fractionated with 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis. The proteins were transferred to polyvinyl difluoride membranes and blocked with blocking solution (5% nonfat dry milk in 1× phosphate-buffered saline Tween-20) at room temperature for 1 hour. The membranes were incubated with either monoclonal antibutulin beta antibodies at 1:2000 (Santa Cruz Biotechnology Inc, Santa Cruz, Calif) or monoclonal anti-eNOS immunoglobulin G antibodies at 1:500 (Transduction Laboratories, Lexington, Ky) for 2 hours at room temperature. The membranes were washed with phosphate-buffered saline Tween-20 6 times, 10 minutes per wash. The tubulin and eNOS proteins were detected with horseradish peroxidase–labeled goat anti-mouse immunoglobulin G secondary antibodies at 1:40 000.

Western blots were quantitatively analyzed with photospectrometry using the Storm protocol. The autoradiographic image is scanned into a Tiff file that is transported to the ImageQuaNT software (Molecular Dynamic, Sunnyvale, Calif). The software uses a volume quantification system that calculates the volume under the surface created by a 3-dimensional plot of pixel locations and pixel values. The individual bands are outlined and identified. By selecting different background correction, the user is able to eliminate the volume percentage generated by the film background. The volume intensities are calculated by taking the volume using a correction value for the amount of protein used within the gel. Data are expressed as intensity unit (IU) per 1 μg tissue±SD.

Statistical Analysis
Data are expressed as mean±SE. In the physiological, echocardiographic, and biochemical measurements. Student t test was used for single comparisons of sham and acute MI at separate time points. For serial comparisons at different time points, we used repeated-measures ANOVA.

The authors had full access to the data and take responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results
In our laboratory, the rat coronary artery ligation model results in 40% to 50% operative mortality to produce large infarcts.11–15 In the present study, we infarcted 75 rats to obtain data on the 48 rats reported here. We did not measure
infarct size in this report, but in our previous work, we measured infarct size using histologically defined endocardial/epicardial infarct circumferences. In those studies, we showed that rats with LVEDPs in the range reported here had infarct sizes ranging from 35% to 40% of the left ventricle.

**Hemodynamic Changes**

Figure 1 is a recording with a 1-mm micromanometer-tipped catheter during the coronary ligation. The arrow depicts the time of the coronary occlusion; the immediate elevation of LVEDP and the decreases in LV systolic pressure and LV dP/dt are evident. The hemodynamic data in the untreated rats (Table 1) show that LVEDP increased (P < 0.05) at 1 minute after MI, LV systolic pressure decreased (P < 0.05) after 3 minutes, and LV dP/dt decreased (P < 0.05) immediately at 1 minute after the MI. Hemodynamics for those rats pretreated with candesartan revealed similar findings, confirming postinfarction LV failure (Table 2), except that the pretreated rats had lower LV systolic pressure and LV dP/dt at all time points as a result of treatment with candesartan. Treatment with candesartan did not alter the increases in LVEDP during the acute MI.

**Changes in LV Function With MI**

The data on global and regional changes in LV function are seen in Table 3 and Figures 2 and 3. LV fractional shortening and ejection fraction are decreased (P < 0.05) with acute MI in untreated animals; this decrease is attenuated (P < 0.05) with the candesartan-pretreated group compared with the untreated group. Interestingly, there is also a significant reduction in the systolic displacement in the posterior noninfarcted wall with candesartan pretreatment (Figure 3).

**Changes in LV Filling With Acute MI**

We evaluated changes in LV filling by recording the pressure-diameter relationship from peak systolic pressure to the lowest LVEDP continuously throughout the cardiac cycle (Figure 4). At 6 minutes after an acute MI, the curve is shifted to the right, with a greater increase in LV short-axis diameter at each pressure point beginning at end systole and proceeding to end diastole. This indicates that acute injury to the left ventricle results immediately in chamber dilation. Pretreatment with candesartan results in a smaller LV short-axis diameter at each pressure, demonstrating that ARB prevents LV remodeling by limiting changes in the LV short-axis diameter in acute MI. The curve of the acute infarct animals pretreated with candesartan clearly shifts left toward normal.

**Changes in eNOS With Acute MI**

Western blot analysis showed that tissue eNOS levels decreased (P < 0.05) in the infarcted wall of the left ventricle 6 minutes after an acute MI; pretreatment with candesartan did not alter eNOS expression in the infarct.

### TABLE 1. LV Pressures in Control Rats After MI

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 Minute</th>
<th>3 Minutes</th>
<th>6 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV SP, mm Hg</td>
<td>127.1±6.1</td>
<td>115.4±11.2</td>
<td>101.7±10.1*</td>
<td>100.6±8.2*</td>
</tr>
<tr>
<td>LV EDP, mm Hg</td>
<td>5.8±0.5</td>
<td>4.9±2.9*</td>
<td>18±2.9*</td>
<td>20.4±4.2*</td>
</tr>
<tr>
<td>+LV dP/dt, mm Hg/s</td>
<td>7106±520</td>
<td>4671±350</td>
<td>4156±667*</td>
<td>4173±874*</td>
</tr>
</tbody>
</table>

LV SP indicates LV systolic pressure. Values are mean±SEM. n=10 in each group.

*P<0.05 vs baseline.

### TABLE 2. LV Pressures in Candesartan-Pretreated Rats After MI

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 Minute</th>
<th>3 Minutes</th>
<th>6 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV SP, mm Hg</td>
<td>87.1±5.4</td>
<td>62.9±6.7*</td>
<td>63.5±6.2*</td>
<td>66.1±6.0*</td>
</tr>
<tr>
<td>LV EDP, mm Hg</td>
<td>5.1±1.4</td>
<td>15.9±1.9*</td>
<td>15±1.7*</td>
<td>18.3±3*</td>
</tr>
<tr>
<td>+LV dP/dt, mm Hg/s</td>
<td>2930±364</td>
<td>2883±273</td>
<td>3011±272</td>
<td>2708±312</td>
</tr>
</tbody>
</table>

LV SP indicates LV systolic pressure. Values are mean±SEM. n=8 in each group.

*P<0.05 vs baseline.
not change this (Figure 5). There were similar decreases in eNOS in the noninfarcted walls (26.7 ± 4.8 versus 8.14 ± 1.6 intensity unit/50 μg; P = 0.002), with no apparent effect with candesartan.

Changes in CM With Acute MI
Western blot analysis of free tubulins showed an increase in the free tubulin level at 6 minutes after an acute MI in the infarcted wall; pretreatment with candesartan blunted or decreased this response (P < 0.05) (Figure 6). There were similar changes in the noninfarcted wall with an increase in CM with acute MI and a return toward normal with pretreatment with candesartan.

Discussion
The results from this study suggest that pretreatment with ARB prevents cardiac dysfunction and attenuates the LV dilatation seen in acute MI. These beneficial effects of ARB pretreatment in the acute MI setting are associated with a decrease in myocardial eNOS protein abundance and a blockade of MI-induced increase in CM protein level. One may speculate that the prevention of MI-induced cardiac dysfunction with ARB treatment is partly due to the decrease in myocardial eNOS levels and that the decrease in LV remodeling is mediated by limiting the increase in CM protein levels. Our laboratory findings are complementary to the clinical data looking at the role of ACEI and ARBs in the reduction of cardiovascular mortality in patients at risk for coronary events.1–3 Because many patients currently on ACEIs and ARBs go on to develop myocardial infarctions, our data support the concept of using ARBs to limit LV dysfunction and remodeling in patients at risk for acute MI.

TABLE 3. Global Hemodynamic Changes in Rats After MI

<table>
<thead>
<tr>
<th>Fractional Shortening, %</th>
<th>LV EF, %</th>
<th>LV EDD, cm</th>
<th>τ, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>44.2 ± 7.3</td>
<td>80 ± 7.2</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>MI</td>
<td>19.3 ± 3.5*</td>
<td>43.7 ± 7.3*</td>
<td>0.54 ± 0.03*</td>
</tr>
<tr>
<td>MI + candesartan</td>
<td>28.9 ± 7.7†</td>
<td>60.8 ± 11†</td>
<td>0.41 ± 0.03†</td>
</tr>
</tbody>
</table>

LV EDD indicates LV end-diastolic diameter; LV EF, left ventricular ejection fraction. n = 15 in each group.
* P < 0.05 vs baseline.
† P < 0.05 vs Baseline and MI with no treatment.

Figure 2. The time sequence of changes in regional systolic displacement of the anterior wall after anterior MI. These are echocardiographic measurements of systolic displacement of the anterior LV wall at baseline and 1, 3, and 6 minutes after MI. Note that within 1 minute of the MI, regional systolic displacement of the anterior wall is decreased. Pretreatment with candesartan decreases systolic displacement at each time point (1, 3, and 6 minutes) after myocardial infarction. Values are mean ± SEM. n = 10 in each group. * P < 0.05, untreated MI vs MI with candesartan pretreatment; † P < 0.05, baseline no MI vs candesartan pretreatment MI.

Figure 3. Time sequence of changes in regional systolic displacement of the posterior noninfarcted wall after anterior MI. These are echocardiographic measurements of systolic displacement of the anterior LV wall at baseline and 1, 3, and 6 minutes after MI. Note that the decreases in systolic displacement occur within 1 minute of the MI and that pretreatment with candesartan (Can) decreases systolic displacement at 3 and 6 minutes after MI. Values are mean ± SEM. n = 8 in each group. * P < 0.05 vs untreated and candesartan treated, all time points; ** P < 0.05 vs no treatment at 3 minutes; + P < 0.05 vs no treatment at 6 minutes.

Figure 4. Concurrent LV pressure and short-axis-diameter curves plotted from peak systolic pressure to end-diastolic pressure. These data are obtained from simultaneous recordings of echocardiography-derived LV diastolic diameters and 1-mm micromanometer recordings of LV pressure at 6 minutes after the acute MI. With the acute MI, the curve is shifted to the right, with a greater increase in the diastolic diameter at each pressure beginning at end systole and proceeding to end diastole. This indicates that the acute MI results immediately in a dilated left ventricle. Pretreatment with candesartan in both the noninfarcted and infarcted rats results in a smaller diastolic diameter at each pressure.
Our data show that acute MI leads to immediate changes in LV hemodynamics and global and segmental systolic function in the infarcted and noninfarcted regions of the left ventricle. These changes occur simultaneously with alterations in LV relaxation and are accompanied by decreases in LV tissue eNOS and increases in CM. Pretreatment with the angiotensin II receptor blocker candesartan results in a lower LV systolic pressure but has no effect on LVEDP, regional systolic dysfunction, LV relaxation, or eNOS production. However, pretreatment with candesartan improves global LV function and LV filling while attenuating the increases in CM and LV chamber size. Thus, it appears that after an acute MI, the immediate changes in hemodynamics, tissue eNOS, and LV systolic and diastolic function may not be mediated exclusively through activation of the RAS because preinfarction ARB did not alter any of these early changes. However, the data suggest that an interaction between the RAS and the intracellular cytoskeletal matrix may exist because preinfarction ARB improves global LV function, prevents LV dilation, and blunts the increase in CM in both the infarcted and noninfarcted left ventricle.

The design of our experiment allows us to examine the effects of preinfarction blockade of the RAS on changes in LV remodeling and function in acute heart failure. This is more than an experimental curiosity in light of the data showing both positive and neutral effects of primary prevention of cardiovascular mortality with ACEIs and ARBs in patients at risk for coronary events.1-3

Although it is clear that an acute MI causes regional LV contractile dysfunction, we measured regional systolic displacement to define the time course of these changes in the infarcted and noninfarcted walls. The increases in LV wall stress, myocardial necrosis in the infarct zone, and LV afterload burden occurring soon after an MI are thought to be responsible for the changes in regional function. Interestingly, the afterload reduction effects of candesartan appear to improve global LV function but fail to alter the impaired regional LV function. The explanation for this is not clear, but afterload reduction can improve LV function, ie, can increase ejection fraction in a damaged heart by decreasing wall stress. Changes in LV geometry and decreases in LV size could also explain the benefit of candesartan. The argument that geometry is one of the most important determinants of LV function in the infarcted heart is based on studies of papillary muscles or isolated myocytes in which the data show no evidence of intrinsic contractile abnormalities in the muscles or myocytes themselves if they are remote from the infarction.13,18 The conclusion is that increases in load and/or changes in geometry, as opposed to problems intrinsic in the myocyte, are responsible for the global LV dysfunction in chronic ischemic heart failure. Uninjured myocytes can potentially increase their contractile reserve when presented with a change in load. From our data, decreases in the systolic displacement of the infarcted and noninfarcted myocardium occur because they are both subjected to the same increase in wall stress and afterload. This problem is compounded by the acute injury to myocardial tissue in the infarct/ischemic zone. Candesartan unexpectedly worsens regional systolic displacement for the infarcted wall and has no effect on the noninfarcted wall; there is no clear explanation for this finding. One possibility is that the decrease in systolic pressure in the candesartan-treated rats may hamper collateral blood flow into the peri-infarct zone, perpetuating the ischemic burden. The restoration of global LV function is in agreement with the concept that reduction in afterload and LV wall stress and normalization of LV geometry act to restore LV function.

One obvious question is, What are the chronic effects of candesartan treatment in this setting? Although this study was not designed to examine long-term outcome data in rats pretreated with candesartan before an MI, our laboratory has data on rats treated at the time of MI with candesartan and followed up for 7 days and 8 weeks.19 At both time points, candesartan lowers LV systolic pressure and LV dP/dt, but LVEDP is only lowered with candesartan at 8 weeks. These data suggest that at least for global hemodynamics, the only difference between the immediate pretreatment and long-term

Figure 5. Changes in eNOS in the anterior infarcted region of the left ventricle at 6 minutes after the acute MI. Note that eNOS decreases with the acute MI and decreases further with 2 weeks of candesartan (Can) treatment before the MI. Values are mean±SEM. n=10 in each group. *P<0.05 vs MI and MI plus candesartan; **P<0.05 vs MI and normal.

Figure 6. Changes in LV infarcted wall CM at 6 minutes after the acute MI. Note that the CM increases with the acute MI and decreases with treatment with candesartan for 2 weeks before the MI. Values are mean±SEM. n=10 in each group. *P<0.05 vs normal; **P<0.05 vs MI.
We have data on the chronic effects of angiotensin receptor blockade with candesartan on LV tissue eNOS or CM, but from our previous work, we know that LV tissue nitric oxide (NO) influences hemodynamics in chronic heart failure because, when we block the effects of myocardial NO with L-arginine methyl ester in rats 3 weeks after infarction, heart rate and LV dp/dt decrease, systemic vascular resistance increases, and τ is prolonged with no change in mean arterial pressure or LVEDP.

LV Tissue eNOS

Our data demonstrate that downregulation of eNOS occurs immediately during an acute MI. To the best of our knowledge, the time frame of these changes has not been reported previously. In chronic ischemia-induced heart failure, tissue and vascular eNOS is downregulated.4–6,20 The importance of changes in eNOS, at least in enhancing vasomotor relaxation, has been illustrated by our previous work that shows that with angiotensin receptor blockade with candesartan, there is upregulation of eNOS with enhanced vasomotor relaxation in heart failure.19 Our current data show that changes in eNOS occur immediately during an acute MI, suggesting that it is possible to rapidly deplete intracellular stores of eNOS in the myocardium. Interestingly, the decrease in eNOS immediately during the MI was not restored with pretreatment with candesartan. Although we did not measure candesartan blood levels, the evidence that the rats received the intended treatment is that in the candesartan-pretreated rats, LV systolic pressure and LV dp/dt were lower at baseline and at every time point compared with the untreated rats (Tables 1 and 2). However, it is possible that the lack of change in LV tissue eNOS with candesartan is due to the fact that upregulation of eNOS cannot occur within minutes of an infarction. The changes in eNOS from baseline before the MI compared with 1 minute after coronary artery ligation suggests that eNOS depletion can occur immediately.

Microtubulin

We showed that free tubulins, which are small subunits that polymerize to form microtubules, are upregulated during an acute MI. Changes in microtubules and the cytoskeleton of the heart have been well documented in animal models and in patients with chronic heart failure.8,9 The mechanisms responsible for this are not clear. Most of the previous work has focused on pressure-overload LV hypertrophy and shown that microtubule upregulation may be one of the underlying mechanisms responsible for reduced contractility leading to heart failure.10,21–24 The α-tubulins in chronic heart failure undergo posttranslational modifications involving detyrosinated Glu-tubulin and deglutamyated delta2-tubulin.10 The thought is that these changes enhance the stability of the microtubule network, leading to increased stiffness in the left ventricle. Most of this work has been done in chronic disease states, so there are few data on potential dynamic changes in the microtubule network similar to what we have seen with an acute MI. The increase in free tubulin level that we report is most likely due to depolymerization of existing microtubules. This uncontrolled depolymerization of existing microtubules potentially alters the intracellular architecture of cardiomyocytes. These changes in the cytoskeleton may contribute the impairments in regional systolic function and LV relaxation with uncontrolled depolymerization as opposed to microtubule excessive bundling in chronic heart failure or chronic pressure-overload LV hypertrophy.

The potential relationship between NO and cytoskeletal function raises some interesting prospects. NO can affect axonal microtubule configuration to S-nitrosylate the cytoskeletal proteins actin and tubulin and to downregulate muscle LIM protein MLP.25–27 These findings suggest that NO, eNOS, and the cytoskeletal network may be part of a common signaling transduction pathway. Our data support this concept because the levels of both eNOS and free tubulins are altered after an acute MI, suggesting that eNOS and microtubules may participate in an early remodeling process after an acute ischemic event. Whether eNOS and microtubules directly or indirectly interact during this process remains an intriguing question; for example, could the stability of eNOS be microtubule dependent? In this ischemic setting, the downstream signaling cascade could involve the breakdown of eNOS proteins and microtubules, leading to a decrease in eNOS and an increase in free tubulins. The result is a decrease in vasomotor relaxation of the left ventricle, a prolongation of τ, and the derangement of the microtubule network that could disrupt the cytoarchitectural organization of cardiomyocytes. Potential signaling molecules in this cascade include heat shock proteins, which have been shown to protect microtubules during simulated ischemia in cardiac myocytes.28

Study Limitations

Like all animal studies, one possible limitation of this work is that the animal model of disease may not accurately represent the human pathology. However, the rat coronary artery ligation model is commonly used to study clinical outcomes of MI and heart failure. The best example is the use of captopril to alter LV remodeling and survival after an acute MI.14,29,30 Another possible limitation in this study is the use of open chest echocardiography to study changes in LV function with an acute MI. The fact that the rat is anesthetized and that the chest is open means that the measurements may not provide the same quantitative data as in the conscious intact animal. Nevertheless, the important point is directional changes in hemodynamics and systolic/diastolic function as opposed to the absolute changes.

Acknowledgments

We acknowledge Howard Byrne, Maribeth Stansifer, and Nicholle Johnson for technical assistance.

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Disclosures

None.
References


CLINICAL PERSPECTIVE

An interest of current therapy for primary and secondary prevention of cardiovascular morbidity and mortality is the inhibition of the renin-angiotensin system with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Although these agents are being used more frequently among patients at high risk for atherothrombotic heart disease, it is important to note that although these agents are effective in reducing the cardiovascular event rates, some patients will have a myocardial infarction (MI) despite alteration of their renin-angiotensin system. This study examines the effects of prior renin-angiotensin system inhibition with an angiotensin receptor blocker on left ventricular (LV) function and LV remodeling during an acute MI. Because such a study is difficult to do clinically, it is appropriate to examine this question in an animal model of MI. This study showed that with an acute MI, there are immediate changes in regional and global LV function. Pretreatment with an angiotensin receptor blocker preserves global LV function, prevents LV dilation, and blunts the increase in constitutive microtubules. This occurs with minimal effects on LV hemodynamics, regional function, or tissue endothelial nitric oxide synthase. In effect, angiotensin receptor blockade before an ischemic insult attenuates LV remodeling and alters the cytoskeleton matrix of the left ventricle. These data are novel and need further investigation. One possible implication from this study is that hypertensive patients, already at increased risk for MI, may derive further benefit if they are on a renin-angiotensin inhibitor as part of their antihypertensive therapy.
Pretreatment With Angiotensin Receptor Blockade Prevents Left Ventricular Dysfunction and Blunts Left Ventricular Remodeling Associated With Acute Myocardial Infarction

Hoang Thai, Lisa Castellano, Elizabeth Juneman, Huy Phan, Rose Do, Mohamed A. Gaballa and Steven Goldman

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