Reverse Remodeling of Cardiac Myocyte Hypertrophy in Hypertension and Failure by Targeting of the Renin-Angiotensin System

Tetsutaro Tamura, MD; Suleman Said, MS; Jennifer Harris, BS; Wenyan Lu, MD; A. Martin Gerdes, PhD

Background—ACE inhibitors (ACEIs) and angiotensin II type 1 (AT1) receptor blockers are effective in reducing left ventricular mass in hypertension and heart failure. However, the ability of these drugs to reverse excessive myocyte lengthening and transverse growth in heart failure is unknown.

Methods and Results—L-158,809 (an AT1 blocker; AT1), enalapril (an ACEI), and hydralazine (a vasodilator) were administered to spontaneously hypertensive heart failure rats between 6 and 10 months of age (early treatment) and between 18 and 22 months of age (late treatment). After 4 months of treatment, hemodynamics and chamber dimensions were collected before left ventricular myocyte isolation and subsequent analysis of myocyte shape. Each drug reduced systolic blood pressures to normal values. In the early and late studies, the ACEI reduced myocyte volume. Myocyte length was also reduced in the late study. However, the AT1 was most effective in reversing myocyte dimensions to near-normal values in both studies. Hydralazine was ineffective in reducing cell size but arrested progression of myocyte lengthening in the late study. Changes in myocyte shape reflected alterations in chamber dimensions and wall thickness.

Conclusions—Reversal of myocyte hypertrophy was produced in hypertensive/heart failure rats with an AT1. The ACEI was effective but to a lesser extent. Results indicate that it is possible to significantly reverse myocyte remodeling pharmacologically even if therapy is initiated near the onset of failure. Further work is needed to determine whether similar results can be obtained in humans. (Circulation. 2000;102:253-259.)

Key Words: hypertension ■ drugs ■ heart failure ■ remodeling ■ myocytes

It is estimated that 4 to 5 million individuals living in the United States have heart failure. Hypertension is one of the major underlying diseases leading to heart failure. For >20 years, regression of hypertrophy has been a major goal of clinical trials and of hypertension research. Among other factors, the renin-angiotensin system plays an important role in the regulation of cardiac myocyte growth. ACE inhibitors have been shown to reduce left ventricular (LV) weight significantly. For the past decade, efforts have been made to develop new angiotensin II receptor blockers and to investigate their effectiveness on hypertrophy reversal and chamber dilatation. Some reports have demonstrated a reduction of heart weight or LV weight after treatment with angiotensin II type 1 receptor (AT1) blockers. It is important, however, to know the effects of AT1 blockers at the myocyte level. Our previous study, using lean female spontaneously hypertensive heart failure rats (SHHF; a genetic model predisposed to hypertension and failure), showed that the cross-sectional area (CSA) of myocytes was approximately twice normal by 3 months of age but did not change after this age. Myocyte lengthening, which underlies chamber dilatation, was detected by 12 months of age and continued until failure at ≈22 to 24 months of age. Although the temporal development of these important cellular changes in humans is not known, it was shown that LV myocyte dimensions in hypertensive patients with compensated hypertrophy and failure are identical to those observed in SHHF rats at similar stages of the disease. Thus, the SHHF rat should represent a relevant animal model to test the efficacy of various drugs on hypertension and associated ventricular remodeling. In this study, L-158,809, enalapril, and hydralazine were administered to SHHF rats with early compensated hypertrophy (treatment from 6 to 10 months of age) and also just before rats developed symptoms of failure (18 to 22 months of age). The primary objective was to determine the effects of these drugs on cardiac myocyte remodeling and whether echo data on chamber dimensions and wall thickness reflect underlying myocyte remodeling.

Methods

Experimental Animals and Drugs

Lean female 6-month-old SHHF rats were obtained from Genetic Models Inc (Indianapolis, Ind). Body weight and systolic blood
pressures (tail-cuff method) were collected from all rats before initiation of drug treatment. The early-treatment study had 6- and 10-month-old untreated groups, plus 6-month-old rats treated for 4 months with hydralazine, enalapril, or an AT1 blocker. The late-treatment study had a similar design with 18- and 22-month-old untreated groups plus 18-month-old rats treated for 4 months with the same 3 drugs. In both the early and late studies, mean body weights and mean blood pressures for each group were matched initially as closely as possible. L-158,809 (AT1 blocker) and enalapril were provided as a gift from Merck & Co, Inc (Rahway, NJ), and hydralazine was obtained from Sigma Chemical Co (St Louis, Mo). Each drug was dissolved in drinking water. Concentrations of the drugs were 1 mg·kg⁻¹·d⁻¹ for L-158,809, 15 mg·kg⁻¹·d⁻¹ for enalapril (initial dose of 3 mg·kg⁻¹·d⁻¹ was raised to the higher dose after 1-month treatment in the early study to reduce blood pressure to the same level as in other treatment groups), and 20 mg·kg⁻¹·d⁻¹ for hydralazine. Systemic blood pressure was collected at baseline and after 1, 2, and 3 months of treatment with an RTBP1001 Rat Tail Blood Pressure System (Kent Scientific Corp).

**Echocardiography and Hemodynamics**

The animals were anesthetized with an intramuscular injection of ketamine HCl (30 mg/kg) and xylazine (5 mg/kg) at the time of terminal experiments. Standard echocardiography techniques were used to obtain M-mode echocardiograms from short-axis views of the left ventricle below the tip of the mitral valve leaflets with a General Electric RT5000 echo machine with a 7-MHz transducer. LV hemodynamics were collected by catheterization during terminal experiments as described previously.

**Myocyte Isolation and Morphometry**

The hearts were quickly removed, trimmed of excess tissue, blotted, and weighed. The procedure for isolating myocytes by use of retrograde aortic perfusion with collagenase has been described previously. Myocyte volume was measured with a Coulter Channelizer (model Z2, Coulter Corp). Myocyte length, defined as the longest length parallel to the longitudinal axis of the myocyte, was measured in 50 cells from each sample with a Jandel Video Analysis System.

### TABLE 1. Body Weight and Blood Pressure

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW, g</th>
<th>BP, mm Hg</th>
<th>1-month Tx</th>
<th>2-month Tx</th>
<th>3-month Tx</th>
<th>4-month Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early-treatment study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>6</td>
<td>221±11</td>
<td>174±16</td>
<td>184±16</td>
<td>...</td>
<td>183±33</td>
<td>169±32</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>6</td>
<td>216±9</td>
<td>175±15</td>
<td>119±23*</td>
<td>...</td>
<td>127±28*</td>
<td>115±9*</td>
</tr>
<tr>
<td>Enalapril</td>
<td>6</td>
<td>219±13</td>
<td>175±15</td>
<td>175±21</td>
<td>127±36†</td>
<td>136±26*</td>
<td>113±16*</td>
</tr>
<tr>
<td>L-158,809</td>
<td>6</td>
<td>214±9</td>
<td>175±14</td>
<td>133±24*</td>
<td>129±23†</td>
<td>129±21*</td>
<td>113±16*</td>
</tr>
<tr>
<td>Late-treatment study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mo</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>18</td>
<td>253±14</td>
<td>211±44</td>
<td>210±39</td>
<td>197±32</td>
<td>...</td>
<td>128±34</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>18</td>
<td>256±14</td>
<td>214±26</td>
<td>107±19*</td>
<td>110±26*</td>
<td>...</td>
<td>116±20</td>
</tr>
<tr>
<td>Enalapril</td>
<td>18</td>
<td>247±17</td>
<td>202±56</td>
<td>107±18*</td>
<td>109±23†</td>
<td>...</td>
<td>121±34</td>
</tr>
<tr>
<td>L-158,809</td>
<td>18</td>
<td>253±15</td>
<td>199±23</td>
<td>109±19*</td>
<td>107±20*</td>
<td>...</td>
<td>122±22</td>
</tr>
</tbody>
</table>

**TABLE 2. Echocardiographic Data**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LVDd</th>
<th>LVDs</th>
<th>AWd</th>
<th>AWs</th>
<th>PWd</th>
<th>PWs</th>
<th>FS</th>
<th>WS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early-treatment study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6M</td>
<td>10</td>
<td>5.8±0.8</td>
<td>3.7±0.3</td>
<td>2.1±0.1</td>
<td>3.0±0.2</td>
<td>2.0±0.2</td>
<td>3.0±0.2</td>
<td>37±3</td>
<td>110±13</td>
</tr>
<tr>
<td>10M</td>
<td>10</td>
<td>6.1±0.4</td>
<td>3.6±0.5</td>
<td>2.1±0.1</td>
<td>2.9±0.2</td>
<td>1.9±0.3</td>
<td>3.0±0.3</td>
<td>41±6</td>
<td>110±30</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>10</td>
<td>6.1±0.4</td>
<td>3.5±0.3</td>
<td>2.0±0.2</td>
<td>3.0±0.3</td>
<td>1.9±0.2</td>
<td>2.8±0.3</td>
<td>42±5</td>
<td>76±16†</td>
</tr>
<tr>
<td>Enalapril</td>
<td>10</td>
<td>6.0±0.4</td>
<td>3.6±0.5</td>
<td>1.6±0.2†</td>
<td>2.5±0.2†</td>
<td>1.4±0.2†</td>
<td>2.5±0.2†</td>
<td>39±6</td>
<td>83±22</td>
</tr>
<tr>
<td>L-158,809</td>
<td>10</td>
<td>6.0±0.6</td>
<td>3.5±0.4</td>
<td>1.4±0.1†</td>
<td>2.4±0.2†</td>
<td>1.2±0.3†</td>
<td>2.4±0.5†</td>
<td>42±4</td>
<td>79±21†</td>
</tr>
<tr>
<td>Late-treatment study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18M</td>
<td>7</td>
<td>6.9±0.2</td>
<td>4.4±0.2</td>
<td>1.7±0.2</td>
<td>2.4±0.2</td>
<td>1.6±0.2</td>
<td>2.6±0.2</td>
<td>36±2</td>
<td>144±21</td>
</tr>
<tr>
<td>22M</td>
<td>11</td>
<td>7.5±0.7*</td>
<td>4.7±0.8</td>
<td>1.7±0.1</td>
<td>2.8±0.4*</td>
<td>1.8±0.1</td>
<td>2.7±0.4</td>
<td>37±9</td>
<td>136±46</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>7</td>
<td>6.7±0.3†</td>
<td>4.1±0.4</td>
<td>2.0±0.2†</td>
<td>3.2±0.6*</td>
<td>1.8±0.3</td>
<td>2.6±0.2</td>
<td>39±5</td>
<td>101±21*</td>
</tr>
<tr>
<td>Enalapril</td>
<td>9</td>
<td>6.3±0.7†</td>
<td>3.5±0.5*</td>
<td>1.5±0.2†</td>
<td>2.6±0.3</td>
<td>1.5±0.2†</td>
<td>2.9±0.3</td>
<td>41±7</td>
<td>78±33†</td>
</tr>
<tr>
<td>L-158,809</td>
<td>13</td>
<td>6.2±0.5*</td>
<td>3.6±0.5*</td>
<td>1.4±0.2†</td>
<td>2.4±0.2</td>
<td>1.4±0.2†</td>
<td>2.5±0.4</td>
<td>43±6*</td>
<td>88±27†</td>
</tr>
</tbody>
</table>

6M, 10M, 18M, and 22M indicate 6-month-old, 10-month-old, 18-month-old, and 22-month-old untreated SHHF rats, respectively; LVDd, LV chamber diameter in diastole; LVDs, LV chamber diameter in systole; AWd, anterior wall thickness in diastole; AWs, anterior wall thickness in systole; PWd, posterior wall thickness in diastole; PWs, posterior wall thickness in systole; FS, fractional shortening (%); and WS, systolic wall stress in kdyn/cm². All chamber dimensions are in mm. All values are mean±SD.

*P<0.05 vs baseline control (6M or 18M); †P<0.05 vs terminal control (10M or 22M).
System (Jandel Scientific). CSA was calculated from myocyte volume/myocyte length.

Data Analysis
Results are presented as mean±SD for animal data and mean±SEM for cellular data. ANOVA was used to compare data in each group. The Bonferroni test was used to examine statistically significant differences observed with the ANOVA.15

Results
There were no significant differences in body weights and blood pressures between each group before the treatments were started (Table 1). Because the initial dosage of enalapril (3 mg · kg⁻¹ · d⁻¹) failed to decrease blood pressure significantly, the dosage was increased to 15 mg · kg⁻¹ · d⁻¹ for the final 3 months of treatment. This higher dosage was used throughout the late treatment study and quickly lowered pressures to the same extent as hydralazine and the AT1 blocker. The dose adjustment for enalapril in the early study should not affect remodeling data, because we have found that maximum reversal in cell size is achieved within 1 month after therapy is begun (A.M.G., unpublished data, 1999). In the late-treatment study, blood pressure was also normal in untreated rats because of the onset of congestive heart failure.

Table 2, top, shows comparative echocardiographic and systolic wall stress data from the early treatment study. Enalapril and the AT1 blocker significantly reduced the thickness of both the anterior and posterior walls in diastole and systole. Although hydralazine reduced systolic blood pressure and LV systolic wall stress to the same extent as the other drugs, it failed to reduce wall thickness significantly. Table 2, bottom, shows echo and wall stress data from the late-treatment groups. Hydralazine prevented the progressive chamber dilatation between 18 and 22 months of age. Enalapril significantly reversed LV diastolic diameter to values lower than those in untreated 18-month-old rats. Diastolic wall thickness was less than that observed in 22-month-old untreated rats in failure. The AT1 blocker, however, significantly reduced most wall thickness and chamber diameter values below those found in 18-month-old untreated rats.

Figure 1A shows heart weight/body weight ratios of animals from each group. There were no significant differences in body weight at the time of terminal experiments. Compared with untreated SHHF controls, enalapril and the AT1 blocker decreased heart weight/body weight ratios, whereas hydralazine did not affect heart mass.
LV isolated myocyte data from the early-treatment study are shown in Figure 1, B through D. Cell volume was significantly less than in 10-month-old but not 6-month-old untreated SHHF rats after enalapril treatment. The AT1 blocker, however, reduced cell volume and CSA to values significantly lower than those of 6-month-old untreated SHHF rats. In the hydralazine group, neither cell volume nor CSA was significantly reduced. Cell length values were within the range of values typically found in normal rats.

Changes in heart weight/body weight ratios in the late-treatment study are shown in Figure 2A. L-158,809 reversed heart weight/body weight ratios to values significantly lower than those in 18-month-old untreated rats. Heart weight/body weight ratio with enalapril treatment was less than in 18-month-old untreated rats but did not reach statistical significance. Hydralazine prevented the progressive increase in heart mass between 18 and 22 months of age (22M). Only AT1 blocker reduced myocyte CSA below values at 18M. P<0.05 vs 18M (*), 22M (†), and hydralazine (‡).

Discussion

The most important finding from this study was that administration of L-158,809, a nonpeptide AT1 receptor–selective blocker,16,17 to hypertensive rats just before the development of symptomatic heart failure produced significant reverse remodeling of myocyte volume, length, and CSA. Myocyte dimensions were reduced below pretreatment values. Thus,
true reverse remodeling, rather than simply arrested progression of myocyte hypertrophy, was shown. To the best of our knowledge, these are the first comprehensive data demonstrating that a noninvasive treatment initiated just before the onset of symptoms of heart failure was able to dramatically reverse the maladaptive changes in cardiac myocyte dimensions to near-normal values. To date, the most significant reversal of myocyte shape alterations due to heart failure was reported in patients on LV assist devices awaiting heart transplants.\textsuperscript{18} Because recent data suggest that excessive series sarcomere addition can account for most of the chamber dilatation in the progression to failure,\textsuperscript{19} this mechanism is of considerable importance. The extent of reverse remodeling shown in the present study was so impressive after AT\textsubscript{1} blockade that values were indistinguishable from those found in normal adult male rats (which typically have larger myocytes than females).\textsuperscript{20} Enalapril was as effective as the AT\textsubscript{1} blocker in reversing LV myocyte length but was not as effective in reversing CSA. Hydralazine was able to prevent progressive myocyte lengthening and chamber dilatation when administered late. In general, echocardiographically derived changes in chamber diameter and wall thickness accurately reflected changes in myocyte length and myocyte thickness (CSA), respectively. This observation suggests that noninvasive imaging may be helpful in predicting underlying cellular remodeling.

Clinical studies have demonstrated that increased LV mass predicts an adverse outcome in patients with hypertension.\textsuperscript{21,22} In addition, treatment that reduces cardiac mass in these patients has been shown to improve outcome.\textsuperscript{23} It has not been clear, however, which drugs or drug combinations are most effective in reducing cardiac mass, improving chamber geometry, and reversing maladaptive myocyte remodeling. Although a new technique allows accurate assess-
ment of myocyte length from cardiac biopsies,\textsuperscript{24} the inherent limitations of such studies in humans make it difficult to assess the effects of antihypertensive drugs on patients at the cardiac myocyte level.

The results of the early-treatment study demonstrated that the AT\textsubscript{1} blocker produced the greatest reduction in LV myocyte volume and CSA in adult SHHF rats with compensated hypertension. These data confirm the findings of Kojima et al.,\textsuperscript{25} who noted a good correlation between a crude measure of myocyte diameter and wall thickness after administering an AT\textsubscript{1} blocker to spontaneously hypertensive rats. Myocyte length and volume changes were not examined in that study. As expected, none of the drugs affected myocyte length in the early-treatment study, which is normal during this phase of the disease process.\textsuperscript{10} Cumulative data from the early and late studies suggest that hypertrophy regression may be more complete if treatment is initiated earlier. Although a good normotensive control for SHHF rats has not been clearly identified, it should be noted that the AT\textsubscript{1} blocker reduced LV myocyte dimensions in the early-treatment study to values typically found in normal female rats.\textsuperscript{20} Such comparisons can be readily made with the techniques used here. In fact, data collected with these methods have shown that LV myocyte dimension in normal rats, cats, guinea pigs, hamsters, and humans are virtually indistinguishable.\textsuperscript{26}

Angiotensin II has been reported to accelerate the hypertrophy of myocytes as well as the development of interstitial fibrosis.\textsuperscript{27} Miyata and Haneda\textsuperscript{28} reported that losartan inhibits increases in the protein-to-DNA and RNA-to-DNA ratios, rates of protein synthesis, and activity of protein kinase C in cultured neonatal cardiac myocytes. It is likely that angiotensin II–induced hypertrophic growth is, at least in part, mediated through AT\textsubscript{1} receptors. Results from these studies suggest that blocking the growth-promoting effects of angiotensin II at the AT\textsubscript{1} receptor level may be more effective than reducing its production by inhibition of converting enzyme.

The role of AT\textsubscript{1} receptors in cardiac hypertrophy and hypertension is poorly understood. Recent data suggest that these receptors may have a growth-inhibitory effect on cardiac myocytes.\textsuperscript{30,31} Thus, it is possible that some of the reverse remodeling observed with AT\textsubscript{1} receptor blockade may be due to growth inhibition through the AT\textsubscript{1} receptor. Antigrowth effects mediated through the AT\textsubscript{1} receptor may result from ACE inhibition and subsequent effects on kinin levels.\textsuperscript{30,31} Human and animal studies also suggest that potentiation of bradykinin may mediate part of the beneficial effects of ACE inhibitors in heart disease.\textsuperscript{32,33} It is clear from recent publications, however, that much more work is needed to fully understand the contributions of bradykinin in ACE inhibitor and AT\textsubscript{1} blocker therapy.\textsuperscript{34,35}

Although hydralazine, an arteriolar dilator, reduced systolic blood pressure and wall stress to an extent similar to that of the other drugs, it failed to reduce myocyte CSA. The basis for this discrepancy between load and mass reduction is not clear. Unlike drugs targeting the renin-angiotensin system, hydralazine does not affect the increased collagen content in spontaneously hypertensive rats, a strain closely related to the SHHF rats used here.\textsuperscript{36} Thus, it is possible that mechanical signaling mediated through extracellular matrix–integrin pathways remains in an activated state with hydralazine treatment. An alternative possibility is that hydralazine does not correct the AT\textsubscript{1} signaling defect that may maintain myocyte hypertrophy in this model. Further work will be needed to clarify these issues. It should be noted that hydralazine-treated rats did not develop a reflex tachycardia, so this can be excluded as a possible means of maintaining mechanical drive and myocyte hypertrophy (data not shown). Although AT\textsubscript{1} receptor stimulation is not mandatory for mechanically induced myocyte hypertrophy,\textsuperscript{37,38} the relative contributions of load and angiotensin II–mediated cellular hypertrophy have not been completely resolved in hypertension.

The degree of heart failure in the 22-month-old “failure” group was not as severe as that reported in 24-month-old SHHF rats in our previous study.\textsuperscript{11} Although LV pressure had declined to normal values and 22-month-old untreated animals had symptoms of heart failure (eg, dyspnea, lethargy, orthopnea), dP/dt\textsubscript{max} (not reported here) had not yet declined as in the previous 24-month-old group. It should be pointed out that female SHHF rats were used in this study, and they tend to have better preservation of function than males with heart failure.\textsuperscript{39} This sex difference is also true in humans with heart failure, in whom ejection fraction is normal in a much greater percentage of women than men.\textsuperscript{40,41} Although systolic function was better preserved in the female SHHF rats used in our studies, more recently we have detected significant diastolic dysfunction in 22-month-old female SHHF by echocardiographic method (A.M.G., unpublished data, 1999). Considering the importance of better understanding diastolic dysfunction in patients with heart failure and apparent similarities in SHHF rats, it may prove helpful to characterize pharmacologically related changes in diastolic function more thoroughly in future studies. Although data reported here are promising, further work is needed to determine whether AT\textsubscript{1} blockers can produce similar beneficial myocyte remodeling in humans.

Acknowledgments
This work was supported by grant HL-30696 from the National Institutes of Health. Support was also provided by the South Dakota Health Research Foundation, which is a partnership between the University of South Dakota School of Medicine and Sioux Valley Hospitals and Health System in Sioux Falls, SD. We would like to acknowledge technical support provided by Susan Andersen.

References
Reverse Remodeling of Cardiac Myocyte Hypertrophy in Hypertension and Failure by Targeting of the Renin-Angiotensin System
Tetsutaro Tamura, Suleman Said, Jennifer Harris, Wenyan Lu and A. Martin Gerdes

Circulation. 2000;102:253-259
doi: 10.1161/01.CIR.102.2.253

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/102/2/253

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/