Postinfarction Survival and Inducibility of Ventricular Arrhythmias in the Spontaneously Hypertensive Rat: Effects of Ramipril and Hydralazine

Tan Nguyen, BSc; Elias El Salibi, BSc; Jean L. Rouleau, MD

Background—Hypertensive patients with left ventricular hypertrophy (LVH) have been found to have greater peri–myocardial infarction (MI) and postinfarction mortality. In this study, we evaluated the postinfarction survival, susceptibility to ventricular arrhythmias, and degree of LVH and cardiac fibrosis in the spontaneously hypertensive rat (SHR) and the effects of the ACE inhibitor ramipril and the direct vasodilator hydralazine on these characteristics.

Methods and Results—An acute myocardial infarction (MI) was produced by ligation of the left anterior descending coronary artery. Rats were randomized to either control (n=50), hydralazine (n=41), or ramipril (n=45). Treatments were started 4 hours after infarction and continued for 8 weeks. Ramipril and hydralazine reduced arterial pressure similarly. Medications were stopped 72 hours before euthanasia, at which time hemodynamic, programmed electrophysiological stimulation (PES), and morphological studies were performed. Mortality was decreased in ramipril (56%) compared with hydralazine (78%) and control (82%) SHRs (P=0.008). This was accompanied by a decrease in myocardial hypertrophy and fibrosis and a decrease in inducibility of ventricular arrhythmias by PES in the ramipril group regardless of MI size. Treatment with hydralazine had little or no effect on LVH and cardiac fibrosis and did not modify inducibility of ventricular arrhythmias by PES. Ramipril but not hydralazine prevented the increase in LV end-diastolic pressure in rats with large MIs.

Conclusions—In the SHR, the ACE inhibitor ramipril reduces LVH, cardiac fibrosis, and susceptibility to ventricular arrhythmias by PES and improves survival and LV function. Despite a similar decrease in arterial pressure, hydralazine does not have these beneficial effects. (Circulation. 1998;98:2074-2080.)

Key Words: hypertrophy ■ myocardial infarction ■ ramipril ■ arrhythmias ■ fibrosis

Patients with hypertension have been found to have a greater peri–myocardial infarction (MI) and postinfarction mortality rates than normotensive patients. Why this occurs is uncertain and is probably related to a number of different factors. The increased mortality rate may be related to increased risk of myocardial ischemia due to vascular changes and increased afterload2 and/or to an increase in susceptibility to ventricular arrhythmias3 resulting from the increased myocardial fibrosis and hypertrophy associated with hypertension.4-8 Also, ventricular remodeling after infarction and its effects on ventricular function may be different in patients with hypertension and left ventricular hypertrophy (LVH).9,10

ACE inhibitors have been found to be the most effective drugs in attenuating ventricular remodeling, preserving ventricular function, and improving survival after large myocardial infarction.11,12 At this time, few data on postinfarction ventricular remodeling and the effects of therapy in hypertensive patients exist. Two studies done in the spontaneously hypertensive rat (SHR) suggest that postinfarction ventricular dilatation in the SHR is at least as marked if not more so than in normal rats and that the decrease in ventricular function that normally occurs in this setting may be greater.9,10 A study by Nishikimi et al9 suggests that ACE inhibitors may attenuate the loss of ventricular function and the changes in ventricular remodeling normally found after infarction in the SHR. Nevertheless, many important questions regarding survival, the characteristics of LV remodeling (such as the degree of cardiac fibrosis), changes in susceptibility to ventricular arrhythmias, and the effects of therapeutic interventions on these changes remain to be explored or resolved.

This study was thus undertaken in the SHR to answer the following questions: (1) What are some of the characteristics of LV remodeling after infarction, such as cardiac fibrosis and hypertrophy, in the setting of hypertension and LVH; (2) does the control of blood pressure after infarction in the SHR significantly modify remodeling and prognosis after infarction; (3) are these changes associated with an increased or decreased susceptibility to ventricular arrhythmias as assessed by programmed electrophysiological stimulation...
(PES); and (4) what are the effects of hydralazine and the ACE inhibitor ramipril on these variables in the SHR?

**Methods**

**Preparation of Animals**
A total of 136 male SHRs (body weight, 250 to 300 g; 13 weeks old) were obtained from Charles River Breeding Laboratories (Saint-Constant, Quebec, Canada). Their care and all procedures were in accordance with the Canadian Council for Animal Care and the Animal Care Committee of the Montreal Heart Institute.

**Systolic Blood Pressure and Heart Rate Monitoring**
Indirect systolic blood pressure and heart rate were determined by the tail-cuff method (Harvard Apparatus). The reported values are the mean of at least 3 recordings taken at the same time of day at baseline, before the infarction, and at 4 and 7 weeks after infarction.

**Myocardial Infarction**
Myocardial infarction was induced in all SHRs after the baseline measurement of systolic blood pressure and heart rate (week 0) according to methods previously described.13

**Drug Randomization**
Rats were randomly divided into 3 groups according to their therapeutic intervention 4 hours after infarction. One group received an intraperitoneal injection of normal saline solution and normal drinking water thereafter (control group, n=50). A second group received an intraperitoneal injection of ramipril (Hoechst-Marion-Roussel) (37.5 μg/kg body wt) followed by 7.5 mg/L in the drinking water14 (ramipril group, n=45). A third group received an intraperitoneal injection of hydralazine (Sigma Chemical Co) (0.4 mg/kg body wt) followed by 80 mg/L in the drinking water15 (hydralazine group, n=41). Only rats that survived for at least 72 hours after infarction were classified according to infarction size (at the end of the study).

**Hemodynamic Measurements**
After 8 weeks of antihypertensive therapy, all drugs were stopped 72 hours before the hemodynamic measurements to permit adequate washout. The rats were anesthetized with an injection of a ketamine-HCl (87 mg/kg IM) and rompun-xylazine (15 mg/kg IM) mixture. The trachea was intubated by a noninvasive method via the mouth and mechanically ventilated with room air supplemented with low-flow oxygen with a small-rodent ventilator (Harvard Apparatus). A 2F micropip pressure transducer catheter, SPR-407 (Millar Instruments Inc), was used to measure LV and right ventricular (RV) hemodynamics, as previously described.13 Hemodynamic parameters were recorded on a Gould 2600S recorder (Gould Inc). Because of equipment problems or death during the procedure, hemodynamic measurements could not be performed in 2 control, 2 hydralazine-treated, and 3 ramipril-treated rats.

**Programmed Electrophysiological Stimulation**
At the end of the hemodynamic measurements, the thorax was opened by sternotomy and PES was done through Biomed electrodes (Cooner Wire Co) sewn onto the epicardial surface of the RV outflow tract, and recordings were made at the LV apex. Pacing was performed by means of a Bloom programmable stimulator (World Precision Instruments). The protocol for PES used in this study was similar to that described by Bélichard et al.13 The effective refractory period was determined by premature stimulation with a single extrastimulus after 20 paced beats at a basic cycle length of 100 ms. Induction of ventricular arrhythmias was then attempted by ventricular stimulation at a basic cycle length of 100 ms (S1) with single (S2), double (S3), and triple (S4) extrastimuli delivered after 20 paced beats (Figure 1).

The end point of PES was induction of a ventricular tachyarrhythmia consisting of at least 6 consecutive non driven ventricular extrastimulus beats. A preparation was considered noninducible when PES produced either no ventricular premature beats or only self-terminating salvos of <6 beats. Distinction was not made between ventricular tachycardia and ventricular fibrillation. A ventricular tachyarrhythmia was considered nonsustained when it lasted ≤15 beats and sustained when it lasted >15 beats before terminating spontaneously or by overdrive pacing.

An arrhythmia scoring system was used: 0, noninducible preparations; 1, nonsustained tachyarrhythmias induced with 3 extrastimuli; 2, sustained tachyarrhythmias induced with 3 extrastimuli; 3, nonsustained tachyarrhythmias induced with 2 extrastimuli; 4, sustained tachyarrhythmias induced with 2 extrastimuli; 5, nonsustained tachyarrhythmias induced with 1 extrastimulus; 6, sustained tachyarrhythmias induced with 1 extrastimulus; and 7, tachyarrhythmias induced during the 20 paced beats at a basic cycle length of 100 ms. If the heart stopped before the PES, the arrhythmia score assigned to that heart was 8.

**Morphological Studies**
The heart was then stopped in diastole with a saturated potassium chloride solution, removed, and rinsed in saline solution. For rats that died during the PES preparation or during the 8 weeks of antihypertensive therapy, the heart was simply removed and rinsed in saline solution. The LV was then filled with saline solution to a pressure of 15 mm Hg, sealed, and fixed in its distended form in formalin. Two cross sections were obtained at 1-mm intervals midway between the base and the apex of the LV. Myocardial infarction size was determined from the 2 cross sections of the LV as previously described.13 A large MI was defined as involving ≥35% of LV circumference, and a small-to-moderate infarction as involving <35%. Collagen was quantified by computer-assisted image analysis of samples from both cross sections cut into 8-μm-thick slices and stained with Sirius red F3BA as a 0.1% solution in saturated aqueous picric acid. The details of the methodology have been described previously.13 The heavily fibrotic scar was excluded. An approximation of total collagen volume was obtained by multiplying the LV weight/body weight (LVW/BW) ratio by collagen volume density percent.

**Statistics**
All values are expressed as mean±SD. A χ² test was used to evaluate the effects of different drugs on inducibility of ventricular arrhythmias and final mortality figures. One-way ANOVA was used to assess the effects of multiple comparisons, followed by a 2-sided
TABLE 1. Effect of MI and Pharmacological Interventions on Heart Rate and Arterial Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>Baseline (Week 0)</th>
<th>Week 4 After Infarction</th>
<th>Week 7 After Infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart Rate,</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>472±37 (n=50)</td>
<td>455±58 (n=11)</td>
<td>455±46 (n=9)</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>472±39 (n=41)</td>
<td>506±16† (n=12)</td>
<td>531±20*† (n=9)</td>
</tr>
<tr>
<td>Ramipril</td>
<td>476±34 (n=45)</td>
<td>506±16† (n=12)</td>
<td>531±20*† (n=9)</td>
</tr>
<tr>
<td><strong>Arterial Pressure,</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>190±13 (n=50)</td>
<td>141±45† (n=11)</td>
<td>151±38† (n=9)</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>191±24 (n=41)</td>
<td>115±13† (n=12)</td>
<td>110±16† (n=9)</td>
</tr>
<tr>
<td>Ramipril</td>
<td>189±16 (n=45)</td>
<td>122±12† (n=29)</td>
<td>112±14† (n=20)</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.05 vs control.
†P<0.05 vs baseline.
fibrosis were even more marked. Results from rats that the LV was adjusted for, these differences in cardiac hypertrophy of small-to-moderate MIs. When the degree of hypertrophy of treated group (Table 5). As a general rule, fibrosis was decreased in both hydralazine- and ramipril-treated groups, the decrease being greatest in the ramipril-treated group. Despite this, in hydralazine-treated hearts compared with controls. In general, hearts from hydralazine-treated rats had changes similar to those with large MIs had no decrease in LVW/BW ratio. All 3 large-MI groups had a significant increase in RVW/BW ratio, the increase tending to be less in the ramipril-treated group. Because of the very high postinfarction mortality in this study, the hearts of rats that died prematurely showed essentially the same results for ramipril-treated hearts but showed no decrease in fibrosis in hydralazine-treated hearts compared with controls.

**Discussion**

Hypertension is a major risk factor for peri-infarction and postinfarction survival.1 ACE inhibitors improve survival in such patients.12 In this study, we demonstrate that postinfarction mortality in the SHR is extremely elevated and that this may be related to increased susceptibility to ventricular arrhythmias and to an increase in cardiac fibrosis and hypertrophy above the increase in these variables already known to exist in the SHR. Our results suggest that the poor postinfarction survival, further development of morphological abnormalities, and increased susceptibility of ventricular arrhythmia in the SHR appear to be largely independent of arterial pressure, because hydralazine had little effect on these characteristics despite normalizing arterial pressure. However, this study indicates that the ACE inhibitor ramipril improves postinfarction survival in the SHR and provides several mechanisms, independent of its hypotensive effect, by which it may do this. These include improved LV hemodynamic parameters, decreased cardiac fibrosis and hypertrophy, and decreased inducibility of ventricular arrhythmias by PES.

### Table 2. Hemodynamic Measurements

<table>
<thead>
<tr>
<th></th>
<th>HR, bpm</th>
<th>LVSP, mm Hg</th>
<th>LVEDP, mm Hg</th>
<th>LV + dP/dt, mm Hg/s</th>
<th>RVSP, mm Hg</th>
<th>MI, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small-to-moderate MI (≤35%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=5)</td>
<td>254±45</td>
<td>103±20</td>
<td>10±4</td>
<td>3831±2138</td>
<td>29±7</td>
<td>17±13</td>
</tr>
<tr>
<td>Hydralazine (n=4)</td>
<td>328±13†</td>
<td>111±25</td>
<td>10±8</td>
<td>3525±1021</td>
<td>30±11</td>
<td>24±7</td>
</tr>
<tr>
<td>Ramipril (n=11)</td>
<td>260±39</td>
<td>108±16</td>
<td>9±5</td>
<td>3913±1589</td>
<td>34±10</td>
<td>20±13</td>
</tr>
<tr>
<td><strong>Large MI (&gt;35%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=2)</td>
<td>283±4</td>
<td>98±1</td>
<td>20±3</td>
<td>3800±989</td>
<td>41±4</td>
<td>53±21</td>
</tr>
<tr>
<td>Hydralazine (n=3)</td>
<td>298±32</td>
<td>99±21</td>
<td>27±5‡</td>
<td>2617±1446</td>
<td>35±8</td>
<td>39±4*</td>
</tr>
<tr>
<td>Ramipril (n=6)</td>
<td>242±44*</td>
<td>88±14</td>
<td>12±2*‡</td>
<td>2428±1209</td>
<td>32±9</td>
<td>55±12</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LVSP, LV systolic pressure; and RVSP, RV systolic pressure. Values are mean±SD.

*P<0.05 treated large MI vs control large MI.
†P<0.05 treated small-to-moderate MI vs control small-to-moderate MI.
‡P<0.10 treated large MI vs control large MI.

### Table 3. Ventricular Arrhythmias Induced With PES

<table>
<thead>
<tr>
<th></th>
<th>Control (n=9, n(%))</th>
<th>Ramipril (n=20, n (%))</th>
<th>Hydralazine (n=9, n)</th>
<th>Global χ² P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninducible</td>
<td>1/8 (12.5)†</td>
<td>13/18 (72.2)</td>
<td>1/9</td>
<td>0.01</td>
</tr>
<tr>
<td>Nonsustained VT or VF</td>
<td>2/8 (25)</td>
<td>1/8 (5.6)</td>
<td>1/9</td>
<td>0.36</td>
</tr>
<tr>
<td>Sustained VT or VF</td>
<td>5/8 (62.5)†</td>
<td>4/18 (22.2)</td>
<td>7/9</td>
<td>0.01</td>
</tr>
<tr>
<td>Death during surgical preparation</td>
<td>1/9 (11.1)</td>
<td>2/20 (10.0)</td>
<td>0/9</td>
<td>0.60</td>
</tr>
<tr>
<td>Noninducible (including death during surgical preparation)</td>
<td>1/9 (11.1)‡</td>
<td>11/20 (55.0)</td>
<td>1/9</td>
<td>0.02</td>
</tr>
</tbody>
</table>

VF indicates ventricular fibrillation; VT, ventricular tachycardia.

*P<0.05 vs control.
†P<0.05 vs ramipril.
‡P<0.1 vs ramipril.
§P<0.1 vs control.
Control and Hydralazine-Treated SHRs

Postinfarction survival in the control MI group was very poor. Most of these deaths occurred early, with 60% of all rats dying in the first 72 hours after infarction. Late mortality was particularly elevated in the control large-MI group, with only 2 of 11 (18%) 72-hour-postinfarction survivors with large MIs surviving the full 8 weeks. Such poor early and late postinfarction survivals in the SHR are compatible with studies in humans in which hypertensive patients were found to have a poor early and late postinfarction survival.1

Results from this study would suggest that when LVH is present, simple control of arterial pressure, such as that obtained with hydralazine, is insufficient to reduce early mortality. However, because hydralazine also increased heart rate, presumably due to reflex activation of the adrenergic system, in this study it cannot be determined whether another hypotensive agent that did not activate the adrenergic system would have fared better. The effects on late mortality of controlling arterial pressure with hydralazine are less certain because of the small number that survived 72 hours after infarction; however, the data that we do have suggest that it is no better than ramipril and may even be a bit worse.

The poor survival of these rats, coupled with the marked inducibility of ventricular arrhythmias by PES in the rats that survived, is consistent with an arrhythmic substrate being present in these hearts and contributing to their high mortality rate. Previous studies in the SHR,4,5,8 in other animal models of LVH,6,7 and in patients with LVH8 have all documented a relationship between LVH and susceptibility to ventricular arrhythmias. This susceptibility has been attributed to both increased cardiac fibrosis4,8 and cardiac hypertrophy.5 In this study, cardiac fibrosis and hypertrophy were greater in control and hydralazine-treated hearts than in ramipril-treated hearts and thus may have contributed to the high inducibility rate of ventricular arrhythmias by PES and mortality in these rats.

Effects of Ramipril

Treatment with the ACE inhibitor ramipril was associated with improved early and late survival. The beneficial effects of ramipril were most marked on early postinfarction survival, but late mortality in rats with large MIs receiving ramipril was also better.

Postinfarction mortality that occurs between 4 and 9 hours after infarction in this SHR model is due to ventricular arrhythmias with or without hemodynamic compromise.16 The origin of ventricular arrhythmias early after infarction appears to be the interface between dead and still viable myocardium, where depolarized myocytes can develop abnormal automaticity.17 How ramipril modifies this early arrhythmic phase, if indeed it does at all, is unknown but may involve a reduction in local as well as systemic neurohumoral activation. Also, although the infarction process is thought to be complete in normal rats by...
4 hours after infarction, recent results by Anversa et al. suggest that progressive cell loss can occur for up to 7 days after infarction, such that another potential mechanism is reduction of cell loss in the peri-infarction region due to reduced myocardial energy requirements and improved coronary blood flow due to ramipril. Against this possibility is the greater number of ramipril-treated rats with large MIs (16 out of a possible 32 ramipril-treated rats [50%]) that survived >72 hours compared with the control group (11 out of a possible 41 rats [27%]).

The cardioprotective effects of the ACE inhibitor ramipril on long-term postinfarction survival in SHRs appear to be multifactorial. In the SHR, ACE inhibitors have been shown to attenuate postinfarction LV dilatation, an effect that should contribute to improved survival. In this study, ramipril was also found to reduce cardiac hypertrophy and fibrosis, improved postinfarction prognosis, and reduced susceptibility to ventricular arrhythmias by PES. In a previous study of normal rats after infarction, we documented a similar beneficial effect of an ACE inhibitor, suggesting that regression of LVH and fibrosis is a major mechanism by which ACE inhibitors reduce susceptibility to ventricular arrhythmias after infarction.

The effects of ACE inhibitors on abnormalities in coronary blood flow in hypertension, in LVH, and after infarction have been evaluated in a number of other studies, but not in this one. In those studies, ACE inhibitors were found to improve coronary vascular reserve, effects that, coupled with the decrease in myocardial oxygen consumption associated with the use of ACE inhibitors, could contribute to the improved prognosis of these rats. Because of the lack of effect of hydralazine on prognosis, ventricular remodeling, susceptibility to ventricular arrhythmias by PES, and hemodynamic parameters, it would appear that in this as well as other settings, the control of arterial pressure and a borderline decrease in cardiac hypertrophy and fibrosis are not enough to reproduce the cardioprotective effects of ACE inhibitors.

The use of ramipril appeared to be associated with the preservation of ventricular function in hearts with large MIs. However, the difference in ventricular function between control rats and ramipril-treated rats with large MIs was less marked than that described in normal rats by Nishikimi et al. in the SHR. This may have been the result of the small number of control SHRs with large MIs that survived until the hemodynamic portion of the study. Presumably, the rats that died before the end of the study had hemodynamic abnormalities that were at least as important as those that survived, such that our findings may underestimate the true level of abnormalities present in control SHRs with large MIs compared with their ramipril counterparts.

### Acknowledgments
This work was supported by the Medical Research Council of Canada and Hoechst-Marion-Roussel of Canada.

### References


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Circulation. 1998;98:2074-2080
doi: 10.1161/01.CIR.98.19.2074

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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