β-Blockade improves adjacent regional sympathetic innervation during postinfarction remodeling

CHRISTOPHER M. KRAMER, PHILIP D. NICOL, WALTER J. ROGERS, PHILIP S. SEIBEL, CHONG S. PARK, AND NATHANIEL REICHEK

1Division of Cardiology, Department of Medicine, and 2Division of Cardiothoracic Surgery, Allegheny General Hospital, Pittsburgh, Pennsylvania 15212

Kramer, Christopher M., Philip D. Nicol, Walter J. Rogers, Philip S. Seibel, Chong S. Park, and Nathaniel Reichek. β-Blockade improves adjacent regional sympathetic innervation during postinfarction remodeling. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1429–H1434, 1999.—The effect of β-blockade on left ventricular (LV) remodeling, when added to angiotensin-converting enzyme inhibition (ACEI) after anterior myocardial infarction (MI), is incompletely understood. On day 2 after coronary ligation-induced anterointerapical infarction, 17 sheep were randomized to ramipril (ACEI, n = 8) or ramipril and metoprolol (ACEI-β, n = 9). Magnetic resonance imaging was performed before and 8 wk after MI to measure changes in LV end-diastolic, end-systolic, and stroke volume indexes, LV mass index, ejection fraction (EF), and regional percent intramyocardial circumferential shortening. 123I-labeled m-iodobenzylguanidine (MIBG) and fluorescent microspheres before and after adenosine were infused before death at 8 wk post-MI for quantitation of sympathetic innervation, blood flow, and blood flow reserve in adjacent and remote noninfarcted regions. Infarct size, regional blood flow, blood flow reserve, and the increase in LV mass and LV end-diastolic and end-systolic volume indexes were similar between groups. However, EF fell less over the 8-wk study period in the ACEI-β group (–13 ± 11 vs. –22 ± 4% in ACEI, P < 0.05). The ratio of adjacent to remote region 123I-MIBG uptake was greater in ACEI-β animals than in the ACEI group (0.93 ± 0.06 vs. 0.86 ± 0.07, P < 0.04). When added to ACE inhibition after transmural anterointerapical MI, β-blockade improves EF and adjacent regional sympathetic innervation but does not alter LV size.

METHODS

Seventeen female Dorsett sheep were studied. All underwent magnetic resonance imaging (MRI) before MI (see protocol below). Left thoracotomy was performed as previously described (12–15) with ligation of the left anterior descending coronary artery and its second diagonal branch to create a moderate-sized transmural anterointerapical infarction. On day 2 post-MI, animals were randomized to ramipril (10 mg once/day) (ACEI, n = 8) or to combination therapy with ramipril (10 mg once/day) and metoprolol (50 mg twice/day) (ACEI-β, n = 9). The dose of ramipril is one that has been demonstrated to limit LV remodeling in this model in previous studies (12). The dose of metoprolol was chosen as a dose that reduced resting heart rate by 30 beats/min in normal animals. Heart rate and blood pressure, by automated pediatric cuff in the left forelock of the standing animal, were measured at weekly intervals. Repeat MRI was performed 8 wk later.

MRI. The anesthetized, ventilated animal was placed in the right lateral decubitus position on a phased array surface coil in a Siemens 1.5T scanner, and electrocardiographic gating was initiated. Intravenous 5% guaifenesin and 500 mg of ketamine provided heavy sedation during imaging. Localizing scout images were performed followed by breath hold, segmented k-space, and multiphase gradient echo cine series [for measurement of LV mass, volumes, and ejection fraction (EF)] spanning the LV from apex to base (Fig. 1A). Imaging parameters were repetition time (TR) of 60 ms (with view-sharing, yielding a 30-ms temporal resolution), echo time (TE) of 4.8 ms, slice thickness of 7 mm, 128 × 256 matrix, and 25-cm field of view producing a final interpolated pixel size of 0.95 mm². For analysis of regional intramyocardial function, short-axis breath hold, segmented k-space, and multiphase gradient echo-tagged images (1, 36) were obtained spanning the LV from apex to base (Fig. 1B). Imaging parameters included 7-mm thick images, 7-mm tag stripe separation, TR of 70 ms (with view sharing, yielding a 35-ms temporal resolution), TE of 4 ms, 128 × 256 matrix, 25-cm field of view.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
from the same animal at the same location as in one

A tagged magnetic resonance apical short-axis image at end systole. The ventricular apex can also be seen from 1 o'clock to 5 o'clock in image. The septum can be seen from 1 o'clock to 5 o'clock in this image. Right therapy at 8 wk after infarction. Thinned transmurally infarcted

Guanidine (MIBG) was labeled with 123I with methods as previously described (14, 33). At 8 wk post-MI, the thorax was opened, and left atrium and descending aorta were cannulated. All animals were infused with two different sets of fluorescent latex microspheres (3 × 10^6, 15 μm in diameter), one before and one during adenosine infusion (0.14 mg·kg^{-1}·min^{-1}). Blood samples were withdrawn for a total of 3 min (1 min before and 1.5 min after a 30-s injection of microspheres) at a constant rate (5 ml/min) from the descending aorta for control measurement of fluorescence. One hour before death, sheep were injected with 1.4 ± 0.1 mCi 123I-MIBG. After 60 min, the heart was excised and cut in 1-cm-thick slices. The sharply defined infarct borders were identified; tissue within 2 cm of the transmural infarct was termed adjacent, and tissue beyond 2 cm was termed remote as previously defined (12–15). Infarcted tissue was weighed and percent infarct calculated as infarct weight per total LV weight. Slices were further sectioned into 1- to 2-g samples with regions marked and counted on a gamma counter to quantify 123I-MIBG uptake per gram of tissue. The MIBG myocardial uptake was expressed in counts per gram, and with the use of the specific activity, absolute molar MIBG uptake was determined for each piece in adjacent and remote noninfarcted regions.

Microsphere blood flow. The same 1- to 2-g pieces were digested as previously described (14), and their fluorescence was determined by an automated fluorescence spectrophotometer and compared with reference blood samples. Fluorescence from tissue samples before and after adenosine infusion as a measure of flow reserve was compared in adjacent and remote noninfarcted regions.

Image analysis. LV mass index (LVMi), end-diastolic and end-systolic volume indexes (EDVI and ESVi, respectively), and EF were measured from stacked short-axis cine slices by a single observer blinded to therapy (A. L. Shaffer) (see Fig. 1A). Regional percent intramyocardial segment shortening (%S) was measured from stacked short-axis tagged images (see Fig. 1B) using a software package (VIDA, Univ. of Iowa) on a SUN workstation by a different blinded observer (T. M. Theobald) using a previously described methodology (12–15). Interstripe distances were measured at end systole (L_{es}) and end diastole (L_{ed}), and %S was calculated as %S = (100(L_{ed} – L_{es})/L_{ed}). %S was measured at endocardial and epicardial sites in segments located within infarcted, adjacent, and remote regions as previously defined.

Statistical analysis. Changes between baseline and 8 wk post-MI within each treatment group in heart rate, blood pressure, LVMi, EDVI, ESVi, stroke volume index (SVi), EF, and regional %S were compared by Student’s paired t-test. Between-group differences in the change in any of these parameters over the 8-wk study period were analyzed using two-way ANOVA. Regional differences in blood flow and blood flow reserve between groups at 8 wk post-MI were compared between groups by two-way ANOVA. Regional 123I-MIBG uptake within groups was compared by paired t-test. Because of differential 123I-MIBG dosing and uptake in each animal, absolute 123I-MIBG uptake was not compared between groups. The ratio of adjacent to remote region 123I-MIBG uptake was compared between groups by unpaired t-test.

RESULTS

Mean infarct mass as percentage of total LV mass by pathological analysis at 8 wk after MI was 14 ± 3% in the ACEI-β group and 16 ± 4% in the ACEI group (P = NS). At baseline, heart rate and blood pressure were not different between groups. As expected, the fall in heart rate over the 8-wk study period (Table 1) was greater in the ACEI-β group (−34 ± 16 (SD) beats/min) than that in the ACEI animals (−9 ± 17 beats/min, P < 0.006). The change in blood pressure was similar in both groups (Table 1).

Changes in LVMi, EDVI, SVi, and EF over the 8 wk are shown in Table 2. At baseline, all of these parameters were similar between groups. The increase in LVMi and LV EDVI and ESVi was similar in the two groups. However, EF fell less over the 8-wk study.
period in the ACEI-β group (−13 ± 11 vs. −22 ± 4% in ACEI; P < 0.05). In addition, SVI increased in the ACEI-β group (−0.1 ± 0.3 ml/kg) but fell slightly in the ACEI group (−0.1 ± 0.1, P < 0.05).

At baseline, %S in adjacent and remote noninfarcted regions was not different between groups, and the expected transmural heterogeneity was noted with subendocardial regions demonstrating greater %S than subepicardial regions (13, 14) (Table 3). By 8 wk, there was a trend toward better preservation of %S in the ACEI-β group in the adjacent subendocardium (10 ± 2 vs. 8 ± 4% in the ACEI group, P = 0.11), but the change over the 8-wk period was not different between groups. The decline in adjacent noninfarcted %S during the remodeling period was −10 ± 3% in ACEI and −8 ± 3% in ACEI-β (P = NS). As expected, no significant fall in remote noninfarcted %S was seen in either group (0 ± 3% in ACEI and −1 ± 6% in ACEI-β, P = not significant (NS)).

Blood flow at 8 wk post-MI was not different between groups in adjacent noninfarcted regions (1.0 ± 0.4 ml·min⁻¹·g⁻¹ in ACEI and 1.0 ± 0.3 ml·min⁻¹·g⁻¹ in ACEI-β). In remote regions, blood flow was not different from adjacent regions and likewise was similar between groups (1.0 ± 0.5 and 0.9 ± 0.3 ml·min⁻¹·g⁻¹, respectively). Blood flow reserve was also similar between groups in both adjacent and remote noninfarcted regions. In adjacent regions, blood flow reserve was 2.4 ± 1.0 in ACEI and 2.2 ± 0.9 in ACEI-β (P = NS), and in remote regions, blood flow reserve was 2.6 ± 1.0 in ACEI and 2.2 ± 0.9 in ACEI-β (P = NS).

In both groups, quantitative ¹²³I-MIBG uptake was less in adjacent noninfarcted regions than in remote. In ACEI animals, ¹²³I-MIBG uptake was 0.87 ± 0.37 nmol/g in adjacent regions and 1.03 ± 0.48 nmol/g in remote regions (P < 0.02). In ACEI-β animals, ¹²³I-MIBG uptake was 0.78 ± 0.31 nmol/g in adjacent regions and 0.85 ± 0.35 nmol/g in remote (P < 0.02). The ratio of adjacent to remote region ¹²³I-MIBG uptake was greater in ACEI-β animals than in the ACEI group (0.93 ± 0.06 vs. 0.86 ± 0.07, P < 0.04) (Fig. 2).

### DISCUSSION

When added to ACEI after transmural anteroapical MI, β-blockade lowers heart rate and improves EF but does not alter blood pressure, LV mass, or cavity size. Neither regional blood flow nor flow reserve is altered. Sympathetic innervation is improved in adjacent noninfarcted regions, and regional function tends to be better preserved in those regions. Restored sympathetic innervation in adjacent noninfarcted regions by β-blockade may contribute to improved regional and global function in the remodeled postinfarct LV. In addition, the improvement in innervation may reduce the arrhythmias generated from these adjacent regions, contributing to mortality reduction seen with β-blockade after MI.

Comparison with previous studies. The lack of additive effect of β-blockade on LV end-diastolic or end-systolic volume is consistent with prior animal studies of postinfarct remodeling. In rat models of MI, the efficacy of nonselective β-blockers in limiting remodeling has been controversial. Fishbein et al. (6) showed that propranolol therapy in a rat model of transmural infarction blunted the increase in myocardial hypertrophy yet was associated with increased ventricular dilatation. In a rat model of reperfused nontransmural infarction, propranolol therapy was associated with increased LV cavity area and reduced wall thickness in infarcted and noninfarcted regions, suggesting proremodeling effects (21). Using β₁-selective blockade, Hu et al. (9) have shown that infarct size and timing of the start of therapy impact on its effect on LV remodeling. Early β-blockade (begun 30 min after infarction) limited mortality, and early or late (14 days post-MI)
β-blockade had favorable effects on LV volumes only in the setting of large infarction.

β₁-Selective β-adrenergic blockade favorably modulated remodeling in two animal models of myocardial damage in which it was compared directly with ACEI. Sabbah et al. (25) used a model of congestive heart failure in the dog induced by intracoronary microembolizations. They demonstrated that in dogs treated with enalapril or metoprolol, LV EF did not decrease over time, and LV end-diastolic and end-systolic volumes did not change, whereas in control animals or digoxin-treated animals, LV volumes increased. McDonald et al. (16) have likewise shown that therapy with either captopril or metoprolol reduced LV mass and end-diastolic volume in a canine model of chronic LV remodeling from previous transmyocardial direct cur-

Table 3. Changes in regional percent intramyocardial circumferential shortening during the 8-wk time period

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Week 8</th>
<th>Δ</th>
<th>Baseline</th>
<th>Week 8</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adj Subendo</td>
<td>20±4</td>
<td>8±4*</td>
<td>-12±4</td>
<td>21±3</td>
<td>10±2*</td>
<td>-10±2</td>
</tr>
<tr>
<td>Adj Subepi</td>
<td>15±1</td>
<td>7±5*</td>
<td>-8±5</td>
<td>16±2</td>
<td>10±3*</td>
<td>-6±3</td>
</tr>
<tr>
<td>Adj Average</td>
<td>17±3</td>
<td>7±5*</td>
<td>-10±3</td>
<td>18±2</td>
<td>10±2*</td>
<td>-8±3</td>
</tr>
<tr>
<td>Rem Subendo</td>
<td>21±4</td>
<td>18±6</td>
<td>-2±5</td>
<td>19±4</td>
<td>18±4</td>
<td>-1±6</td>
</tr>
<tr>
<td>Rem Subepi</td>
<td>15±2</td>
<td>16±4</td>
<td>0±3</td>
<td>15±3</td>
<td>14±4</td>
<td>-1±4</td>
</tr>
<tr>
<td>Rem Average</td>
<td>18±3</td>
<td>17±4</td>
<td>0±3</td>
<td>17±4</td>
<td>16±4</td>
<td>-1±6</td>
</tr>
</tbody>
</table>

Values are means ± SD. Subendo, subendocardium; Subepi, subepicardium; Δ, difference between week 8 and baseline. *P < 0.008 vs. baseline.

Fig. 2. Graph demonstrating ratio of 123I-labeled m-iodobenzylguanidine (MIBG) uptake in adjacent (Adj) noninfarcted regions compared with remote (Rem) noninfarcted regions in 2 groups of animals. ACEI, angiotensin-converting enzyme inhibitor; ACEI + β, ACEI plus β-blocker. Ratio is significantly closer to unity in ACEI–β group.
Limitations. No group of animals was studied with \(\beta\)-blockade alone. However, ACE inhibitors are likely to be background therapy for all patients with LV dysfunction after anterior MI based on previous large clinical trials (10, 23, 30), and relevant questions remain regarding the role of additive \(\beta\)-blockade in these patients. The two groups studied were limited in size. However, despite the small n, significant intergroup differences were found in regional sympathetic innervation and global systolic function. Only one time point in the course of postinfarct remodeling was studied. The 8-wk time point post-MI was chosen because of the extent of remodeling that occurs and because of our previous experience using \(^{123}\)I-MIBG at this time point in this model in untreated postinfarct animals (14).

The difference in EF between the groups was greater than the difference found in regional function as measured by %SY. %SY is only one direction of measured strain within the myocardium, namely, circumferential, and EF is a measure of the result of myocardial deformation in three dimensions. The trend to improved adjacent subendocardial function in the ACEI-\(\beta\)-block group was in the same direction as the differences in global function.

\(^{123}\)I-MIBG uptake was expressed as regional ratios within animals. The absolute value of \(^{123}\)I-MIBG uptake could not be compared directly between animals or groups because of expected differences in \(^{123}\)I-MIBG labeling before infusion in each animal. Changes in nonneuronal uptake of \(^{123}\)I-MIBG may account in part for reduced \(^{123}\)I-MIBG uptake in noninfarcted tissues (29), although most reductions in nonneuronal uptake have been demonstrated within nonviable infarcted myocardium. In addition to changes in presynaptic sympathetic nervous system function in the post-MI setting, significant changes may occur in the cascade of postsynaptic events (11). ACEI has been demonstrated to upregulate \(\beta\)-adrenergic receptors (34), which also occurs with \(\beta\)-blockade (8). The combination of ACEI and \(\beta\)-blockade would therefore be expected to further enhance \(\beta\)-receptor responsiveness. Postsynaptic events will be a direction for future investigation in this model.

We gratefully acknowledge the technical and analytical support of Amy L. Shaffer and Therese M. Theobald. This study was supported by American Heart Association, National Office, Grant-in-Aid 96014830. Address for reprint requests and other correspondence: C. M. Kramer, Univ. of Virginia Health Systems, Departments of Radiology and Medicine, Box 170, Charlottesville, VA 22908.

Received 5 February 1999; accepted in final form 28 May 1999.

REFERENCES


