Comparative Effects of Carvedilol and Losartan Alone and in Combination for Preventing Left Ventricular Remodeling After Acute Myocardial Infarction In Rats

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It has been verified that losartan has beneficial effects on ventricular remodeling (VRM) after acute myocardial infarction (AMI), but the effects of carvedilol alone or in combination with losartan on this condition have not been defined. The present study used rats to compare the effects of carvedilol and losartan alone and in combination for preventing VRM after AMI. After ligation of the left coronary artery, 100 surviving female Sprague-Dawley rats were randomly assigned to 1 of 4 groups: (1) AMI control (n=25), (2) carvedilol (Car, 1 mg·kg–1·day–1) (n=25), (3) losartan (Los, 3 mg·kg–1·day–1) (n=25), and (4) Car (1 mg·kg–1·day–1)+Los (3 mg·kg–1·day–1) (n=25). A sham-operated group (n=17) was also randomly selected. Drugs were administered by gastric gavage for 4 weeks. After hemodynamic studies, the hearts were fixed and analyzed pathologically. Exclusive of the rats that had died or had an infarct size <35% or >55%, complete data were obtained for 65 rats, comprising AMI control (n=13), Car (n=12), Los (n=13), combination (n=14), and sham (n=13) groups. There were no significant differences in the size of infarct among the 4 AMI groups (45.8~46.7%, all p>0.05). Compared with the sham group, left ventricular (LV) end-diastolic pressure (LVEDP), volume (LVV), weight (LVW) and septal thickness (STh) were all significantly increased (all p<0.001), whereas ±dp/dt was significantly decreased (both p<0.001) in the AMI group. In comparison with the AMI group, LVEDP, LVV, LVW and STh were all significantly decreased (LVEDP: 12.7±2.3, 9.7±2.8, and 8.6±3.5 mmHg vs 20.6±2.7 mmHg, all p<0.001; LVV: 0.74±0.07, 0.76±0.07, and 0.70±0.09 ml vs 0.86±0.05 ml, all p<0.05; LVW: 668.4±52.0, 702.6±45.4, and 683.9±67.7 mg vs 787.3±76.7 mg, p<0.05–0.001; STh: 1.57±0.05, 1.48±0.07, and 1.46±0.07 mm vs 1.71±0.04 mm, all p<0.05), whereas ±dp/dt was significantly increased (all p<0.05) in the Car, Los, and combination groups, with LVEDP decreasing more in both Los and the combination groups than in the Car group alone (p<0.05) and STh decreasing more in the combination group than in the Car group alone (p<0.05). Carvedilol and losartan alone and in combination all prevent VRM after AMI in rats, with almost equivalent effect. (Circ J 2003; 67: 159–162)

Key Words: Acute myocardial infarction; Carvedilol; Left ventricular remodeling; Losartan; Rat
the sham-operated rats, a suture was tied loosely around the left coronary artery, but the vessel was not ligated.

From 24h after AMI, the rats that had survived were given drugs twice daily by gastric gavage. Carvedilol (donated by Roche Pharmaceutical Company) and losartan (donated by MSD Pharmaceutical Company) were dissolved in distilled water and the drug solutions were constituted at a concentration of 12.5 mg% and 37.3 mg%, respectively. When the solution was delivered in the same volume of 8 ml·kg⁻¹·d⁻¹, namely 2 ml per 250 g body weight, it approximated the target doses of losartan and carvedilol in the 3 treatment groups, and the combination group was given both drugs. The AMI controls as well as the sham-operated controls were fed the same amount of distilled drinking water at the same time.

**Hemodynamic Measurement** Four weeks after the initiation of therapy, hemodynamic studies were performed in each group using the methods described by Pfeffer et al: Each rat was weighed and then anesthetized. The right carotid artery was separated and cannulated with a 20G sheathed needle. The needle was extracted and the end of the sheath was connected to the energy exchanger of an 8-channel physiological recorder via a heparin–saline-filled plastic tube. After balancing with the air pressure, the ascending aortic systolic pressure (SBP), diastolic pressure (DBP) and mean pressure (MBP) were recorded. Subsequently, the sheath was reversed into the left ventricle and the LV systolic pressure (LVSP), end-diastolic pressure (LVEDP) and the maximal rate of rise and fall (±dp/dt) were recorded. The heart rate (HR) was also recorded synchronously.

**Pathological Analysis** After the hemodynamic studies, the heart was arrested in diastole by intravenous injection of 2–3 ml 10% KCl through the femoral vein. The thorax was rapidly opened and the aortic arch was ligated with the head of the sheath fixed in the ascending aorta. The end of the sheath was connected to an irrigating bottle of 10% formalin and then the coronary arteries were perfused with the fixative and the right atrium was cut to allow the drainage of blood and fixative. The procedure lasted for 20–30 min and the heart was fixed, after which it was excised and preserved in 10% formalin after the atria and great vessels were trimmed away.

**Weighing of the Heart** The 2 ventricles were separated by incising the right ventricle (RV) along the septum and each was weighed by an electronic balance. When the weight of the LV and RV (LVAW and RVAW) was calculated (LVRW and RVRW).

**LV Volume (LVV) and Length of Longitudinal Axis (L) Measurement** These measurements were performed directly with a cutaneous syringe and vernier callipers, respectively. The mean value of 3 examinations was the experimental data of one heart sample. The coefficient of variability of 3 measurements of LVV by one examiner was 1% (0.95±0.85%), intraobserver variability was 2.1% (2.06±2.10%) and interobserver variability was 2.7% (2.68±1.29%).

**LV Transverse Diameter (D) and Size of Infarction** Perpendicular to L, the LV was cut midway into 2 parts. The apical half was imbedded in paraffin and a 10µm transverse section was cut from its bottom and mounted on plastic, which was then processed by a BHEC microscope–computer colour imaging processor. The septal thickness (STh), maximum and minimal transverse diameter of LV (Dmax and Dmin) were then measured and their geometric mean value was calculated according to the following formula: \[ D = \sqrt{D_{\text{max}} \times D_{\text{min}}} \]

The epicardial and endocardial circumferences, as well as the circumference of the infarct, were also measured, and MI size was computed according to the following formula: \[ \text{MI size} = \frac{\text{infarcted circumference}}{\left(\text{epicardial circumference} + \text{endocardial circumference}\right)/2} \times 100\% \]

### Statistical Analysis
All variables are expressed as mean±SD. Differences among groups were assessed by ANOVA with a Scheffe’s test, and the differences between 2 groups were assessed by q test. A value of p<0.05 in a two-tailed distribution was considered statistical significance.

### Results

Exclusive of the rats that died and those with MI size <35% or >55%, complete data were obtained from 65 rats, which comprised 13 AMI controls, 12 from the carvedilol group, 13 from the losartan group, 14 from the combination group and 13 from the sham-operated group. There were no significant differences in MI size among the 4 AMI groups (all p>0.05) (Table 1).

**Effect of Carvedilol and Losartan Alone and in Combination on Hemodynamics (Table 1)**

Compared with the sham-operated group, the LVEDP was significantly increased (p<0.001) and SBP, DBP, MBP and LVSP were all significantly decreased (p<0.05–0.01)

### Table 1 Effect of Carvedilol and Losartan Alone and in Combination on Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=13)</th>
<th>AMI (n=13)</th>
<th>Carvedilol (n=12)</th>
<th>Losartan (n=13)</th>
<th>Combination (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI size (%)</td>
<td>0</td>
<td>46.2±3.74</td>
<td>46.6±4.54</td>
<td>45.8±4.79</td>
<td>45.9±4.44</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>392.6±45.71</td>
<td>392.3±43.00</td>
<td>349.5±43.37</td>
<td>386.5±31.75</td>
<td>365.4±36.42</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>127.5±14.83</td>
<td>102.5±9.41</td>
<td>92.7±16.50</td>
<td>99.4±16.62</td>
<td>98.3±14.40</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>102.1±16.20</td>
<td>76.7±13.52</td>
<td>72.9±15.90</td>
<td>76.3±13.78</td>
<td>77.3±16.04</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>112.8±15.61</td>
<td>88.0±11.64</td>
<td>81.4±16.79</td>
<td>85.7±18.13</td>
<td>85.7±15.24</td>
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<tr>
<td>LVSP (mmHg)</td>
<td>131.1±14.16</td>
<td>106.7±10.28</td>
<td>97.9±15.84</td>
<td>102.6±15.92</td>
<td>103.7±13.54</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>2.4±0.61</td>
<td>20.5±0.61</td>
<td>14.2±2.5</td>
<td>17.6±2.5</td>
<td>18.1±3.0</td>
</tr>
</tbody>
</table>

Compared with sham group: *p<0.05, **p<0.01, ***p<0.001.
Compared with AMI group: *p<0.05, **p<0.01, ***p<0.001.
Compared with carvedilol group: *p<0.05, **p<0.01.
AMI, acute myocardial infarction; HR, heart rate; SBP, DBP and MBP, systolic, diastolic and mean blood pressure respectively; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure.
in the AMI group. In comparison with the AMI group, LVEDP, but not blood pressure and LVSP, as significantly decreased in all therapy groups (all p<0.001), and as reduced more in the combination and losartan groups than in the carvedilol group (p<0.001). HR was significantly decreased only in the carvedilol group (p<0.05).

**Effects of Carvedilol and Losartan Alone and in Combination on LVRM and LV Function (Table 2)**

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=13)</th>
<th>AMI (n=13)</th>
<th>Carvedilol (n=12)</th>
<th>Losartan (n=13)</th>
<th>Combination (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDP (mmHg)</td>
<td>16.3±3.3</td>
<td>20.5±3.9</td>
<td>8.9±2.8</td>
<td>13.1±3.5</td>
<td>10.8±3.1</td>
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<tr>
<td>BW (kg)</td>
<td>67.9±7.5</td>
<td>70.3±7.8</td>
<td>68.1±7.8</td>
<td>70.6±7.9</td>
<td>69.5±7.7</td>
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<tr>
<td>LVMI (mg/kg)</td>
<td>15.3±5.2</td>
<td>17.1±5.8</td>
<td>13.1±4.8</td>
<td>15.8±5.6</td>
<td>14.5±5.3</td>
</tr>
<tr>
<td>STh (mm)</td>
<td>1.162±0.26</td>
<td>1.71±0.44</td>
<td>1.57±0.65</td>
<td>1.48±0.71</td>
<td>1.6±0.71</td>
</tr>
<tr>
<td>LVAW (mg)</td>
<td>569.56±52.64</td>
<td>787.32±76.69</td>
<td>665.41±52.01***</td>
<td>702.63±45.39**</td>
<td>683.94±67.70***</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>128±22</td>
<td>135±24</td>
<td>118±20</td>
<td>123±22</td>
<td>119±22</td>
</tr>
<tr>
<td>dp/dt (mmHg/s)</td>
<td>5900±820</td>
<td>396±413***</td>
<td>3750±399***</td>
<td>3815±363***</td>
<td>4014±682***</td>
</tr>
<tr>
<td>LVV (ml)</td>
<td>0.43±0.06</td>
<td>0.86±0.05***</td>
<td>0.74±0.07****</td>
<td>0.76±0.07******</td>
<td>0.70±0.09******</td>
</tr>
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</table>

**Discussion**

It has been shown that excessive activation of the sympathetic nervous system (SNS) and renin–angiotensin system (RAS) after AMI plays a key role in initiating and maintaining LVRM. Therefore, inhibiting these systems might attenuate the remodelling process. Experimental studies including ours have shown that losartan can prevent LVRM because of its inhibition of RAS via AT1 receptor blockade. Clinical studies have showed also that carvedilol has beneficial effects in preventing LVRM in patients with ischemic heart failure and AMI partly because of inhibition of the SNS. Theoretically, carvedilol should be as effective as losartan in attenuating post-infarction LVRM, and their combination should be better. The results of our comparison of the effects of carvedilol and losartan alone and in combination on post-infarction LVRM are encouraging.

**Effect of Carvedilol and Losartan Alone and in Combination on Hemodynamics**

This study showed that LVEDP after AMI was significantly increased, and elevated LVEDP was significantly decreased with the treatment of carvedilol, losartan and their combination by 38%, 52% and 58% respectively, with almost equivalent effect. This result strongly suggests that carvedilol is almost as effective as losartan, and its combination with losartan, in reducing LVEDP. The mechanisms for the improved hemodynamics by carvedilol have not been elucidated, but it is probably associated with the reduction of wall stress via blockade of excessive sympathetic activation1,11,12 whereas the mechanism of losartan’s effect is reducing LV preload, afterload and wall stress and improving LV relaxation1,13 via AngII blockade effects on the AT1 receptor.

**Effect of Carvedilol and Losartan Alone and in Combination on LVRM**

The study showed that LVV, LVAW and STh were significantly increased after AMI, indicating that LVRM occurred, which is consistent with the results of Pfeffer et al and Litwin and Katz. The major finding of this study is that carvedilol, losartan and their combination all can prevent LV dilatation (LVV was decreased by 14%, 12% and 19%, respectively), but can also attenuate the hypertrophy of the non-infarcted myocardium, indicating that post-infarction LVRM is attenuated by these drugs. This strongly suggests that carvedilol is as effective as losartan, and its combination with losartan, in preventing post-infarction LVRM.

No similar reports were found in the literature. LV dilatation after AMI is one of the key characteristics of LVRM and is the most powerful predictor of poor prognosis. So the attenuation of LV dilatation by carvedilol is of clinical significance because it indicates that carvedilol not only can be used for the treatment of heart failure, as demonstrated in clinical studies, but also can probably be used to prevent heart failure via the attenuating of LV dilatation after AMI.

Hypertrophy of the non-infarcted myocardium is the main pathologic basis of LVRM and is initiated and promoted by hemodynamic abnormality and activation of the SNS and RAS. This hypertrophy can improve LV contractility and reduce LV wall stress, which compensates for the reduced LV function in the early stage of AMI.
however, it also has a detrimental effect through the enlargement of the LV, because the increase in wall stress may in turn promote further LV dilatation and finally lead to progressive LV dysfunction and heart failure. In addition, hypertrophy itself can also result in myocardial dysfunction and heart failure at the end stage because of collagen deposition and severe fibrosis. So attenuation of the hypertrophy of the non-infarcted myocardium by carvedilol, losartan and their combination is very important in preventing LVRM and heart failure.

The mechanisms of carvedilol’s beneficial effects are not quite clear, but are probably associated with blockade of norepinephrine on cardiomyocytes and attenuation of collagen deposition in the non-infarcted zone. It has been verified that AngII exerts deleterious effects as a potent vasoconstrictor and growth-promoter by inducing myocyte hypertrophy, myocardial fibroblast proliferation and collagen synthesis, and its AT1 receptor appears to mediate most of the adverse effects of AngII. So the AT1 receptor antagonist, losartan, not only attenuates the reactive cardiac hypertrophy by its favorable hemodynamic profile, but also directly inhibits myocyte hypertrophy, fibroblast proliferation and collagen synthesis through AT1 receptor blockade. The present study has shown that the combination of carvedilol and losartan is no more effective in attenuating cardiac hypertrophy than either drug alone, but further studies are needed to verify this apparent result.

Effect of Carvedilol and Losartan Alone and in Combination on LV Function

The study showed that ±dp/dt and ±(dp/dt) - LVSP were significantly decreased after AMI, indicating that LV function was impaired, and carvedilol or losartan alone and their combination all improved LV function to an equivalent extent. Carvedilol improves LV function by not only blockade of adrenergic receptors, which has direct protective effects on cardiomyocytes against catecholamines and improves regional wall motion, but also its potent antioxidant and vasodilating effects protect cardiomyocytes from injury by oxygen free radicals and reduce the LV preload and afterload, which then furthur reduces the oxygen consumption of cardiomyocytes. The improvement of LV function by losartan is probably by reduction of preload, afterload and wall stress and improvement of the oxygenation and systolic function of cardiomyocytes.

References