Angiotensin-(1–7) Attenuates the Development of Heart Failure After Myocardial Infarction in Rats

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**Background**—The renin-angiotensin system (RAS) is a key player in the progression of heart failure. Angiotensin-(1–7) is thought to modulate the activity of the RAS. Furthermore, this peptide may play a part in the beneficial effects of angiotensin-converting enzyme inhibitors in cardiovascular disease. We assessed the effects of angiotensin-(1–7) on the progression of heart failure.

**Methods and Results**—Male Sprague-Dawley rats underwent either coronary ligation or sham surgery. Two weeks after induction of myocardial infarction, intravenous infusion of angiotensin-(1–7) (24 μg/kg per hour) or saline was started by minipump. After 8 weeks of treatment, hemodynamic parameters were measured, endothelial function was assessed in isolated aortic rings, and plasma angiotensin-(1–7) levels were determined. Myocardial infarction resulted in a significant deterioration of left ventricular systolic and diastolic pressure, dP/dt, and coronary flow. Raising plasma levels 40-fold, angiotensin-(1–7) infusion attenuated this impairment to a nonsignificant level, markedly illustrated by a 40% reduction in left ventricular end-diastolic pressure. Furthermore, angiotensin-(1–7) completely preserved aortic endothelial function, whereas endothelium-dependent relaxation in aortas of saline-treated infarcted rats was significantly decreased.

**Conclusions**—Angiotensin-(1–7) preserved cardiac function, coronary perfusion, and aortic endothelial function in a rat model for heart failure. (*Circulation*. 2002;105:1548-1550.)

Key Words: angiotensin | heart failure | hemodynamics | myocardial infarction

It has been well established that activation of the renin-angiotensin system (RAS) plays a detrimental role in the progression of heart failure. Consequently, one of the most successful pharmacotherapeutic interventions in patients, as well as in experimental models of heart failure, consists of angiotensin-converting enzyme (ACE) inhibitor therapy.1,2

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Although ACE inhibitors were originally developed to suppress the formation of angiotensin (Ang) II, recent studies suggest that part of their beneficial effect in cardiovascular diseases may be attributed to the elevation of plasma Ang-(1–7) levels.3 The heptapeptide Ang-(1–7) is a biologically active metabolite of Ang I and Ang II that is formed through cleavage of endopeptidases and is inactivated by ACE.4 Under normal conditions, tissue and plasma levels of Ang-(1–7) are similar to those of Ang II. ACE inhibitor treatment, although having limited effects on the circulating amount of Ang II, increases Ang-(1–7) levels 10- to 25-fold.5,6

Ang-(1–7) antagonizes the RAS at various levels. Being a substrate for ACE, Ang-(1–7) competes with Ang I and bradykinin (BK) for degradation, thereby inhibiting Ang II formation and augmenting BK activity.7,8 Furthermore, Ang-(1–7) antagonizes the vasoconstrictive effects of Ang II in various species,9 and is a vasodilator in canine and porcine coronary arteries,9,10 either by blocking the AT1-receptor11 or by releasing nitric oxide and vasodilating prostaglandins via an as yet unidentified receptor.10,12,13

In this study, we investigated the specific effects of Ang-(1–7) on the development of heart failure using continuous intravenous infusion in the rat myocardial infarction (MI) model.

**Methods**

**Experimental Design**

The Animal Research Committee of the University of Groningen approved this study. Left coronary artery ligations were performed in 39 male Sprague-Dawley rats weighing 250 to 300 g (Harlan; Zeist, the Netherlands).14 Perioperative mortality was 49%. Two weeks after induction of MI, rats were randomly allocated to intravenous infusion of either Ang-(1–7) (24 μg/kg per hour; n=10) or saline (n=10) by osmotic minipumps (Alzet 2004). Sham-operated controls (n=10) received saline. After 8 weeks of treatment, hemodynamic studies were performed after isoflurane anesthesia with a microtip pressure transducer,15 coronary flow was measured in a
Langendorff setup, endothelial function was tested in isolated aortic rings, and plasma Ang-(1–7) levels were measured by radioimmunoassay. In the remainder of the text, the maximal rate of change in ventricular pressure is indicated as dP/dt.

Histology
Midventricular slices were processed for histochemical analysis. Infarct size was determined on picrosirius red/fast green-stained sections and was expressed as the percentage of 8×3 cm length of total left ventricular circumference. Rats with infarcts smaller than 20% were excluded (n = 3 for Ang-(1–7) and n = 1 for saline-treated rats). Capillary density was determined on sections stained with biotin-labeled Griffonia simplicifolia lectin I (GSL-I) and hematoxylin and was expressed as the number of capillaries per mm². Myocyte cross-sectional area was measured on hematoxylin/eosin-stained sections.

Statistical Analysis
Data are presented as mean ± SEM. Statistical analysis between the groups was performed by 1-way ANOVA followed by Bonferroni’s t test. Differences in dose-response curves were tested by ANOVA for repeated measures with Greenhouse-Geisser correction for asphericity. Differences were considered significant at P < 0.05.

Results

General Characteristics
General parameters at the end of treatment are shown in Table 1. There was no difference in body weight among the 3 groups. Infarct size did not differ between the Ang-(1–7) and saline-treated groups, with an average of 33%. Left ventricular weight to body weight ratio was equally increased in both MI groups compared with sham-operated controls (17%, P < 0.05). Myocyte cross-sectional area was significantly increased after infarction, and the increase was attenuated to a nonsignificant level by Ang-(1–7). Capillary density was diminished in infarcted rats, but did not differ between the Ang-(1–7) and saline-treated groups.

To confirm delivery of the peptide, Ang-(1–7) plasma levels were measured at the end of treatment. Intravenous infusion of Ang-(1–7) increased plasma levels of the peptide 40-fold compared with MI controls to 917.8 ±194.1 pmol/L (Table 1).

Hemodynamics
After 8 weeks of treatment, cardiac function was measured in vivo in anesthetized rats. As expected, cardiac function was significantly impaired in untreated MI rats compared with sham-operated rats. In contrast, in Ang-(1–7)–treated rats, none of these parameters were significantly deteriorated, except the systolic dP/dt (Figure 1A and 1E).

Coronary flow was measured ex vivo in a Langendorff setup. When compared with sham-operated hearts, baseline coronary flow was decreased in untreated MI rats, whereas in Ang-(1–7)–treated rats, baseline coronary flow was almost completely preserved (Figure 1F). Coronary endothelial function and maximal coronary flow were tested by a 2-minute infusion of 3 ×10⁻⁸ mol/L bradykinin and 10⁻⁵ mol/L adenosine, respectively. Bradykinin infusion evoked an increase in coronary flow in all 3 groups, but flow was still significantly lower in untreated infarcted hearts than in sham-operated hearts. Bradykinin-dependent flow in Ang-(1–7)–treated hearts was not significantly impaired. Maximal flow after adenosine infusion was significantly decreased in untreated MI rats, as well. In Ang-(1–7)–treated MI rats, the difference did not reach statistical significance (Table 2).

Endothelial Function
Endothelial dysfunction is a key feature in heart failure. To examine the effects of Ang-(1–7) treatment on this aspect of cardiac failure, we investigated endothelium-dependent relaxation in isolated aortic rings. Phenylephrine elicited similar contractile responses in all 3 groups (data not shown). The response of aortic rings from infarcted animals to the endothelium-dependent vasodi-

**TABLE 1. General Characteristics After 8 Weeks of Treatment**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI Control</th>
<th>MI Ang-(1–7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>432.9 ± 6.8</td>
<td>418.0 ± 6.1</td>
<td>414.0 ± 9.0</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>...</td>
<td>35.5 ± 2.2</td>
<td>29.6 ± 3.3</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>2.88 ± 0.08</td>
<td>3.46 ± 0.14*</td>
<td>3.42 ± 0.10*</td>
</tr>
<tr>
<td>Ang-(1–7), pmol/L</td>
<td>9.9 ± 1.9</td>
<td>22.9 ± 7.8</td>
<td>917.8 ± 194.1†</td>
</tr>
<tr>
<td>Capillary density, N/mm²</td>
<td>3104 ± 142</td>
<td>2531 ± 179*</td>
<td>2578 ± 176*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. BW indicates body weight; LVW/BW, left ventricular weight to body weight ratio; and Ang-(1–7), plasma concentration of angiotensin-(1–7).

"P < 0.05 vs sham; †P < 0.05 vs MI control.

![Image](http://circ.ahajournals.org/1549.png)

**Figure 1. Effects of myocardial infarction (MI) and Ang-(1–7) treatment on hemodynamic parameters. LVP indicates left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; MAP, mean arterial pressure; +dP/dt and –dP/dt, maximal rate of increase and decrease of ventricular pressure, respectively; and flow, baseline coronary flow. *P < 0.05 versus sham.

Coronary flow was measured ex vivo in a Langendorff setup. When compared with sham-operated hearts, baseline coronary flow was decreased in untreated MI rats, whereas in Ang-(1–7)–treated rats, baseline coronary flow was almost completely preserved (Figure 1F). Coronary endothelial function and maximal coronary flow were tested by a 2-minute infusion of 3 ×10⁻⁸ mol/L bradykinin and 10⁻⁵ mol/L adenosine, respectively. Bradykinin infusion evoked an increase in coronary flow in all 3 groups, but flow was still significantly lower in untreated infarcted hearts than in sham-operated hearts. Bradykinin-dependent flow in Ang-(1–7)–treated hearts was not significantly impaired. Maximal flow after adenosine infusion was significantly decreased in untreated MI rats, as well. In Ang-(1–7)–treated MI rats, the difference did not reach statistical significance (Table 2).

**TABLE 2. Ex Vivo Coronary Flow**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI Control</th>
<th>MI Ang-(1–7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>8.7 ± 0.6</td>
<td>6.8 ± 0.3*</td>
<td>8.2 ± 0.6</td>
</tr>
<tr>
<td>Bradykinin 3 ×10⁻⁸ mol/L</td>
<td>12.1 ± 0.6</td>
<td>9.9 ± 0.4*</td>
<td>11.1 ± 0.7</td>
</tr>
<tr>
<td>Adenosine 10⁻⁵ mol/L</td>
<td>14.0 ± 0.5</td>
<td>10.8 ± 0.5*</td>
<td>12.3 ± 0.8</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM in mL/min per gram ventricular weight. *P < 0.05.
Figure 2. Metacholine-dependent relaxation of phenylephrine (PE) precontracted aortic rings. *P<0.05 versus sham and versus Ang-(1–7).

Discussion

In the present study, the effects of intravenous infusion of Ang-(1–7) on the development of heart failure were examined in a rat coronary artery ligation model. We found that 8 weeks of Ang-(1–7) treatment prevented the deterioration of cardiac function, as shown by a 40% reduction in left ventricular end-diastolic pressure, an almost full preservation of coronary flow, and preserved aortic endothelial function. Although Ang-(1–7) has weak vasodilator activities, an increase in mean arterial pressure was found in the group infused with Ang-(1–7). Moreover, myocyte hypertrophy was attenuated by Ang-(1–7) infusion. Both results may be indicative of an intracardiac mode of action for Ang-(1–7).

A putative local effect of Ang-(1–7) would be in line with a previous study, in which 12 days of Ang-(1–7) infusion was found to inhibit restenosis after balloon-catheter injury of carotid arteries.

Interestingly, infusing Ang-(1–7) to levels obtained with ACE inhibition yields similar beneficial effects, including reduction of left ventricular end-diastolic pressure, preservation of aortic endothelial function, improvement of coronary flow, and reduction of myocyte hypertrophy. On the other hand, differences seem to exist between Ang-(1–7) infusion and ACE inhibition. ACE inhibitors fail to exhibit a positive effect on left ventricular systolic pressure and lower blood pressure even further, whereas Ang-(1–7) augments left ventricular systolic pressure and mean arterial pressure. In addition, Ang-(1–7), unlike ACE inhibitors, did not improve capillary density. The similarity between Ang-(1–7) infusion and ACE inhibitor treatment may be explained by the fact that ACE inhibitors increase Ang-(1–7) levels. Further, Ang-(1–7), like ACE inhibitors, potentiates bradykinin by acting as an ACE inhibitor, which may contribute to similar therapeutic effects of these compounds in cardiac failure. This study shows that Ang-(1–7) is an effective agent in the attenuation of the development of heart failure after MI.

Acknowledgment

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References

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