Chapter 2

Angiotensin-(1-7) attenuates the development of heart failure after myocardial infarction in rats


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Abstract

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 ACE 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 hr) or saline was started by minipump. After eight 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 and dP/dt, and coronary flow. Raising plasma levels 40-fold, angiotensin-(1-7) infusion attenuated this impairment to a non-significant 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.
**Introduction**

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 ACE inhibitor therapy.\(^1,2\)

Although ACE inhibitors were originally developed to suppress the formation of angiotensin (Ang) II, recent studies suggest that part of their beneficial effects in cardiovascular diseases may be attributed to the elevation of plasma Ang-(1-7) levels.\(^3\) Ang-(1-7) is a biologically active metabolite of Ang I and Ang II that is formed through cleavage by endopeptidases, and that 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, while having limited effects on the circulating amount of Ang II, increases Ang-(1-7) levels 10-25 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,\(^8\) and is a vasodilator in canine and porcine coronary arteries,\(^9,10\) either by blocking the AT1-receptor\(^11\) or by releasing nitric oxide and vasodilating prostaglandins via a 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 model.

**Methods**

**Experimental design**

The study was approved by the Animal Research Committee of the University of Groningen. Left coronary artery ligations were performed in 39 male Sprague-Dawley rats weighing 250-300 g (Harlan Zeist, The Netherlands).\(^14\) Peri-operative mortality was 49%. Two weeks after induction of myocardial infarction, rats were randomly allocated to intravenous infusion of either Ang-(1-7) (24 µg/kg per hr, \(n = 10\)) 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 under isoflurane anesthesia with a microtip pressure transducer,\(^15\) coronary flow was measured in a Langendorff set-up,\(^14\) endothelial function was tested in isolated aortic rings,\(^14\) and plasma Ang-(1-7) levels were measured by radioimmunoassay.\(^16\)

**Histology**

Mid-ventricular slices were processed for histochemical analysis. Infarct size was determined on picrosirisius red/fast green stained sections and was expressed as the percentage of scar length of total left ventricular circumference.\(^14\) 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 biotine-labeled Griffonia simplicifolia lectin I (GSL-I) and hematoxylin and expressed as the number of capillaries per mm². Myocyte cross-sectional area was measured on hematoxylin/eosine stained sections.

Statistical analysis
Data are presented as mean ± s.e.m. Statistical analysis between the groups was performed by one-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 in the three groups. Infarct size did not differ between the Ang-(1-7) and saline treated group, and averaged 33%. Left ventricular weight to body weight ratios were equally increased in both MI groups compared to sham-operated controls (+17%, p < 0.05). Myocyte cross-sectional area was significantly increased after infarction and the increase was attenuated to a non-significant 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) significantly increased plasma levels of the peptide 40-fold compared to MI control to 917.8 ± 194.1 pmol/L (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>General characteristics after 8 weeks of treatment</th>
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<tbody>
<tr>
<td></td>
<td>sham</td>
</tr>
<tr>
<td>BW (g)</td>
<td>432.9 ± 6.8</td>
</tr>
<tr>
<td>infarct size (%)</td>
<td>-</td>
</tr>
<tr>
<td>LVW/BW (mg/g)</td>
<td>2.88 ± 0.08</td>
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<tr>
<td>[Ang-(1-7)] (pmol/l)</td>
<td>9.9 ± 1.9</td>
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<tr>
<td>MCSA</td>
<td>341 ± 17</td>
</tr>
<tr>
<td>capillary density (N/mm²)</td>
<td>3104 ± 142</td>
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</tbody>
</table>

Results are expressed as mean ± s.e.m., BW: body weight, LVW/BW: left ventricular weight body weight ratio, [Ang-(1-7)]: plasma concentration of angiotensin-(1-7); MCSA: myocyte cross-sectional area * p < 0.05 vs. sham, † p < 0.05 vs. MI control.
**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 to sham-operated rats. In contrast, in Ang-(1-7) treated rats none of these parameters, were significantly deteriorated, except the systolic dP/dt (Figure 1).

Coronary flow was measured ex vivo in a Langendorff set-up. When compared to 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 1). Coronary endothelial function and maximal coronary flow were tested by a two-minute infusion of $3 \times 10^{-8}$ M bradykinin and $10^{-5}$ M adenosine, respectively. Bradykinin infusion evoked an increase in coronary flow in all three 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 compared to sham-operated rats, as well. In Ang-(1-7)-treated MI the difference did not reach statistical significance (Table 2).

![Figure 1](image)

**Figure 1 | Hemodynamic parameters.** Effects of myocardial infarction (MI) and Ang-(1-7) treatment on hemodynamic parameters. LVP: 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, flow: baseline coronary flow; * p < 0.05 vs. sham.
## 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 M</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 M</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 ± s.e.m. in ml/min per g ventricular weight, *p < 0.05.

### 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 three groups (data not shown). The response to the endothelium-dependent vasodilator metacholine was markedly decreased in rings from infarcted animals to 58.4% ± 11.7% (p < 0.05) of sham-operated rats. In Ang-(1-7)-treated rats, however, metacholine-induced relaxation was identical to sham rats (p < 0.05 vs. MI control). (Figure 2) The relaxation in response to the endothelium-independent vasodilator NaNO₂ (10⁻² M) was equal in the three groups (data not shown).

![Figure 2 | Endothelial function. Metacholine-dependent relaxation of phenylephrine (PE) precontracted aortic rings; *p < 0.05 vs. sham and p < 0.05 vs. 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 LVEDP, an almost full preservation of coronary flow and a preserved aortic endothelial function. Although Ang-(1-7) has weak vasodilator activities,\textsuperscript{9,10} an increase in MAP was found in the group infused with Ang-(1-7). Moreover, myocyte hypertrophy was attenuated by Ang-(1-7) infusion. Both may be indicative of an intra-cardiac 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 Ang-(1–7) infusion was found to inhibit restenosis following balloon-catheter injury of carotid arteries.\textsuperscript{17}

Interestingly, infusing Ang-(1-7) to levels obtained with ACE inhibition\textsuperscript{6} yields similar beneficial effects: Reduction of LVEDP,\textsuperscript{15} preservation of aortic endothelial function,\textsuperscript{14,18} improvement of coronary flow,\textsuperscript{14} and reduction of myocyte hypertrophy.\textsuperscript{19} On the other hand, differences appear to exist between Ang-(1-7) infusion and ACE inhibition, as ACE inhibitors fail to exhibit a positive effect on LVP and lower blood pressure even further,\textsuperscript{20} whereas Ang-(1-7) augmented LVP and MAP. In addition, Ang-(1-7), unlike ACE inhibitors,\textsuperscript{20} 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,\textsuperscript{7} which may contribute to similar therapeutic effects of these compounds in cardiac failure.

In conclusion, this study shows that Ang-(1-7) is an effective agent to attenuate the development of heart failure after myocardial infarction.

**Acknowledgements**

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Chapter 2

References


Addendum (Circulation 2002;106:e147)

To the Editor:
We read with interest the report by Loot et al1 and the accompanying editorial2 on the possible role of angiotensin-(1–7) in heart failure and cardiovascular regulation in general. It is also commonly stated (and repeated by Ferrario3) that angiotensin converting-enzyme (ACE) inhibitors increase angiotensin-(1–7) and that angiotensin-(1–7) may contribute to the actions of ACE inhibitors. We believe that neither of these views may be valid.

Although it is true that angiotensin-(1–7) may have pharmacological and even physiological effects in experimental animals, the same cannot be said for humans. We have shown that even high concentrations of angiotensin-(1–7) given by brachial arterial infusion do not cause forearm vasodilatation in patients with chronic heart failure.3 Neither were we able to show any potentiation of the vasodilator action of bradykinin, as reported in animal studies and discussed by Ferrario.2 The only other study in humans that we know of showed that high doses of angiotensin-(1–7) actually had a pressor effect, rather than the vasodepressor effect predicted on the basis of experiments in animals.4

The view that ACE inhibitors increase plasma angiotensin-(1–7) concentrations is based on 1 study in humans.5 Although that study showed that angiotensin-(1–7) levels were increased by a last dose of captopril at the end of 6 months of treatment with captopril, even that increased level was not as high as the pretreatment level, which was itself unaffected by the first dose of captopril. This was in marked contrast to the effects of captopril on angiotensin I levels.

Consequently, we would caution readers that the role of angiotensin-(1–7) both in cardiovascular regulation and in the action of ACE inhibitors is far from clear in humans. What evidence there is suggests that angiotensin-(1–7) may be physiologically and pathophysiologically irrelevant.

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References
Response

We fully agree with McMurray and Davie that the effects of angiotensin (Ang)-(1–7) in humans are poorly documented. Nevertheless, it seems premature to conclude only from their study that Ang-(1–7) is devoid of effects in humans. In this particular study in heart failure patients on chronic angiotensin-converting enzyme (ACE) inhibitor therapy, they observed that Ang-(1–7) does not affect the vasodilator actions of bradykinin in human forearm. These results may be explained by the fact that the potentiating effect of Ang-(1–7) on bradykinin is mediated solely through ACE inhibition. In contrast, in similar experiments in healthy, untreated volunteers, Ueda et al3 did show a clear potentiation of bradykinin-induced forearm vasodilatation by Ang-(1–7). In addition, we have previously shown that Ang-(1–7) inhibits Ang II-induced vasoconstriction in isolated human vessels.

Moreover, the above mentioned studies all focus on acute hemodynamic effects of Ang-(1–7). Our present study, however, shows that chronic infusion of the peptide is effective in preventing heart failure and preserves endothelial function. Correspondingly, no one will dispute the long-term benefits of ACE inhibitors in heart failure, but their short-term hemodynamic effects are limited.

In conclusion, the current (sparse) literature rather supports a role for Ang-(1–7) in regulation of cardiovascular function in humans. Further studies, in particular those using long-term administration of Ang-(1–7) in patients, will be needed to provide conclusive answers.

References
