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Angiotensin-Converting Enzyme Inhibition Restores Hepatocyte Growth Factor Production in Patients With Congestive Heart Failure

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Abstract—Endothelium-dependent vasodilation is impaired in patients with congestive heart failure. For vascular endothelium, hepatocyte growth factor (HGF) is one of the most potent and specific growth factors, which acts protectively against endothelial dysfunction. HGF production is downregulated by angiotensin II (Ang II) in vitro. We hypothesized that HGF production is impaired as the result of increased Ang II in patients with congestive heart failure, and that if so, the impaired production should be restored with angiotensin-converting enzyme inhibitors (ACE-I). We studied 16 patients with congestive heart failure caused by previous anterior myocardial infarction in whom left ventricular ejection fraction was 35±8% (mean±SD). Before and 4 weeks after the treatment with ACE-I, blood samples were collected to measure the levels of HGF, Ang II, and brain natriuretic peptide as a biochemical marker for severity of heart failure. We also studied 5 control subjects, in whom heparin increased HGF production to 48±5-fold. However, in patients with heart failure, HGF response to heparin was significantly attenuated (24±5-fold, P<0.05 vs control). Therapy with ACE-I decreased the levels of Ang II and brain natriuretic peptide and restored HGF production in response to heparin by 43±7-fold, comparable to the control response. In conclusion, impaired HGF production was restored after the treatment with ACE-I probably by the mechanism of Ang II suppression. This novel effect of ACE-I may contribute to the clinical improvement in patients with heart failure and thereby may have an important therapeutic implication. (Hypertension. 1999;33:1374-1378.)

Key Words: angiotensin | endothelium | growth factors | heart failure | natriuretic peptides

Hepatocyte growth factor (HGF) is a disulfide-linked heterodimeric molecule composed of a 69-kDa kringle-containing α-chain and a 34-kDa β-chain. The HGF system (HGF and its receptor c-Met) has been found in various tissues and is considered to integrate complex biological processes. This system is currently being given increasing attention in cardiovascular diseases. Of importance, HGF has been characterized as one of the most potent growth factors specific to endothelium, which may act protectively against endothelial dysfunction.

In patients with congestive heart failure (CHF), endothelium-dependent vasodilation is attenuated. Endothelial dysfunction observed in CHF patients is related to symptoms and exercise intolerance and thereby is pathophysiologically important. Angiotensin-converting enzyme inhibitors (ACE-I) improve the vasodilatory response in patients with CHF in several months, which suggests that there may be an angiotensin II (Ang II)–mediated mechanism for endothelial impairment. Possibly, this mechanism would be related to deficiency of a vascular modulator of HGF, because Ang II is known to suppress HGF production in vascular cells in vitro.

We found in the present study that impaired HGF production was restored with ACE-I treatment through Ang II inhibition in patients with CHF. This finding provides a novel therapeutic implication of ACE-I–modulating growth factors in cardiovascular diseases.

Methods

Study Population
Sixteen patients with CHF, clinically stable without acute decompensations, were studied (13 men and 3 women). Their age averaged 64±9 (mean±SD) years, and mean body weight was 62±12 kg. Their clinical profiles are summarized in Table 1. All patients had left ventricular dysfunction caused by previous anterior myocardial infarction. Nine patients were in New York Heart Association functional class II and the remaining 7 patients were in functional class III. No patients had liver, kidney, or lung dysfunction. Four patients had a mild degree of diabetes mellitus and 5 patients had hypertension. All patients were receiving digoxin and diuretics, and 11 patients were receiving calcium antagonists and/or nitrates for postinfarction angina pectoris. However, no patients had ever been treated with ACE-I.

The control group included 5 subjects admitted for the workup of chest pain (3 men and 2 women, 58±12 [mean±SD] years old).
Cardiac catheterization revealed that all control subjects had normal cardiac function and angiographically normal coronary arteries. None of the control subjects received medication.

The study was approved by the institutional ethics committee, and all patients and control subjects gave their written informed consent. The procedures followed were in accordance with institutional guidelines.

**Study Design**

During this study, no drugs other than ACE-I were given newly to the CHF patients. Before starting ACE-I treatment, antecubital venous blood was drawn with the patient in a supine position after an overnight fast to measure levels of HGF, Ang II, and brain natriuretic peptide (BNP). Blood was collected again after intravenous bolus injection of heparin (5000 U), which is known to stimulate HGF production.19,20 Blood was centrifuged immediately at 4°C and the sample was stored at −80°C until assay. These procedures were repeated 28 ± 3 (mean ± SD) days after ACE-I treatment in patients with CHF.

Left ventriculography was performed to determine left ventricular end-systolic and end-diastolic volume index and ejection fraction by use of the ANCHOR analysis system (Siemens-Elema).

**Neurohormonal Measurements**

HGF levels were determined with specific enzyme-linked immunosorbent assay kits (Otsuka Assay Laboratories). Microtiter plates coated with anti-HGF murine monoclonal antibody were incubated with standard HGF or serum samples, and anti-HGF rabbit polyclonal antibody was added. After the addition first of anti-rabbit goat immunoglobulin G-peroxidase conjugate and then clonal antibody was added. After the addition first of anti-rabbit goat immunoglobulin G-peroxidase conjugate and then clonal antibody was added. After the addition first of anti-rabbit goat immunoglobulin G-peroxidase conjugate and then clonal antibody was added. After the addition first of anti-rabbit goat immunoglobulin G-peroxidase conjugate and then clonal antibody was added.

BNP levels were measured with the specific immunoradiometric assay kits (Shionogi Co), as previously reported.22 The sensitivity of this BNP kit was 2 pg/mL (≈0.5 pmol/L). BNP has been considered as a biochemical marker for ventricular dysfunction after myocardial infarction.22–24 Ang II levels were measured by radioimmunoassay (Osaka Kessei Laboratories).

**Statistical Analysis**

Data are presented as mean ± SD. For comparisons within a group over time or comparisons among 3 groups, ANOVA for repeated measures was applied, followed by a Student-Newman-Keuls post hoc test. In all cases, differences were considered significant at P < 0.05.

**Results**

In patients with CHF, left ventricular ejection fraction was 35 ± 8%, end-diastolic volume index was 94 ± 23 mL/m², and end-diastolic pressure was 20 ± 6 mm Hg. All patients remained stable during the entire study.

**HGF Response to Heparin in Control Subjects**

In 5 control subjects, HGF levels at baseline (before heparin) were 2.4 ± 0.8 pmol/L (Table 2). Figure 1 shows the fold increase in HGF production in response to intravenous administration of 5000 U heparin. After heparin injection, HGF levels increased remarkably in 10 minutes, remained stable over the next 10 minutes, and then gradually decreased. Thus in all subsequent protocols, HGF response was assessed 10 to 20 minutes after heparin injection, when steady-state HGF levels should be maintained.

**Attenuated HGF Production in CHF Patients**

In 16 patients with CHF, HGF levels at baseline were comparable to those in control subjects (Table 2). However, as shown in Figure 2, HGF production in response to heparin was significantly attenuated (CHF 24 ± 5 versus control 48 ± 5 fold, P < 0.05).

**TABLE 1. Clinical Profiles of Patients With CHF Caused by Previous Anterior Myocardial Infarction**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Gender</th>
<th>BW, kg</th>
<th>NYHA</th>
<th>Q in ECG</th>
<th>EF, %</th>
<th>EDVI, mL/m²</th>
<th>ACE-I, daily</th>
<th>Tchol, mmol/L</th>
<th>BP, mm Hg</th>
<th>FBS, mmol/L</th>
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<tr>
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<td>67</td>
<td>F</td>
<td>51</td>
<td>2</td>
<td>V1–4</td>
<td>42</td>
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<td>5.17</td>
<td>150/70</td>
<td>6.10*</td>
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<tr>
<td>2</td>
<td>77</td>
<td>M</td>
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<td>V2–4</td>
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<td>4.61</td>
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<tr>
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<td>77</td>
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<td>V1–5</td>
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<td>130/80</td>
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<td>5</td>
<td>63</td>
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<td>59</td>
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<tr>
<td>7</td>
<td>48</td>
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<td>67</td>
<td>3</td>
<td>V1–5</td>
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<td>130/80</td>
<td>4.66</td>
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<td>I, aVL, V1–4</td>
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<tr>
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<td>2</td>
<td>I, aVL, V1–5</td>
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<td>90</td>
<td>Enalapril 5 mg</td>
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<td>V1–4</td>
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<td>53</td>
<td>3</td>
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<td>V1–6</td>
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<td>102</td>
<td>Impidapril 5 mg</td>
<td>5.68</td>
<td>100/60</td>
<td>6.21</td>
</tr>
</tbody>
</table>

BW indicates body weight; NYHA, New York Heart Association classification; ECG, electrocardiography; EF, left ventricular ejection fraction; EDVI, left ventricular end-diastolic volume index; ACE-I, angiotensin-converting enzyme inhibitors; Tchol, total cholesterol; BP, blood pressure (before ACE-I treatment); and FBS, fasting blood sugar.

*Treated with 2.5 mg of glibenclamide per day.
Restored HGF Production With ACE-I

Four-week ACE-I treatment decreased Ang II (from 72±16 to 25±8 pmol/L, P<0.05) and BNP levels (from 85±29 to 39±11 pmol/L, P<0.05), in patients with CHF, as shown in Figure 3. In addition, ACE-I decreased systolic (from 130±16 to 110±15 mm Hg, P<0.01) and diastolic blood pressure (from 76±7 to 66±7 mm Hg, P<0.01), respectively.

ACE-I treatment did not alter HGF levels at baseline (P=NS versus before ACE-I) in patients with CHF (Table 2). However, as shown in Figure 2, fold increase in HGF production in response to heparin was restored by 43±7-fold (P<0.05 versus before ACE-I), comparable to the control response. This improvement of HGF production was not observed during the first 48 to 72 hours of the treatment (24±8-fold increase after heparin, n=10).

The subanalysis of HGF production in 13 male patients is also summarized in Table 2.

Discussion

The major finding of this study is that impaired HGF production was restored with ACE-I treatment in association with Ang II inhibition, which indicates a novel aspect of ACE-I therapy modulating growth factors in patients with CHF.

HGF is synthesized in large amount in the liver and secreted into the blood. HGF exerts multipotent actions through its receptor c-Met in various target organs that include the heart and vessels. Intriguingly, HGF was recently characterized as one of the most potent mitogens specific to endothelial cells and may contribute to the vascular protection or repair. HGF was also found to have a vasodilatory effect in conscious animals. Thus HGF, a potent vascular modulator with cardioprotective activity, is now emerging as a potential therapeutic target in cardiovascular diseases. Because a recent in vitro study has demonstrated that Ang II is a strong suppressor of HGF, we hypothesized that HGF production would be suppressed in patients with CHF where rennin-angiotensin system is activated.

Ang II is a major contributor to the pathophysiology of heart failure, increasing vascular resistance, enhancing sympathetic activity, or promoting water retention. In addition, Ang II is rather important at the tissue level as a modulation of remodeling of the heart and vessels. Thus interference with the renin-angiotensin system by ACE-I has been shown to produce significant hemodynamic, symptomatic, and prognostic benefits in patients with CHF. In the present study, ACE-I decreased plasma levels of BNP (Figure 3), a sensitive biochemical marker for the severity of heart failure. The decrease in BNP after ACE-I treatment was reported also in the previous studies for patients with acute myocardial infarction.

In the present study, we assessed HGF increase in response to its stimulant, heparin. This response may reflect the capability or functional reserve of the vascular system to produce HGF and potentially may be related to the severity of endothelial dysfunction in pathophysiological conditions such as CHF. As shown in Figure 2, we demonstrated that HGF production in response to heparin was significantly impaired in CHF patients with elevated Ang II levels. The attenuated HGF production was completely restored by ACE-I to the control level. While ACE-I produces a variety of beneficial effects in cardiovascular diseases, the present finding provides another implication of ACE-I therapy modulating growth factors such as HGF.

| TABLE 2. Levels of HGF Before (Baseline) and After Intravenous Administration of Heparin |
|-------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                              | CHF, All (n=16) |                |                |                |                |                |
|                              | Before ACE-I    | After ACE-I    | Before ACE-I   | After ACE-I    | Before ACE-I   | After ACE-I    |
| Baseline                      | 2.4±0.8         | 3.3±1.4        | 3.0±1.4        | 3.4±1.5        | 2.9±1.5        |
| After heparin                 | 121.8±21.8      | 74.3±21.5*     | 122.2±36.2†    | 74.3±23.4*     | 121.2±40.0†    |

*P<0.05 vs control, †P<0.01 vs before ACE-I.

Figure 1. HGF production in response to heparin (5000 U IV injection) in control subjects (n=5). Points show mean±SD data of the fold increase in HGF after heparin compared with baseline (by definition, 1.0-fold before heparin). *P<0.05 vs baseline by ANOVA.

Figure 2. Effect of ACE-I on HGF production in patients with CHF (n=16). Bars show mean±SD data of the fold increase in HGF after heparin. *P<0.05 by ANOVA. HGF production in control subjects (n=5) is also presented.
The restoration of HGF production was realized ∼4 weeks after the treatment with ACE-I, which may precede the improvement in clinical status of heart failure usually obtained many weeks after ACE-I treatment. As an explanation for this sequence, we would speculate that Ang II–related endothelial dysfunction or myocardial damage in CHF would be ameliorated by restored HGF that has potential activity for vascular and cardiac protection.

The present study has several limitations. First, this study was performed in a limited number of patients treated with different ACE-I. Therefore the obtained results might not be considered to be conclusive. Second, the mechanism of the impaired HGF production with ACE-I remains unknown because this was not a mechanistic study. As a potential mechanism, we would speculate that the tissue Ang II may be effectively suppressed even with a relatively low dose of ACE-I as well as circulating Ang II (Figure 3). However, a possibility remains that HGF restoration might have occurred secondary to the hemodynamic improvement. Taken together with the role of an ACE-independent pathway (the chymase pathway) in humans, further studies are needed to determine the exact mechanisms.

In conclusion, we have found that ACE-I therapy restores the attenuated HGF production in patients with CHF. When taking the recently reported biological actions of HGF into account, the restoration of HGF production may play a role in mediating functional repair processes induced by ACE-I therapy, which may add a new therapeutic implication to CHF treatment.

Acknowledgments

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References


