Serum Hepatocyte Growth Factor Predicts Ventricular Remodeling Following Myocardial Infarction

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Hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) stimulate endothelial cell proliferation and induce angiogenesis, but the timing and significance of their release in patients with acute myocardial infarction (AMI) are unknown in relation to future left ventricular remodeling. Venous blood samples were obtained at admission and up to 3 weeks later in 40 patients with AMI and in 40 age- and sex-matched control subjects. Blood samples were also taken from the coronary sinus (CS) in 20 patients on day 7 following AMI. Left ventricular end-diastolic volume in the subacute (1 week) and chronic (3 months) phases was assessed by left ventriculography to identify the remodeling group (n=15), which was defined as an increase in left ventricular end-diastolic volume index ≥5 ml/m² relative to the baseline value. Serum HGF and VEGF concentrations were higher in newly admitted patients with AMI than in the controls (HGF, 0.33±0.09 vs 0.24±0.08 ng/ml, p<0.01; VEGF, 92.2±43.1 vs 67.2±29.8 pg/ml, p<0.01), peaking on day 7 (HGF, 0.41±0.12; VEGF, 161.7±76.9), and gradually decreasing between days 14 and 21. The HGF concentration in the CS did not differ from the concentration in the periphery, but the VEGF concentration was significantly more abundant in the CS than in the peripheral sample on day 7 (p<0.05). The serum HGF concentration on day 7 was higher in the remodeling group than in the nonremodeling group (0.47±0.13 vs 0.36±0.09 ng/ml, p<0.01), but there was no difference between the groups on admission, day 14 and day 21. The serum VEGF concentration did not differ between the remodeling and nonremodeling groups at any time. Thus, the serum HGF concentration on day 7 after AMI is mostly from noncardiac sources and predicts left ventricular remodeling.

Key Words: Hepatocyte growth factor; Myocardial infarction; Vascular endothelial growth factor; Ventricular remodeling

Methods

Patients

We studied 40 consecutive patients (31 men; age, 65.2±11.6 years) who presented with their first AMI within 24 h of symptom onset and whose infarct-related coronary artery was the left anterior descending artery. They also underwent successful percutaneous transluminal coronary angioplasty; that is, repeat coronary angiography 3 months after onset of AMI demonstrated no significant coronary restenosis. AMI was diagnosed based on typical chest pain lasting >30 min with ST-T segment elevation >0.2 mV in 2 or more contiguous leads on a standard 12-lead electrocardiogram and an increased plasma creatine kinase concentration representing at least twice the upper limit of normal. The control group consisted of 40 age- and sex-matched patients (31 men; age, 65.3±9.4 years) with atypical chest pain who proved to have angiographically normal coronary arteries.

In all AMI patients, angiotensin-converting enzyme inhibitors were administered routinely from the 2nd hospital day onward. Patients with the following disorders were excluded: malignant disease, inflammatory disease, and hepatic or renal failure. All patients gave their written informed consent prior to participation in the study. The institutional committee on human research approved the study protocol.
The concentration of HGF on day 21 remained higher than the control, peaked on day 7, and gradually decreased between days 14 and 21. The serum concentration of VEGF was elevated at the time of admission, peaked on day 7, and gradually decreased between days 14 and 21. The serum VEGF concentration on day 21 was similar to the control value.

**Blood Samples**

Venous blood samples were obtained immediately before the administration of heparin at the time of admission, and on days 7, 14, and 21 for the patients with AMI. Clotted cellular elements were removed by centrifugation at 4°C (2,000 G, 10 min), and the serum samples were stored at –70°C until the time of HGF and VEGF assays. We also collected peripheral blood samples from the AMI patients at the time of admission and every 4 h until maximum plasma creatine kinase (CK) concentration was determined.

To examine whether HGF and VEGF in serum is primarily secreted from the heart in patients with AMI, blood samples were also taken from the coronary sinus (CS) before administration of heparin in 20 patients on day 7 following AMI and from 20 control subjects, using a coronary sinus catheter (6F; Goodman, Nagoya, Japan). These subjects were randomly selected.

**Biochemical Analysis**

Serum HGF and VEGF concentrations were measured by enzyme-linked immunosorbent assay (HGF, Otsuka Assay Laboratories, Tokyo, Japan; VEGF, Immuno-Biological Laboratories, Gunma, Japan). Sensitivities of the HGF and VEGF kits were 0.010 ng/ml and 15.6 pg/ml, respectively.

**Right-Sided Cardiac Catheterization**

Right-sided cardiac catheterization using a 7F thermodilution catheter (Nihon Kohden, Tokyo, Japan) was performed in all patients for measurement of pulmonary capillary wedge pressure (PCWP) and cardiac output immediately after successful percutaneous transluminal coronary angioplasty, and also in the control subjects. Cardiac index was then calculated as the ratio of cardiac output to body surface area.

**Left Ventriculography**

Angiographic evaluation of the left ventricular volume was performed by a cardiologist unaware of the results of the serum HGF and VEGF assays. Ventricular silhouettes in a 30° right anterior oblique projection were digitized with a ventricular analysis system (Integris H3000; Philips Medical Systems, Tokyo, Japan). Using the area–length method, the left ventricular end-diastolic volume index and ejection fraction were calculated in the subacute phase (1 week) and chronic phase (3 months). The remodeling group (n=15) was defined as showing an increase in left ventricular end-diastolic volume index ≥5 ml/m² in the chronic phase relative to the baseline value in the subacute phase.

**Statistical Analysis**

All results are expressed as mean±standard deviation. Comparison between 2 groups was by Student’s t test. Differences in ratios were compared using the chi-squared test. The statistical significance of changes in the time course of serum HGF and VEGF concentrations were evaluated by repeated measure analysis of variance and the Bonferroni/Dunn test. Simple linear regression was used for assessment of the relation between 2 variables. A value of p<0.05 was considered to indicate statistical significance.

**Results**

**Patient Characteristics**

Table 1 shows the clinical characteristics of each group. No significant differences presented in age, gender ratio, incidence of smoking, hypertension, diabetes mellitus, or total cholesterol and triglyceride concentrations between patients with AMI and control subjects. The high-density lipoprotein-cholesterol concentration was lower in patients with AMI than in control subjects.

**Serial Changes in Serum Concentrations of HGF and VEGF During AMI**

Serum concentrations of both HGF and VEGF were higher at the time of admission in patients with AMI than in control subjects (HGF, 0.33±0.09 vs 0.24±0.8 ng/ml, p<0.01; VEGF, 92.2±43.1 vs 67.2±29.8 pg/ml, p<0.01), peaking on day 7 (HGF, 0.41±0.12 ng/ml; VEGF, 161.7±

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Table 1 Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>AMI group</th>
<th>Control group</th>
<th>p value</th>
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<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>31/9</td>
<td>–</td>
</tr>
<tr>
<td>M/F</td>
<td>31/9</td>
<td>31/9</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.2 ± 11.6</td>
<td>65.3 ± 9.4</td>
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<tr>
<td>Smoking</td>
<td>23 (57.5%)</td>
<td>15 (37.5%)</td>
<td>0.073</td>
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<tr>
<td>Hypertension</td>
<td>13 (32.5%)</td>
<td>10 (25.0%)</td>
<td>0.458</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>10 (25.0%)</td>
<td>8 (20.0%)</td>
<td>0.592</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>204.5 ± 49.4</td>
<td>192.3 ± 38.7</td>
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<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>43.7 ± 10.3</td>
<td>48.5 ± 10.3</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>134.0 ± 71.2</td>
<td>130.2 ± 58.0</td>
<td>0.792</td>
</tr>
</tbody>
</table>

AMI, acute myocardial infarction; HDL, high-density lipoprotein.
76.9 pg/ml) and gradually decreasing from day 14 to just above the control values on day 21 (day 14: HGF, 0.36±0.10 ng/ml, VEGF, 126.2±59.6 pg/ml; day 21: HGF, 0.29±0.09 ng/ml, VEGF, 80.4±32.6 pg/ml). The HGF concentration on day 21 remained higher than control values (Figs 1, 2).

**HGF and VEGF Concentrations in the CS vs Peripheral Circulation**

The concentration of serum HGF (ng/ml) in the CS was not different from that in the periphery on day 7 in the 20 selected patients with AMI, including 8 patients defined as the remodeling group (CS, 0.40±0.11 vs peripheral sample, 0.39±0.11, NS), but the serum VEGF concentration (pg/ml) was significantly higher in the CS than in the peripheral sample on day 7 in the same 20 patients (CS, 169.1±78.4 vs peripheral vessel: 164.6±76.1, p<0.05). In control patients, both serum HGF and VEGF concentrations were similar in the CS and the periphery (HGF: CS, 0.25±0.07 vs peripheral vessel, 0.25±0.08, NS; VEGF: CS, 67.2±31.1 vs peripheral vessel, 66.4±31.2, NS).

**Roles of HGF and VEGF in Left Ventricular Remodeling Following Myocardial Infarction**

Table 2 compares the patients with and without left ventricular remodeling. The groups were comparable with respect to gender distribution, age, infract-related site of the coronary artery, maximum plasma CK concentration, PCWP, and cardiac index. The left ventricular ejection fraction (LVEF) tended to be lower in the remodeling group than in the nonremodeling group, with marginal significance. Serum HGF concentration on day 7 was higher in the remodeling group than in the nonremodeling group, but on days 1, 14, and 21 HGF did not differ between the 2 groups (Table 3). Serum VEGF concentrations did not differ between the remodeling and nonremodeling groups at any time point (Table 3). In addition, the serum HGF concentration on day 7 did not correlate with the maximum plasma CK concentration (r=-0.110, p=0.502), PCWP (r=0.020, p=0.902), cardiac index (r=0.110, p=0.500), or LVEF (r=0.051, p=0.756).

**Discussion**

In the present study both serum HGF and VEGF concentrations on admission in patients with AMI were higher than in controls, peaking on day 7, and gradually decreasing between days 14 and 21. These results are similar to our previous findings, indicating that the increase in the HGF and VEGF concentrations on day 7 of AMI is part of the recovery response to tissue damage in the infarcted myocardium resulting from acute inflammation.

**Ventricular Remodeling and HGF in AMI**

To investigate the significance of HGF and VEGF production in patients with AMI, we focused on the relationship between the serum concentrations and subsequent left ventricular remodeling. The remodeling phenomenon is an important determinant of the patient’s prognosis and of the incidence of heart failure after AMI. We found that HGF on day 7 was higher in the remodeling group than in the nonremodeling group, but did not differ between the 2 groups on admission and on days 14 and 21. The differences might reflect HGF interaction in the wound-healing process associated with inflammation after AMI, because production of HGF is stimulated by inflammatory cytokines, such as interleukin-1 and tumor necrosis factor-α, and it exerts a cytoprotective effect against ischemic injury. Further, HGF correlates with C-reactive protein concentration in patients with AMI. In other words, the more intense acute inflammation after AMI is associated with a greater concentration of circulating HGF, and this response may be a compensatory mechanism that protect cardiomyocytes against inflammation. We also demonstrated that serum HGF concentration on day 7 did not correlate with the maximum plasma CK concentration, PCWP, cardiac index, or the LVEF, which suggests that the effect of HGF on remodeling is independent of the extent of damage in left ventricular function that has been considered to be one of the main causes of left ventricular remodeling. It is not surprising that the serum HGF concentration correlates with the C-reactive protein concentration, but not with the maximum plasma CK concentration, because the correlation between serum peak C-reactive protein and CK concentration is very weak or not significant in patients with successful reperfusion.

Our findings are similar to those of Zhu et al who could not find significant differences in serum HGF concentrations between remodeling and nonremodeling groups in the course of AMI, except for a marginal difference on day 7. Those authors also found that in patients with AMI, the HGF concentration in the medium from cultured monocytes obtained from patients on days 1 and 7 in the remodeling group were significantly higher than in the medium.
from monocytes from the nonremodeling group. This suggests that local HGF production by cardiac monocytes is involved in ventricular remodeling after AMI. In contrast, another report linked enhanced secretion of cardiac HGF from the infarcted region to both attenuation of ventricular remodeling and improvement in cardiac function in patients with AMI. In that study, HGF concentration was measured only at 1 month after the onset of AMI, and subjects included both patients treated with angiotensin-converting enzyme inhibitors and patients not so treated; these drugs can affect HGF concentration. Such considerations make comparisons between the studies difficult. In contrast to results concerning HGF, the VEGF concentration on any day did not differ between our remodeling and nonremodeling groups.

In the present study, we used only the change in the left ventricular end-diastolic volume index as an indicator of the magnitude of left ventricular remodeling in patients with AMI. A previous study has shown that plasma brain natriuretic peptide concentration, an acceptable indicator of ventricular remodeling after AMI, was higher in the remodeling group defined by an increase in left ventricular end-diastolic volume index ≥5 ml/m² relative to the baseline value and correlated positively with an increase in left ventricular end-diastolic volume index from the acute to chronic phase in patients with AMI. Therefore, we believe that the definition of left ventricular remodeling in the present study reflects its true magnitude.

Cardiac Secretion of HGF and VEGF in AMI

The concentration of HGF in the CS was similar to that in peripheral blood on day 7 in patients with AMI, which indicated that the increased amount of HGF in the serum from patients with AMI is secreted from not only the heart but also other organs. It has been suggested that HGF is produced and released by various organs including the heart, lung, liver, kidney, and brain. In rats with myocardial ischemia followed by reperfusion, mRNA encoding c-met, the high-affinity HGF receptor, was up-regulated in the heart, but not in the kidney, liver, lung, or spleen; however, HGF itself was up-regulated in all of these organs. The results of previous basic studies are consistent with the notion that HGF acts via endocrine as well as autocrine and paracrine mechanisms. In patients with AMI, increased HGF activity via the endocrine mechanism might impact on highly expressed c-met in the heart and play an important role in recovery from inflammation.

In contrast to the HGF concentration in the CS, the concentration of VEGF was higher than that in peripheral blood on day 7 of AMI, indicating that VEGF found in serum may be secreted primarily from the heart in patients with AMI. In a previous study of rats with myocardial infarction, an initial rapid rise in VEGF and VEGF receptor (flk-1, flt-1) mRNA expression was observed throughout the heart whereas VEGF expression in the infarct and surrounding area increased gradually over 7 days. Human hearts studied after AMI showed strong expression of VEGF in smooth muscle cells and macrophages surrounding the infarct 3–14 days after AMI. Those findings support our clinical results. However, Kranz et al recently reported that the serum VEGF concentration was similar in samples taken from the CS which disagrees with our results. In their study only 6 patients’ samples were taken simultaneously from the CS, and they did not report whether or not heparin, which can reduce VEGF concentration, was used before cannulation; this impedes analysis of the differences in results between studies.

Recently, it was reported that the release of VEGF from platelets might affect the serum VEGF concentration, but not citrated plasma VEGF concentration during coagulation in vitro. However, one recent study has shown that the serum VEGF concentration is not affected by storage at 4°C prior to centrifugation for up to 48 h. In a preliminary study in our laboratory, the serum VEGF concentration correlated with the plasma VEGF concentration in 12 healthy subjects. Therefore, it is unlikely in this study that the increased serum concentration of VEGF reflects its from platelets in vitro.

Study Limitations

First, all patients with AMI in this study underwent successful percutaneous transluminal coronary angioplasty; we did not evaluate changes in serum HGF or VEGF concentrations in patients who did not undergo angioplasty. Second, we compared concentrations of HGF and VEGF at only 2 sites, the heart (CS) and a peripheral vein. Thus, the main sources of HGF and VEGF in AMI remain uncertain. Third, the remodeling group was defined by an increase in left ventricular end-diastolic volume index in the chronic phase relative to the value in the subacute phase (1 week after the onset of AMI) as opposed to the acute phase; we did not routinely perform left ventriculography on day 1 after the onset of AMI. Further clinical studies are necessary to clarify the precise roles of HGF and VEGF in patients with AMI.

Conclusions

In the present study, serum HGF and VEGF concentrations on admission in patients with AMI were higher than control values and peaked on day 7. In addition, the serum HGF concentration in the CS did not differ from concentrations in the periphery, whereas the VEGF concentration was significantly more abundant in the CS than in the peripheral sample on day 7. The HGF concentration on day 7 was higher in the remodeling group than in the nonremodeling group, but did not differ between the 2 groups on the other sampling days, nor did VEGF concentrations differ at any time point.

These results suggest that the serum HGF concentration on day 7 after AMI arises mostly from noncardiac sources and predicts left ventricular remodeling.

References

HGF and Ventricular Remodeling in AMI


