Hepatocyte growth factor (HGF) is a well-known powerful proliferative factor of vascular endothelial cells and it has been reported that plasma HGF concentrations are increased in acute myocardial infarction (AMI), although the mechanisms are not yet well delineated. Serum HGF levels and C-reactive protein (CRP) were measured in 22 patients with unstable angina pectoris (UAP) (15 males, 7 females; class IIb or IIIb of the Braunwald classification), 60 patients with AMI (37 males, 23 females; average time from the onset of symptoms to admission 4.6±0.7h, range, 0.5–12h), and 20 normal subjects. Immediate angioplasties were performed in 51 patients with AMI, and the time course of the HGF levels were measured in 31 patients among them. Heparin dramatically increased the HGF level and it declined to the normal range 18h after heparin injection. Blood samples were taken before heparin treatment, or at least 24h after. Serum HGF levels on admission was significantly increased in UAP (mean±SE: 0.30±0.03 ng/ml, p<0.01), and AMI (0.27±0.02 ng/ml, p<0.01) compared with the normal subjects (0.19±0.01 ng/ml). Even in the early stage (within 3h of onset of symptoms to admission, average time was 1.8±0.1h), serum HGF levels were already elevated (0.25±0.02 ng/ml, p<0.05). There was no significant difference between the HGF levels in UAP and AMI. Fifty-one of the 60 patients with AMI underwent immediate percutaneous transluminal coronary angioplasty and blood samples were obtained from 31 of them on days 7, 14, and 21 after MI. Serum HGF levels peaked on day 7 (0.34±0.04 ng/ml, p<0.01) and there was a weak relationship between peak creatine kinase and serum HGF levels at that time. A statistically significant correlation was found between peak CRP and serum HGF levels on day 7 (r=0.52; p<0.001). Serum HGF levels decreased to nearly normal by day 21 (0.22±0.01 ng/ml). The study shows that serum HGF levels during the early stage of AMI increased significantly and peaked by day 7 after the onset, at which time there was a strong correlation with peak CRP levels. These data suggest that HGF production may be related to the inflammatory response in AMI. (Circ J 2002; 66: 253–256)

Key Words: Acute myocardial infarction; Biochemical marker; C-reactive protein (CRP); Hepatocyte growth factor; Inflammation

Hepatocyte growth factor (HGF), which was originally isolated from the sera of rats after partial hepatectomy, is a mesenchyme derived pleiotropic factor that has unique biological activities as a mitogen, a motogen, and a morphogen. HGF can stimulate endothelial cell growth without vascular smooth muscle cells replication and can induce angiogenesis in ischemic diseases. In a clinical study, plasma HGF concentrations were increased with acute myocardial infarction (AMI) which may be related to degradation of cardiac tissue, but the mechanisms of HGF production in AMI are not yet well delineated.

AMI results in a local inflammatory reaction that, depending on the magnitude of the injury, may extend to the whole organism. This systemic response is characterized by fever, leukocytosis and changes in neurohumoral activation; increased plasma levels of cytokines and C-reactive protein (CRP) have been reported in patients with AMI and these inflammatory responses may be involved in the HGF synthesis after AMI. Moreover, the HGF level may be affected in the absence of myocardial injury. In order to clarify the production of HGF in patients with AMI, we measured plasma HGF levels in patients with unstable angina pectoris (UAP) and AMI, and followed the change in the plasma HGF and CRP levels in patients with AMI.

Methods

We studied 22 patients with UAP (15 males, 7 females; mean±SE age, 65±2 years), 60 patients with AMI (37 males, 23 females; mean age, 65±1 years), and 20 age-matched normal subjects. The diagnosis of UAP was based on clinical symptoms (typical precordial chest pain) and coronary angiography (documented severe stenosis of more than 90% by the American Heart Association classification in one or more principal coronary arteries) and no
evidence of significant elevation in serum creatine kinase (CK). The diagnosis of AMI was based on clinical symptoms, ECG evidence (ST elevation of at least 0.1 mV in 2 or more leads of ECG), coronary angiography findings (principal coronary artery occlusion: TIMI grade 0 or 1), and increased serum enzyme levels. In this study, we evaluated the patient who was the first to experience a myocardial infarction. In patients with AMI, the average time from the onset of symptoms to admission was 4.6±0.7 h (mean±SE), with a range of 0.5–12 h. Patients with liver disease and lung disease and other active inflammatory disease were excluded.

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

Heparin has been shown to be a potent enhancer of HGF synthesis in various types of cells. Cell-surface binding proteins may be involved in signal transduction, which links the binding of heparin/heparin sulfate to the translational stimulation of HGF synthesis. We tested the influence of heparin injection on the measurement of serum HGF concentration. During routine diagnostic cardiac catheterization, 3,000 units of heparin were injected into the femoral artery or vein of 10 patients and blood samples were collected immediately before the injection, after 30 min, and after 18 h. A marked increase in serum HGF concentration was measured 30 min after injecting the 3,000 units of heparin, and the level declined to its previous range 18 h after the injection (Fig 1). Therefore, in the present study, we took blood samples before or at least 24 h after heparin injection.

Study Protocols

Blood samples were collected at the time of admission from patients with UAP and AMI. All patients were immediately taken to the cardiac catheterization laboratory where coronary arteriography was performed. After angiography, percutaneous transluminal coronary angioplasty (PTCA) was performed in 13 of 22 patients with UAP and 51 of 60 patients with AMI. PTCA was performed in the ischemia-related artery only, with the goal of obtaining less than 25% residual stenosis. Intravenous heparin (5,000–20,000 units/day) was maintained for 24–96 h. We studied the HGF levels of 31 of the 51 AMI patients who underwent PTCA on days 7, 14, and 21 after the onset of AMI. We also studied serum CK-MB isoenzyme every 3 h until the serum levels peaked.

Table 1 Clinical Characteristics of the Patient Groups

<table>
<thead>
<tr>
<th>UAP (n=22)</th>
<th>AMI (n=60)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.0±2.1</td>
<td>66.1±1.5</td>
</tr>
<tr>
<td>Male sex</td>
<td>15 (68.2%)</td>
<td>38 (63.3%)</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>10 (43.5%)</td>
<td>31 (51.7%)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>8 (36.4%)</td>
<td>26 (43.3%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (9.1%)</td>
<td>23 (38.3%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>11 (50.0%)</td>
<td>25 (41.7%)</td>
</tr>
<tr>
<td>Treatment in acute phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTCA</td>
<td>13 (59.0%)</td>
<td>51 (85.0%)</td>
</tr>
<tr>
<td>CABG</td>
<td>0 (0%)</td>
<td>2 (3.3%)</td>
</tr>
<tr>
<td>Medication</td>
<td>9 (40.1%)</td>
<td>7 (11.7%)</td>
</tr>
</tbody>
</table>

Values are mean±SE. UAP, unstable angina pectoris; AMI, acute myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass grafting; NS, not significant.

Statistical Analysis

All data are expressed as mean value±SE. Unpaired t-test was performed to compare with normal subjects. Data were analyzed with Duncan’s multiple-range test for multiple comparisons preceded by a one-way analysis of variance. Correlation between the values of HGF and those of CK and its MB isoenzyme was analyzed by linear regression analysis. A p value of less than 0.05 was considered statistically significant.

Results

The clinical characteristics of the normal, UAP and AMI groups are presented in Table 1. The average time from the onset of AMI symptoms to admission was 4.6±0.7 h (mean±SE), with a range of 0.5–12 h. Serum HGF levels on admission were significantly increased in patients with UAP (0.30±0.03 ng/ml, p<0.05) and with AMI (0.27±0.02 ng/ml, p<0.01) compared with normal subjects (0.19±0.01 ng/ml) (Fig 2A). There was no significant difference be-
between UAP and AMI. Even in the early stage of AMI (21 males, 13 females; within 3h of onset of chest pain), the serum HGF concentration was already elevated (*p<0.05, **p<0.01 vs normal subjects).

HGF is a powerful proliferative factor of vascular endothelial cells, so we assessed the correlation between serum HGF levels and collateral vessel growth. We divided the AMI patients into 4 groups, according to their collateral vessel growth (Rentrop classification 0, 1, 2, 3) on coronary angiography on admission. No statistical difference was found among the groups (Fig 3).

For 51 of the 60 patients with AMI, percutaneous transluminal coronary angioplasties were performed immediately, and we took blood samples from 31 of them on days 7, 14, and 21 after their onset of AMI. Serum HGF levels tended to peak on day 7 (0.34±0.04 ng/ml, p<0.05; compared with the levels of admission) and had decreased by day 14 (0.25±0.01 ng/ml) and further decreased to almost normal by day 21 (0.22±0.01 ng/ml) (Fig 4). A weak correlation was found between peak CK and peak MB isoenzyme levels and serum HGF levels on day 7 (r=0.35; p<0.05, r=0.36; p<0.05, respectively) (Fig 5A,B). A statistically significant correlation was found between peak CRP level and serum HGF levels on day 7 (r=0.62; p<0.001) (Fig 6).

Discussion

Our study has shown that, for the patients with AMI (who undergo PTCA in the acute phase), the serum HGF concentrations tend to peak on day 7, and then gradually decrease to almost normal levels by day 21 after onset. Myocardial damage because of ischemia is affected by alterations of the physiological properties of the muscular fibers, release of cytosolic components (myoglobin and enzymes), and finally cell necrosis. Tissue necrosis caused by ischemia results in a local and systemic inflammatory reaction, depending on the magnitude of the infarct. C-
reactive protein is a non-specific, commonly used marker of the acute inflammatory response; inflammatory cells release cytokines that stimulate hepatocytes to release CRP and inflammatory cytokine reportedly stimulated HGF production in cultured cells. In the present study, peak serum HGF levels in patients with AMI apparently correlated well with peak serum CRP levels, whereas there was only a weak relation between peak HGF levels and peak CK activity. It is possible that the serum CRP level reflects the process of infarct healing rather than the extent of myocardial necrosis. We suspect that the inflammatory reaction may cause the increased HGF levels in patients with AMI.

We observed that the serum HGF concentration was significantly higher in patients with AMI than in normal subjects, even in the early stage of AMI (less than 3 h from onset of chest pain). Several clinical reports have shown that the serum HGF concentration is increased at the time of admission in patients with AMI, which is compatible with our results. Those reports also demonstrated marked increases in circulating HGF during the early stage of AMI; however, the average value for HGF on admission in patients with AMI was more than 20 times the value found in the present AMI group. Those authors did not state whether heparin, which can increase the serum HGF level, had been administered when they took blood samples. We demonstrated that heparin dramatically increases the HGF level, which only declined to its previous range 18 h after heparin injection. Therefore, the HGF levels recorded in the previous studies may have been influenced by heparin. In the present study, the HGF level was increased during the early phase of AMI prior to the administration of heparin.

Interestingly, the serum HGF levels in the UAP patients on admission were similarly elevated to those in the AMI cases. An increased HGF level in UAP patients was reported by Tanaka et al. We cannot propose the precise mechanism of the increased HGF level in UAP or during the early stage of AMI. The pathophysiology of acute coronary syndromes is characterized by plaque disruption and thrombus formation. UAP is characterized by increased levels of the acute phase reactants fibrinogen, CRP, serum amyloid A protein and the cytokine interleukin (IL)-6, the major inducer of CRP production in the liver. Studies of atherectomy samples from human coronary lesions show an association of a variety of cytokines and growth factors, including transforming growth factor β (TGF-β) basic fibroblast growth factor (bFGF) and platelet derived growth factor-I (PDGF-I) with coronary atherogenesis and plaque rupture. There may be a connection between the inflammatory reaction and peptide growth factor, and we suspect that the inflammatory reaction in acute coronary syndromes induces HGF production in the liver or cardiovascular system, although we do not have any direct evidence.

Our data suggest that HGF production may be related to the inflammatory response in AMI and may be increased even before the infarction occurs. Serum HGF may play an important role in the pathophysiology of AMI.

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References