Hepatocyte Growth Factor and Cardiovascular Thrombosis in Patients Admitted to the Intensive Care Unit

Noritake Hata, MD; Akira Matsumori, MD**; Shinya Yokoyama, MD; Takayoshi Ohba, MD; Takuro Shinada, MD; Hiroshi Yoshida, MD; Kenichi Tokuyama, MD; Takahiro Imaizumi, MD; Kyoichi Mizuno, MD*

Background  Hepatocyte growth factor (HGF) has been reported as a marker of atherosclerosis and of thrombi synthesis, but the relationship between HGF and proven coronary thrombosis has not been described. The aim of this study was to investigate this relationship in patients with chest pain.

Methods and Results  The study group comprised 107 patients with chest pain (61 acute myocardial infarction (AMI), 18 unstable angina, 15 stable angina, and 13 others; 65 males, 42 females; 66±11 years old). The presence of thrombi was evaluated by angiography, intravascular ultrasonography, angioscopy, and computed tomography. Serum HGF concentrations were measured using a new enzyme-linked immunosorbent assay. Serum HGF was significantly higher in the patients with AMI (335.0±197.5 pg/ml), unstable angina (269.1±152.7 pg/ml), acute aortic dissection (320.3±116.5 pg/ml), and pulmonary thromboembolism (292.5±101.9 pg/ml), than in those with stable angina (171.2±56.1 pg/ml). Serum HGF concentration was also higher in those patients with proven thrombi than in those patients without (326.7±189.7 pg/ml vs 226.9±110.8 pg/ml).

Conclusion  Increased serum HGF concentrations correlate with the presence of thrombi in patients with acute coronary syndrome, acute aortic dissection, and pulmonary thromboembolism.  (Circ J 2004; 68: 645–649)

Key Words:  Coronary artery disease; Growth substances; Thrombosis; Vascular disease

Human hepatocyte growth factor (HGF) was first identified in the plasma of patients with fulminant hepatic failure and subsequently, the cDNA for HGF was cloned. HGF has been reported to affect many cellular functions including mitogenesis, motogenesis, morphogenesis, and angiogenesis. HGF is a mesenchymal cell-derived cytokine that is produced by fibroblasts, Kupffer cells, and endothelial cells. Recent studies have demonstrated that human HGF may play a role in endothelial cell growth, arterial vascularization, the pathogenesis of coronary artery diseases, and cerebral infarction. Endothelial dysfunction and thrombosis play an important role in cardiovascular diseases including acute coronary syndromes, pulmonary embolism, and acute aortic dissection. Gordon et al and Ueda et al reported the presence of HGF in coronary artery plaques and thrombi. Matsumori et al found that increased HGF had relationship with thrombotic diseases. However, the correlation between HGF and the documented presence of coronary thrombi has remained unclear. The aim of this study was to evaluate serum HGF concentrations in patients with chest pain, and to determine whether angiographically proven thrombi correlate with elevated circulating HGF.

Methods  Serum HGF concentrations were measured in 92 consecutive patients who were admitted to the intensive care unit with chest pain, and 15 patients with stable angina (65 males, 42 females; 66±11 years old) from November 2000 to March 2002. The clinical diagnosis was acute myocardial infarction (AMI) in 61 patients, unstable angina pectoris in 18, chronic stable ischemic heart disease in 15, pulmonary embolism in 4, and acute aortic dissection in 9. AMI was diagnosed when chest pain continued for more than 1 h and increased serum concentrations of cardiac enzyme (creatine kinase (CK) or troponin-T) were confirmed. Unstable angina pectoris was diagnosed when chest pain lasted less than 1 h without elevation of the serum CK concentration. Diagnosis of acute aortic dissection and pulmonary embolism were based on computed tomography and ultrasonography.

On admission, a blood sample was obtained from a peripheral vein, and sera were stored in –20°C. Serum HGF was measured by a new enzyme-linked immunosorbent assay which can detect as little as 10 pg/ml of HGF. The normal value of HGF (250 healthy volunteers) using this method was 201±64 pg/ml. Patients who had received heparin sodium before arrival at the intensive care unit were excluded from the study. Patients gave informed consent to participate in this study, which was approved by the Institutional Ethics Committee.

Coronary artery thrombi were evaluated by coronary angiography, coronary angioscopy, and/or intravascular ultrasonography (IVUS). Fresh coronary thrombi were defined when total or subtotal obstruction (TIMI 0 and 1 flow) was demonstrated in patients with clinically-defined acute coronary syndrome. Totally occluded coronary arte-
ries in patients with stable angina were considered organized old thrombi. Thrombi in other sites, including the aorta, pulmonary artery, deep vein, and heart chambers, were investigated with computed tomography, ultrasonography, aortography, pulmonary arteriography, and venography.

Angiography, angioscopy, ultrasonography, and computed tomography were performed within 12 h of the measurement of serum HGF.

After routine coronary angiography, an additional 100 U/kg heparin was administered, and an 8F guiding catheter was used to engage the coronary artery. Coronary angioscopy was performed with an imaging catheter (Vecmova, Clinical Supply Co, Gifu, Japan). Before use, the white balance was adjusted for color correction. The light power was adjusted to avoid refraction and to determine the color of plaque and thrombi. Coronary thrombus was defined as a red or red-and-white mass within the lumen or adhering to the arterial wall that persisted despite flushing with physiological saline. Angioscopic images were analyzed independently by 2 observers. Both observers had no knowledge of the findings of coronary angiography, IVUS findings, or patient history. Intraobserver agreement was measured by having an observer repeat the assessment of 20 images (presented in random order) after 1 week. The inter-observer agreement was measured by comparing the assessment of 20 images by the 2 observers. Intra- and inter-observer agreements were both 95%. If there was no consensus concerning the presence of coronary thrombi, data were excluded from the study.

A 30 MHz, 3.2F monorail IVUS catheter (Ultra Cross, Boston Scientific Scimed, Inc, Boston, MA, USA) was used. The IVUS catheter was advanced over a guide wire to at least 10 mm distal to the culprit lesion. The IVUS studies were performed immediately after the intracoronary administration of 0.4 mg nitroglycerin to prevent coronary spasm. Coronary thrombus was defined as an intraluminal mass having a layered or lobulated appearance, evidence of blood flow within the mass, and speckling or scintillation. A single observer blinded to the angioscopic findings and clinical history analyzed the IVUS images.

Continuous data are expressed as the mean ± SD.
tical analyses were performed by one-way analysis of variance and Fisher's exact chi-squared test. A p-value <0.05 was considered statistically significant.

Results

Patient Characteristics
The clinical characteristics of the 107 patients are presented in Table 1. Differences in age and gender ratio did not achieve statistical significance. The presence of thrombi could be evaluated in 93 patients by adequate angiography, angioscopy, ultrasonography, and computed tomography. Sixty-one patients were proven to have thrombi (43 AMI, 4 unstable angina, 4 stable angina, 6 acute aortic dissection, and 4 pulmonary thromboembolism).

Serum HGF
Blood samples were obtained 134.2±60.3 min after the onset of the last chest pain episodes in patients with acute coronary syndrome, acute aortic dissection, and pulmonary thromboembolism, and there were no differences between the disease subgroups (133.0±61.3 min in AMI, 140.1±63.1 min in unstable angina, 123.9±59.2 min in acute aortic dissection, and 147.5±37.7 min in pulmonary thromboembolism). Serum HGF concentrations for each group of patients are shown in Fig 1. Serum HGF was significantly higher in patients with AMI (335.0±197.5 pg/ml), unstable angina (269.1±152.7 pg/ml), acute aortic dissection (320.3±116.5 pg/ml), and pulmonary thromboembolism (292.5±101.9 pg/ml) than in those with stable angina (171.2±56.1 pg/ml).

Serum HGF concentration was also higher in patients with fresh thrombi (326.7±189.7 pg/ml) than in those without thrombi (226.9±110.8 pg/ml) (Fig 2).

The relationship between the presence of thrombi and the concentration of serum HGF is shown in Table 2. Serum HGF was greater than 265 pg/ml (mean ±1 SD of healthy volunteers) in 52% of patients with thrombi, but only in 25% of those without thrombi.

Discussion

HGF and Myocardial Ischemia
Although a relationship between atherosclerosis and HGF has been reported,25,34 the serum HGF concentration in patients with stable angina and coronary artery sclerosis did not differ from that in healthy volunteers. So, the high level of circulating HGF seen in the present patients cannot be explained solely by the presence of atherosclerosis. Other investigators found that there is an increase in serum HGF in patients with AMI and unstable angina.26,27,29,35,36 Our results are similar to those reports. Soeki et al suggested that the serum HGF concentration peaked at 7 days after the onset of AMI, and it was related to left ventricular remodeling.35 Shimada et al reported that the serum HGF concentration on the 7th day of AMI had a relationship with C-reactive protein, and that HGF was involved in the inflammatory reaction.36 However, in the present study, the serum HGF concentration was measured only on arrival at the hospital, because Matsumori et al found that the serum HGF concentration increased after heparin administration.37

Several investigators have reported elevations of serum HGF in patients with myocardial ischemia.18–20,23,25,27–29 Only 1 patient with acute aortic dissection or pulmonary thromboembolism presented with myocardial ischemia in the present study. This patient had an acute aortic dissection, and his serum HGF concentration was not elevated (211.2 pg/ml). Therefore, myocardial ischemia alone cannot explain the increase in HGF.

HGF and Thrombi
Gordon et al reported that the highest rates of proliferating cell nuclear antigen, a marker of proliferation in atherosclerotic plaques, occurred in plaques associated with acute thrombosis22 and Ueda et al found HGF in coronary artery thrombi obtained by directional coronary atherecotomy.21 Matsumori et al also reported high concentrations of serum HGF in patients with thrombi, including cerebral infarction and coronary artery diseases, and our data support their finding.18,26,30,31 We took this observation a step further by
using intravascular angioscopy, IVUS, angiography, and computed tomography to prove the presence of cardiovascular thrombi, and this is the first report that demonstrates a relationship between increased serum HGF and proven cardiovascular thrombosis.

In the present patients with AMI or unstable angina, thrombolytic therapy that included heparin and/or tissue-type plasminogen activator was started after the measurement of serum HGF, and the investigation of the presence of thrombi was performed after those treatments. So, our results concerning the presence of thrombi may have been influenced by these interventions, because it is impossible to say whether the thrombi existed before the treatment or not.

Patients with acute aortic dissection may have lesions in the aortic intima, and a thrombosed false lumen was proven in 6 of 9 cases in our study. In all cases of pulmonary thromboembolism, pulmonary artery thrombi were demonstrated by pulmonary arteriography, and aspiration of these thrombi or thrombotic therapy significantly improved these patients’ respiratory status. In these cases, pulmonary thrombi were suspected to have embolized from deep veins in the lower extremities.

We suggest that increased circulating HGF correlates with the presence of fresh thrombi in patients with coronary artery disease, acute aortic dissection, or pulmonary thromboembolism. However, it remains unclear whether the degree of increase in HGF is related to the quantity of thrombi or to endothelial lesions. To determine this, further investigation will be required.

**HGF in Acute Aortic Dissection and Pulmonary Thromboembolism**

In our results, circulating HGF was elevated in patients with acute aortic dissection and pulmonary thromboembolism. We did not research the time course of HGF concentration in patients with pulmonary thromboembolism who received heparin treatment for 3–7 days after the admission, because Matsumori et al. found that serum HGF concentration increased immediately after heparin use. In patients with acute aortic dissection, lesions of the aortic endothelial cells may be responsible for increased serum HGF. On the other hand, it is suspected that vascular lesions in deep veins have a relationship with high concentrations of serum HGF. However, thrombi in the false lumen of an acute aortic dissection and thrombotic lesions in pulmonary arteries were demonstrated in our investigation, so our results suggest that increased serum HGF is related to the presence of thrombi rather than endothelial disruption in these diseases. In our study population, 6 of 9 patients with acute aortic dissection were shown to have a thrombosed false lumen and HGF concentrations were higher in those patients than in patients with a patent false lumen after aortic dissection (360±114 vs 241±89 pg/ml).

In conclusion, increased concentrations of circulating HGF were seen in the early stage of patients with acute coronary syndromes (AMI and unstable angina), acute aortic dissection, and pulmonary thromboembolism, but not in those with stable angina. High concentrations of serum HGF were specifically noted in patients with thrombi demonstrated by angiography, angioscopy, ultrasonography, and tomography. These results suggest that an increased concentration of circulating HGF is a marker of the presence and synthesis of thrombi in coronary arteries, pulmonary arteries, deep veins and the aorta.

**Acknowledgments**

The authors thank all staff in the Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine for scientific suggestions and the measurement of HGF, and all our coworkers for their care of the patients.

**References**


