Circulating Hepatocyte Growth Factor as a Diagnostic Marker of Thrombus Formation in Patients With Cerebral Infarction

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Circulating levels of hepatocyte growth factor (HGF) are increased in the early stage of an acute myocardial infarction because of arterial thrombosis. The purpose of this study was to use a new sensitive enzyme-linked immunosorbent assay to investigate whether circulating HGF is increased in patients with cerebral infarction. Circulating HGF was measured in 32 patients with cerebral infarction on admission to hospital and on days 2, 3, 7 and 14 after the onset of symptoms. Serum HGF levels exceeded the mean value +2SD (329 pg/ml) measured in controls in 10 of 20 patients (50%) within 6 h after onset and in 15 of 32 patients (47%) within 24 h. Plasma D-dimer was increased in more than half of the patients with elevated HGF values. HGF levels in 16 patients who were measured serially were persistently increased throughout the study period. The results suggest that circulating HGF is a reliable early marker of cerebral infarction, and that this new sensitive HGF assay may be useful for diagnosing cerebral thrombosis. (Circ J 2002; 66: 216–218)

Key Words: Cerebral infarction; Hepatocyte growth factor; Thrombosis

Acute ischemic stroke is caused mainly by arteriosclerotic or thromboembolic disorders of the cerebral circulation. Detecting and monitoring activation of the coagulation system in acute ischemic stroke by conventional laboratory methods is difficult because the currently available tests lack sufficient sensitivity and specificity. Hepatocyte growth factor (HGF) was originally regarded as specific to hepatocytes, but has been found to be identical to the scatter factor. The plural activities of HGF/SF are mitogenesis, motogenesis (enhancement of cell motility), morphogenesis (promotion of cell survival, tumor inhibition, and hematopoiesis), and it has also been shown that HGF/SF induces angiogenic activity in vivo, suggesting an important function during ischemic injury. Recently, we showed that circulating levels of HGF were markedly increased in the very early period of acute myocardial infarction, and that these levels correlated with peak levels of creatine kinase. Those studies suggested that measurement of HGF would be a sensitive method of diagnosing incipient acute myocardial infarction and more recently, we have shown that circulating HGF is also increased during the early stage of arterial thrombosis and that its measurement contributes to its early diagnosis.

However, the sensitivity of commercially available enzyme immunoassay kits (Otsuka Pharmaceutical Co, Tokushima, Japan) is 100 pg/ml, which is not sensitive enough to establish normal values and so we developed a new sensitive method of measuring HGF levels as low as 10 pg/ml. According to our method, the mean HGF value in 250 healthy volunteers (aged 18–55 years) was 201±64 pg/ml (mean±SD). No sex- or age-related differences in the levels of circulating HGF were observed (men: 203±61, 214±73, and 192±41 pg/ml; women: 189±59, 204±76, and 195±54 pg/ml; 18–29, 30–39, and 40–49 years of age, respectively). HGF levels did not seem to be influenced by renal function because increased HGF levels were not found in patients with renal disease in the absence of thrombus formation. Using this method, we successfully detected thrombosis in approximately one-third of patients with unstable angina pectoris.

The present study was performed to determine whether the level of circulating HGF would assist in the diagnosis of acute cerebral infarction, and whether it could serve as an indicator of stroke in the very early period of brain ischemia. The study population consisted of 32 consecutive patients with acute cerebral infarction (20 men and 12 women), 57–92 years of age (mean = 74±8.8 years) who were admitted to hospital within 24 h of onset. Each patient was clinically examined by 2 neurologists and all patients underwent brain computed tomography (CT) scan on admission. Excluded from the study were patients whose medical history or neuro-radiological imaging was consistent with intracerebral hemorrhage, hemorrhagic infarction, brain tumor, demyelinating disease, vascular headache, acute or chronic infection, inflammatory disease of the central nervous system, systemic metabolic disorders, or systemic vasculitis. Seven patients with transient ischemic attacks, defined as a sudden episode of focal neurological deficit believed to be secondary to inadequate blood supply and completely resolved in less than 24 h, were also studied.

Brain CT scans were obtained on admission and followed up 7 days later, using a CT scanning system (Asteion, Toshiba, Tokyo, Japan) with slices 10 mm wide. The scans...
were evaluated by a neuroradiologist who identified the site of the lesion and estimated the lesion size as small (≤1/2 lobe), intermediate (1/2–1 lobe), or large (>1 lobe). The protocol was approved by the Institutional Review Boards of the hospitals, and informed consent was obtained from the study participants or an immediate family member. Blood samples were obtained from all patients on admission and in 16 patients, samples were taken serially at 24 h and then 2, 3, 7 and 14 days after admission. Heparin was not administered to patients during the observation period. Sera were stored at −20°C, and transported to the core laboratory.

The wells of a microtiter plates had the addition of 100 μl of a 3 μg/ml anti-human HGF monoclonal antibody solution and were incubated overnight at 4°C. The plates were then incubated with 50 μl of serum or diluted recombinant human HGF antigen at 25°C for 1 h, after which they were washed, and incubated with 100 μl of diluted biotinylated anti-human recombinant HGF IgY at 25°C for 1 h. Finally, the plates were incubated with 100 μl of diluted horseradish peroxidase-conjugated streptavidin (Vector Laboratories, Inc, Burlingame, CA, USA) at 25°C for 1 h and then incubated with 100 μl of 3,3',5,5'-tetramethylbenzidine (TMB) soluble solution (Scytek Laboratories, Logan, UT) for color development at room temperature for 10 min. Optical density was measured at 450 nm using a plate reader (Molecular Devices Vmax, Sunnyvale, CA, USA). The concentrations of the samples measured were calculated using the standard curve constructed from the recombinant human HGF antigen values measured at the same time. The 2 antibodies used for this assay were specific to mature (heterodimeric, biologically active) human HGF and did not cross-react with pro-human HGF (single peptide, biologically inactive). Plasma levels of D-dimer were also measured in 23 patients on admission by enzyme-linked immunosorbent assay (Otsuka Assay Laboratory, Toku-shima, Japan; normal value; <0.5 μg/ml).

Patient characteristics are shown in Table 1. Serum HGF level exceeded the mean value +2SD (329 pg/ml) measured in controls in 10 of 20 patients (50%) with stroke within 6 h.
of onset and in 15 of 32 patients (47%) within 24 h (Table 1). The mean serum levels of HGF measured serially in 16 patients were: 311 ± 160 pg/ml (mean ± SD) at 6 h after the onset of stroke symptoms, 364 ± 135 pg/ml at 24 h, and 333 ± 90 pg/ml, 397 ± 238 pg/ml, 375 ± 149 pg/ml and 403 ± 176 pg/ml at 2, 3, 7, and 14 days after the onset of symptoms, respectively (p < 0.005, ANOVA, vs control value, Fig 1). Of 6 of 16 patients with increased HGF levels on presentation, the serum HGF remained persistently increased in 5 patients and was less than the mean control level in 1 patient (Fig 1). Among 10 patients without elevated HGF levels on admission, the serum HGF concentration exceeded the mean value +2SD measured in controls at least at one time point in 5 patients. Therefore, overall, abnormally high levels of HGF were found during at least 1 time point in 12 of 16 (75%) patients. The circulating HGF level was not increased in patients with transient ischemic attacks (228 ± 84 pg/ml, n=7, within 24 h of onset of symptoms).

In the present study, the concentration of circulating HGF was increased significantly during the very early stage of cerebral infarction, and in some patients, it remained persistently elevated, consistent with persistent thrombus formation. In other patients, the serum HGF level decreased as the disease evolved, suggesting that thrombus formation abated. The different time courses of the changes in the level of circulating of HGF suggest that thrombus formation varies among patients with acute cerebral infarction, but further studies are needed to clarify the mechanism of a persistently increased concentration of circulating HGF. In the present study, infarct size did not correlate with HGF level, and in our recent study of patients with unstable angina pectoris, a significant proportion of patients had high levels of HGF without myocardial infarction, suggesting that thrombus formation does not automatically cause myocardial infarction.

Heparin potentiates the angiogenesis induced by tumor extracts, may raise the ischemia threshold and improve collateral blood flow when administered at the time of exercise stress testing, and improves collateral blood flow after myocardial infarction. In our animal and clinical studies, rapid increases in the level of circulating HGF were confirmed as early as 3 min after the injection of heparin, probably as a result of unbinding from the extracellular matrix in several organs rather than to de novo synthesis. In a more recent study, we showed that the in vivo administration of heparin causes a significant increase in HGF concentration in the systemic circulation, and that the serum obtained from patients treated with heparin had growth-promoting and vascular tube-forming effects on endothelial cells in vitro, effects that were completely inhibited by neutralization of HGF. These findings are consistent with a significant role for HGF in heparin-induced angiogenesis.

Furthermore, we have shown that the level of circulating HGF increases early after arterial thrombosis which suggests that HGF released during thrombus formation participates in the angiogenetic process in ischemic tissue. We consider that HGF might be released from the vessel wall by unknown mechanisms related thrombus formation and we have embarked on further study of those mechanisms of release. D-dimer, which is a fragment specific to the degradation of fibrin, has been reported to be useful in the diagnosis of thrombosis and in the present study, increased levels of D-dimer were found in 6 of 11 patients (55%) with high HGF values, although patients with increased HGF concentration did not consistently have elevated D-dimer levels. Because the mechanisms of increase in the concentrations of HGF and D-dimer may be different, combining the 2 measurements detected cerebral thrombosis more reliably on admission (18 of 23 patients, 78%, Table 1) than either measurement alone. Our observations suggest that the circulating HGF level is a reliable diagnostic marker of cerebral infarction and further studies in larger numbers of patients seem warranted to further define the role of increased HGF in the pathogenesis of cerebral infarction, as well as with the diagnostic and prognosis power of this new laboratory test.

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References