Production of hepatocyte growth factor during acute myocardial infarction

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Abstract

Objective—To investigate the clinical significance of circulating hepatocyte growth factor (HGF) and the role of peripheral blood mononuclear cells (monocytes), which are a possible source of HGF, in patients with acute myocardial infarction.

Design and patients—37 patients with acute myocardial infarction and 13 normal control subjects were recruited. Peripheral venous blood samples were drawn from the infarct patients 1, 7, 14, and 21 days after onset. Monocytes were isolated from peripheral blood at those times. HGF concentrations in serum and in a culture medium of monocytes after incubation for 24 hours (monocyte HGF levels) were measured by enzyme linked immunosorbent assay.

Results—Serum HGF and monocyte HGF values within seven days after onset of myocardial infarction were significantly higher than those of control subjects and decreased by day 14. There were significant positive correlations between serum HGF and monocyte HGF levels on day 7; between maximum plasma creatine phosphokinase levels and serum HGF levels on day 1; between maximum plasma C reactive protein and serum HGF levels; and between maximum C reactive protein and monocyte HGF levels. Monocyte HGF levels were raised in the patients with progression of ventricular enlargement in the course of acute myocardial infarction.

Conclusions—Early serum HGF concentrations reflect the extent of myocardial damage in acute myocardial infarction patients. Inflammation after acute myocardial infarction is supposed to be involved in enhanced HGF production. Monocytes may play an important role in ventricular remodelling after acute myocardial infarction by releasing the cardiovascular protective mitogen HGF.

(Keywords: C reactive protein; cytokines; ischaemic heart disease; remodelling; hepatocyte growth factor)

Hepatocyte growth factor (HGF) is a mesenchyme derived growth factor originally identified in the plasma of partially hepatectomised rats and later isolated from rat platelets and human plasma. HGF was initially known as a specific mitogen for hepatocytes, but several studies have shown that it has marked and varied effects on epithelial cells, endothelial cells, and other cell types, including morphogenic and mitogenic activity.

Plasma HGF concentration has been reported to be increased in response to organ damage, such as in liver and kidney diseases. HGF also acts as a growth factor for vascular tissues, and its mitogenic activity is the most potent among various known growth factors.

Plasma HGF concentrations are reported to be increased in cardiovascular diseases, such as in the early stage of acute myocardial infarction and in hypertension. It has been also found that HGF can induce angiogenesis in ischaemic diseases. The course of acute myocardial infarction is a complex dynamic process involving not only myocardial but also endothelial cells, resulting in a local inflammatory reaction and vascular proliferation. Recent findings suggest that myocardial ischaemia and reperfusion can induce HGF expression in a rat model. Therefore an increase in HGF in response to myocardial infarction may promote collateral formation around the ischaemic area. However, detailed mechanisms of HGF production and the clinical significance of HGF in ischaemic hearts are not yet well delineated. In the present study, we investigated the clinical significance of circulating HGF concentrations in patients with acute myocardial infarction. We focused on the role of peripheral blood mononuclear cells (monocytes) in HGF production because circulating blood cells are reported to produce a substantial amount of HGF.

Methods

Patients
This investigation conformed with the principles outlined in the Declaration of Helsinki. We studied 37 patients with acute myocardial infarction (32 male and five female, mean (SD) age 61.8 (9.8) years, range 40 to 80 years) who were admitted to our hospital between November 1997 and May 1998. The diagnosis of acute myocardial infarction was made according to World Health Organisation criteria. We also studied 13 normal control subjects (eight males and five female; age 54.2 (4.5) years, range 34 to 72 years). If there were no absolute contraindications, all patients underwent cardiac catheterisation and direct percutaneous transluminal coronary angioplasty (PTCA). Heparin and isosorbide dinitrate were given intravenously during PTCA. The use of aspirin, β blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, and nitrate preparations was left to the discretion of the attending physician. None of the patients with acute myocardial infarction had hepatic or renal failure.
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measurements, serum samples were stored at −80°C (1.1) hours, range 1 to 24 hours. For HGF concentrations in the supernatant of cultured peripheral blood mononuclear cells (monocytes) in patients with acute myocardial infarction and in control subjects. Monocytes were isolated and cultured as described in Methods. HGF was determined by enzyme linked immunosorbent assay as described in Methods. Serum HGF concentration in the supernatant of cultured peripheral blood mononuclear cells (monocytes) in patients with acute myocardial infarction and in control subjects. Monocytes were isolated and cultured as described in Methods. HGF was determined by enzyme linked immunosorbent assay (ELISA) kit (Special Immune Research Constitution Co, Tokyo, Japan). Measurement was performed according to the assay protocol. The standard curve was linear between 0.1 and 3.0 ng/ml of HGF. The lower limit of detection of HGF was 0.1 ng/ml.

STATISTICAL ANALYSIS

Data were expressed as means (SEM). Differences in HGF levels were analysed by repeated measures ANOVA followed by Scheffe’s test and unpaired Student’s t test. Simple linear regression was used for assessment of the

BLOOD COLLECTION

Our preliminary study showed that the intravenous administration of 3000 units of heparin markedly increased circulating HGF levels (by about 60-fold), but this effect was transient and decreased to a negligible level 24 hours after heparin administration. This finding is consistent with previous reports.20–22 We also found that HGF levels in plasma isolated using heparin as an anticoagulant were underestimated (~89%) compared with those in plasma or serum isolated without using heparin. We therefore excluded patients who had received systemic heparin within 24 hours before admission, and isolated serum from peripheral blood without using heparin to measure circulating HGF levels.

We collected peripheral blood samples from patients with acute myocardial infarction at the time of admission and every four hours until maximum plasma creatine phosphokinase (CPK) activity was determined. Peripheral blood was also drawn from patients on day 1 (at the time of admission), day 7, day 14, and day 21 after the onset of infarction for measurements of HGF. The mean (SD) time from the onset of symptoms to admission was 6.2 (1.1) hours, range 1 to 24 hours. For HGF measurements, serum samples were stored at −80°C until assay.

PERIPHERAL BLOOD MONONUCLEAR CELL

ISOLATION AND CULTURE

Anticoagulated blood (20 ml total, containing 0.5 ml heparin) was obtained from patients with acute myocardial infarction on day 1, day 7, day 14, and day 21 after the onset, and from normal control subjects. The blood was layered onto Mono-Poly resolving medium (Dainippon Pharmaceutical Co, Osaka, Japan), centrifuged at 400 × g for 20 minutes at room temperature. The monocyte fraction was drawn up and rinsed with RPMI medium containing 10% bovine serum albumin (BSA). After it was centrifuged again at 400 × g for 12 minutes at room temperature, the cells were resuspended in 1 ml of RPMI medium containing 10% BSA. The monocytes were cultured in the same medium with a density of 5 × 10⁶ cells/500 µl in a humidified atmosphere of 5% CO₂ incubator at 37°C. The supernatants were collected 6, 12, and 24 hours after incubation and stored at −80°C until assay.

HGF ASSAY

HGF was measured by a specific enzyme linked immunosorbent assay (ELISA) kit (Special Immune Research Constitution Co, Tokyo, Japan). Measurement was performed according to the assay protocol. The standard curve was linear between 0.1 and 3.0 ng/ml of HGF. The lower limit of detection of HGF was 0.1 ng/ml.

Figure 1 Time course of serum hepatocyte growth factor (HGF) concentrations in patients with acute myocardial infarction. HGF was determined by enzyme linked immunosorbent assay as described in Methods. Serum HGF reached a peak on day 1 and decreased by day 21. Serum HGF in the patients was significantly higher than in the normal controls on day 1 (0.54 (0.11) v 0.12 (0.03) ng/ml, p < 0.05), and remained significantly higher on day 7. Filled circles, patients with acute myocardial infarction; empty circles, control subjects. Error bars = SEM. *p < 0.05 v control subjects.

Figure 2 (A) Hepatocyte growth factor (HGF) concentrations in the supernatant of cultured peripheral blood mononuclear cells (monocytes) in patients with acute myocardial infarction and in control subjects. Monocytes were isolated and cultured as described in Methods. HGF levels in the monocyte culture medium increased time dependently until 24 hours after incubation. (B) Changes in HGF production by monocytes in the course of acute myocardial infarction. HGF levels in the monocyte culture medium after incubation for 24 hours are called “monocyte HGF levels” and used as a marker of the HGF producing ability of monocytes. Monocyte HGF levels were significantly higher than those of control subjects on day 1 but decreased by day 14. Filled circles, patients with acute myocardial infarction; empty circles, control subjects. Error bars = SEM. *p < 0.05; **p < 0.01 v control subjects.
Results

SERUM HGF CONCENTRATIONS
As shown in fig 1, serum HGF concentrations on day 1 (at the time of admission) in patients with acute myocardial infarction were significantly higher than in control subjects (0.54 (0.11) v 0.12 (0.03) ng/ml, p < 0.05). Serum HGF remained higher for seven days and decreased by 21 days after onset of infarction.

HGF PRODUCTION BY MONOCYTES
Figure 2A shows HGF concentrations in the monocyte culture medium on day 1 after the onset of acute myocardial infarction and in the control subjects. HGF levels in the monocyte culture medium increased with incubation time in these subjects. After incubation for 24 hours, HGF levels in the monocyte culture medium were significantly higher in the infarct patients than in the controls (0.20 (0.03) v 0.05 (0.01) ng/ml, p < 0.01). We called the HGF levels in the monocyte culture medium after incubation for 24 hours “monocyte HGF levels” and used them as a marker of the HGF producing ability of the monocytes.

Figure 2B shows the changes in monocyte HGF levels in the course of acute myocardial infarction. The values peaked on day 1 and decreased by day 14. Monocyte HGF concentrations on days 1 and 7 were significantly higher than in control subjects.

CORRELATIONS BETWEEN SERUM HGF AND MONOCYTE HGF CONCENTRATIONS
We then examined correlations between serum HGF and monocyte HGF concentrations. As shown in fig 3A, a weak positive correlation was found between serum HGF and monocyte HGF levels on day 1 after the onset of infarction (r = 0.47, p < 0.05). A significant positive correlation was also found on day 7 (r = 0.54, p < 0.01) (fig 3B).

CORRELATIONS BETWEEN HGF CONCENTRATIONS AND CLINICAL VARIABLES
We next analysed correlations between HGF concentrations and clinical variables in acute myocardial infarction. There was a significant positive correlation between maximum plasma CPK and maximum serum HGF (r = 0.54, p < 0.01) (fig 4A), and between maximum plasma CPK and serum HGF on day 1 (r = 0.61, p < 0.001) (fig 4B). Similarly, there

relation between two variables. Probability (p) values of < 0.05 were considered statistically significant.
were significant positive correlations between maximum plasma CPK-MB and maximum serum HGF \((r = +0.56, p < 0.001)\), and between maximum plasma CPK-MB and serum HGF on day 1 \((r = +0.64, p < 0.001)\). We also observed a weak positive correlation between maximum plasma CPK and maximum monocyte HGF \((r = +0.44, p < 0.01)\).

We found positive correlations between maximum C reactive protein and maximum serum HGF \((r = +0.55, p < 0.01)\), and between maximum C reactive protein and maximum monocyte HGF \((r = +0.65, p < 0.001)\) in the course of acute myocardial infarction. Significant positive correlations were also found between C reactive protein and serum HGF and between C reactive protein and monocyte HGF levels on day 1 \((r = +0.61, p < 0.001 \text{ and } r = +0.68, p < 0.001, \text{ respectively})\) and on day 7 \((r = +0.42, p < 0.05 \text{ and } r = +0.47, p < 0.01, \text{ respectively})\).

We performed left ventriculography at the time of admission and before discharge (mean interval 18.5 (5.2) days) in 16 patients and evaluated the changes in left ventricular end diastolic volume index (LVEDVI) in the course of acute myocardial infarction. Mean LVEDVI did not change significantly in these patients (from 81.9 (3.1) to 82.9 (3.0) ml/m\(^2\)). We divided these patients into two groups according to the changes in LVEDVI (group A: eight patients with increases in LVEDVI during the course of acute myocardial infarction; group B: eight patients without increases in LVEDVI). There were no significant differences in serum HGF between the two groups in the course of acute myocardial infarction except with a marginal significance on day 7 (fig 6A). However, monocyte HGF levels on day 1 \((0.37 (0.07) \text{ v } 0.15 (0.04) \text{ ng/ml, } p < 0.05)\) and on day 7 \((0.30 (0.07) \text{ v } 0.08 (0.03) \text{ ng/ml, } p < 0.05)\) in group A were significantly higher than in group B (fig 6B).

**Figure 5** Correlations between maximum C reactive protein levels and hepatocyte growth factor (HGF) levels: there was a significant positive correlation between maximum plasma C reactive protein and maximum serum HGF (panel A, \(r = +0.55, p < 0.01\)), and between maximum C reactive protein and maximum monocyte HGF (panel B, \(r = +0.65, p < 0.001\)).

**Figure 6** Hepatocyte growth factor (HGF) levels in the patients with or without left ventricular dilatation in the course of acute myocardial infarction. We divided the patients into two groups according to the changes in left ventricular end diastolic volume index (LVEDVI) (group A: eight patients with increases in LVEDVI during the course of acute myocardial infarction; group B: eight patients without increases in LVEDVI). (A) Mean serum HGF on day 7 in group A was higher than in group B, but the difference was not significant \((0.47 (0.10) \text{ v } 0.29 (0.05) \text{ ng/ml, } p = 0.13)\). (B) Monocyte HGF levels on day 7 in group A were significantly higher than in group B \((0.30 (0.07) \text{ v } 0.08 (0.03) \text{ ng/ml, } p < 0.05)\). Error bars = SEM. *\(p < 0.05\).
Discussion
HGF was initially thought to be a liver specific mitogen. Later it was reported to induce angiogenesis through its effects on endothelial migration and proliferation. In addition, HGF has been shown to act as an antiapoptotic agent. Recently, several reports have focused on its function in the heart. Ono et al reported that HGF expression in the heart was upregulated in the ischaemic reperfused region, and plasma HGF levels peaked at three hours and remained elevated for 24 hours. However, they did not study the changes in HGF levels during the course of acute myocardial infarction, nor the effects of therapeutic use of heparin on HGF assay (it has now been shown that intravenous administration of heparin markedly increases circulating HGF levels). We therefore excluded patients who had received systemic heparin before admission and isolated serum from peripheral blood without using heparin to measure circulating HGF levels. We found that from days 1 to 7 after the onset of acute myocardial infarction serum HGF in the infarct patients was significantly higher than in normal subjects.

We speculated that the increased serum HGF immediately after acute myocardial infarction was related to degradation of cardiac tissue, as there was a significant positive correlation between serum HGF on day 1 and maximum CPK levels (fig 4B). Owing to the impact of myocardial infarction, HGF might be released from injured cardiac tissue. To further explore mechanisms of HGF production in acute myocardial infarction, we focused on monocytes because monocytes/macrophages and lymphocytes have been reported to be major HGF producing cells. We observed a significant positive correlation between serum HGF levels and monocyte HGF levels on day 7, and a weak positive correlation on day 1 after onset of acute myocardial infarction (fig 3). These results suggest that monocytes might also contribute to the rise in serum HGF in the subacute phase of acute myocardial infarction. Thus it is possible that both degradation of cardiac tissue and enhanced production by monocytes are involved in the rise in serum HGF levels after acute myocardial infarction. It has been reported that platelets and endothelial cells are other production sources of HGF. However, we did not find a significant correlation between the platelets count and serum HGF levels.

Infarcted myocardium causes a local inflammatory response comprising a complicated interaction between ischaemic myocardial and inflammatory cells. C reactive protein is a reactant of the acute phase of inflammation, and its hepatic synthesis is triggered by many forms of inflammation, infection, and tissue injury. Previous studies have reported that plasma C reactive protein level is related to prognosis of acute myocardial infarction and unstable angina. It is known that inflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumour necrosis factor α, induce HGF gene expression and that C reactive protein synthesized in the liver is also mediated by these cytokines. In our present study, maximum C reactive protein levels were significantly correlated with maximum monocyte HGF levels as well as with serum HGF in the course of acute myocardial infarction (fig 5). This indicates that factors related to the inflammatory response are involved in HGF synthesis after acute myocardial infarction. Further studies are needed to determine the major cell types and which cytokines are involved in HGF production.

We found increased production of HGF by monocytes in patients with acute myocardial infarction who had ventricular enlargement as a feature (fig 6). We only measured HGF production by peripheral monocytes, but if they are representative of monocytes infiltrating the infarcted myocardium then our data suggest that local HGF produced by monocytes in the heart may play an important role in ventricular remodelling after acute myocardial infarction. Further studies should be undertaken to establish that monocytes infiltrating the infarcted myocardium also show increased HGF expressions.

CONCLUSIONS
Our study showed that serum HGF is raised in the acute and subacute stages of acute myocardial infarction. Serum HGF in the early stage might reflect the extent of the infarct. Subsequent inflammation after the acute infarction could contribute to the rise in circulating HGF levels and enhanced HGF production by monocytes. We found that monocyte HGF levels were raised in patients with ventricular enlargement in the course of acute myocardial infarction. Thus increased HGF expression in the subacute phase of acute myocardial infarction could play an important role in ventricular remodelling through regeneration of endothelial cells, promoting angiogenesis and inhibition of apoptosis in the ischaemic myocardium.

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Commentary

An already large, and continually growing, family of peptide growth factors is implicated in a number of physiological and pathophysiological responses in the cardiovascular system. Among these is hepatocyte growth factor (HGF). This paper by Zhu and colleagues provides evidence that circulating HGF concentration correlates with the extent of myocardial injury in acute myocardial infarction which raises the possibility of HGF being a circulating marker with prognostic implications. Clearly, larger and more detailed time course studies are required to assess this potential. At present the cellular source of HGF in acute myocardial infarction remains unclear. Although the paper demonstrates that monocytes may elaborate HGF, there is evidence that tissues, including myocardium, liver, kidney and brain, may produce and release the peptide in response to ischaemia, along with other growth factors including insulin like growth factor and transforming growth factor β. The biological functions of these growth factors in ischaemic and postischaemic myocardium remain uncertain. Growth factors such as HGF are known to exert a number of actions. The control of cell growth, proliferation, and apoptosis (programmed cell death) in healing and remodelling is likely to be only one important role. HGF, in common with several other peptide growth factors, is known to be cytotoxic to cardiomyocytes in ischaemia reperfusion and its production may represent a fundamental endogenous cardioprotective response through activation of cell “survival” signalling pathways.