Early Treatment with Hepatocyte Growth Factor Improves Cardiac Function in Experimental Heart Failure Induced by Myocardial Infarction

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ABSTRACT

Plasma levels of hepatocyte growth factor (HGF) are increased within hours of cardiac ischemia/reperfusion in rats, and HGF has been shown to be cardioprotective toward acute ischemic injury. Myocardial levels of HGF mRNA and protein are increased for several days after myocardial infarction (MI), however, indicating a possible additional protective effect of HGF toward the progression of MI to heart failure. The purpose of this study was to determine whether HGF administration during the time course of endogenous cardiac HGF induction would lead to long-term improvement in cardiac function in rats with MI. MI was induced by 2-h occlusion of the left coronary artery, followed by reperfusion. HGF was given by intravenous infusion at 0.45 mg/kg/day for 6 days beginning on the day after surgery. Cardiac function and hemodynamic parameters were measured by using indwelling catheters and perivascular flow probes in conscious animals 8 weeks post-MI. Myocardial infarcts were approximately 30% of the left ventricle, and there was no difference in infarct size between the vehicle-treated and HGF-treated groups. Compared with untreated sham-operated rats, vehicle-treated MI animals had significantly lower cardiac index and stroke volume index and higher systemic vascular resistance, indicating heart failure developed. Treatment with HGF caused a significant increase in cardiac index and stroke volume index and a reduction in systemic vascular resistance in rats with MI, restoring these parameters close to those observed in sham-operated control animals. These results provide direct evidence that HGF may be of benefit to cardiovascular function in ischemic cardiomyopathy.

Hepatocyte growth factor (HGF) is a disulfide-linked heterodimeric protein initially purified and cloned as a potent mitogen for hepatocytes (Nakamura et al., 1989). Subsequently, several functions have been ascribed to HGF, including motogenesis, morphogenesis, hematopoiesis, anti-apoptosis, and angiogenesis (Bussolino et al., 1992; Grant et al., 1993; Zarnegar and Michalopulos, 1995; Matsumoto and Nakamura, 1996; Kopp, 1998; Balkovetz and Lipschutz, 1999). HGF has also been shown to induce tissue regeneration in a variety of organs, particularly in the liver (Comoglio, 1993; Matsumoto and Nakamura, 1996, 1997). These multipotent activities of HGF are mediated by stimulating the tyrosine kinase activity of its specific receptor, which is encoded by the c-met proto-oncogene (Bottaro et al., 1991; Naldini et al., 1991).

Recently, HGF has been given increasing attention in cardiac diseases (Mastusmori et al., 1996; Ono et al., 1997; Ueda et al., 1999, 2001; Aoki et al., 2000; Nakamura et al., 2000; Yasuda et al., 1999, 2000; Taniyama et al., 2000; Zhu et al., 2000). Circulating levels of HGF are substantially elevated in rats (Ono et al., 1997) and in humans after acute myocardial infarction (MI) (Mastusmori et al., 1996; Molnar et al., 2000; Soeki et al., 2000a,b). In the rat, plasma HGF levels increase in a biphasic manner after cardiac ischemia reperfusion, peaking at 3 h and increasing again at 24 h (Ono et al., 1997), and data from previous studies indicated that HGF can be cardioprotective toward acute ischemia-reperfusion injury. Early administration of HGF in a rat model of cardiac ischemia-reperfusion reduced infarct size and improved short-term cardiac performance (Nakamura et al., 2000).

It is also known that myocardial levels of HGF and e-Met mRNA and protein are elevated in the week after myocardial infarction (Ono et al., 1997; Ueda et al., 2001). Cardiac mRNA abundance for HGF was increased for up to 5 days after MI in a model of 1 h of cardiac ischemia followed by reperfusion (Ono et al., 1997). Immunohistochemical staining for HGF at 24 and 48 h post-MI showed intense staining...
in the cytoplasm of endothelial cells and in interstitial cells, including infiltrating macrophage. c-Met transcript levels were also increased for 5 days in the ischemic heart. Immunohistochemical analyses showed positive staining for c-Met in capillary endothelial cells (Ono et al., 1997). In a recent study, both HGF and c-Met mRNA levels in the left ventricle substantially increased from 6 h after left coronary ligation and continued to increase for up to at least 7 days in rats with MI (Ueda et al., 2001). The c-Met receptor was expressed in cardiomyocytes localized in the border regions of the viable myocardium and in noninfarcted regions, suggesting that myocytes may be a primary target of endogenous HGF.

The observations that HGF and c-Met are persistently induced in the myocardium, well past the acute phase of ischemic injury, led us to hypothesize that HGF may have beneficial effects in ischemic cardiomyopathy, independent of those that influence infarct size. To test this we used a rat model of cardiac ischemia/reperfusion where the duration of ischemia was 2 h, sufficient time to produce infarcts large enough to cause progression to heart failure. HGF was administered to MI rats for 6 days, beginning the day after surgery so as to avoid having a direct effect on infarct size. The long-term effects of HGF treatment on cardiac function were then determined 8 weeks post-MI, because our previous studies have shown that untreated rats with moderate-large myocardial infarction develop evident heart failure at 8 weeks in this model (Yang et al., 1995; Jin et al., 2001). Our results suggest that HGF improves cardiac function in experimental heart failure induced by MI.

**Materials and Methods**

All experimental procedures conformed to the guiding principles of the American Physiology Society and were approved by Genentech’s Institutional Animal Care and Use Committee.

**Animal Model.** Male Sprague-Dawley rats (7–8 weeks of age; Charles River Laboratories, Inc., Wilmington, MA) were acclimated to the facility for at least 1 week before surgery. Rats were fed a pelleted rat chow and water ad libitum, and housed in a light- and temperature-controlled room. The procedure used for left coronary ligation has been described in detail elsewhere (Pfeffer et al., 1979). In brief, the rats were anesthetized with ketamine hydrochloride (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.), intubated via tracheotomy, and ventilated by a respirator (model 683; Harvard Apparatus, Inc., Holliston, MA). After a left-sided thoracotomy, the left coronary artery was ligated approximately 2 mm from its origin with a 7-0 silk suture. Two hours after ligation, the ligature was released for reperfusion.

**Time Course of Cardiac Gene Expression of HGF and c-Met.** The time points used were 3 h, 8 h, 1 day, 3 days, 1 week, 2 weeks, and 1 month after left coronary ligation or sham operation (n = 6 at each time point in each group). ECG analyses were used to identify rats with moderate-to-large infarcts (Yang et al., 1995) for time points 1 day after surgery or longer. For the 3- and 8-h time points serum troponin T levels were used to identify rats with evidence of infarcts. In a pilot study, we found a significant correlation between serum troponin T levels and infarct size at 3 h (r = 0.675, P < 0.001), 4 h (r = 0.849, P < 0.0001), and 24 h (r = 0.916, P < 0.0001) after MI/reperfusion. The serum troponin T levels at 3 and 8 h were markedly different between MI rats (304.32 ± 58.99 mg/dl) and sham controls (1.08 ± 0.13 mg/dl). ECG on days 1 to 30 after surgery showed significant abnormal Q wave in at least three precordial leads in MI rats but no abnormalities in sham-operated animals.

At the various time points the rats were killed and the hearts were removed. The left ventricular free wall and septum were frozen in liquid nitrogen and stored at −70°C until used. Total RNA was isolated from the samples using the RNeasy maxi kit (Qiagen, Valencia, CA). Relative mRNA abundance of the target genes was determined by real-time reverse transcriptase-polymerase chain reaction using a TaqMan model 7700 sequence detector (ABI-PerkinElmer, Foster City, CA) as described previously (Winer et al., 1999). Expression levels for each gene were normalized to ribosomal protein L19, which was unaffected by MI.

**HGF Administration.** One day after left coronary ligation/reperfusion, animals with ECG evidence of moderate-to-large infarcts were randomly assigned to receive either intravenous infusion of saline vehicle or recombinant human HGF (Genentech, Inc., South San Francisco, CA) at 0.45 mg/kg/day for 6 days by implanted osmotic pump (model 2001; Alza, Palo Alto, CA) with a catheter into the right jugular vein. The total dose of HGF was equivalent to the dose that produced beneficial effects in rats with myocardial ischemia/reperfusion (Nakamura et al., 2000). Sham-operated rats did not receive treatment. The animals were followed up for either 1 week or 8 weeks after the initiation of treatment. At 1 week, blood (1 ml) was collected for measurement of plasma HGF and serum biochemistry for liver and kidney function, and rats were killed and the heart and other organs were removed and weighed. At 8 weeks, hemodynamics and cardiac function were measured before blood collection and organ harvest as described above.

**Assessment of Hemodynamics and Cardiac Function.** Under anesthesia with ketamine (80 mg/kg) and xylazine (10 mg/kg) given intraperitoneally, rats were intubated and ventilated with a respirator. A catheter (polyethylene-10 fused with polyethylene-50) filled with heparin-saline (50 U/ml) was implanted into the abdominal aorta via the right femoral artery for measurement of mean arterial pressure (MAP) and heart rate (HR). After a right-sided thoracotomy, an ultrasonic perivascular flowprobe (2.58; Transonic Systems, Inc., Ithaca, NY) was placed around the ascending aorta (Yang et al., 1998). The catheter and flowprobe cable were exteriorized and fixed at the back of the neck. All rats were housed individually after surgery.

One day after implantation, MAP and HR were measured with a model P23 XL pressure transducer (Viggo-Spectramed, Oxnard, CA) coupled to a polygraph, and cardiac output was determined with a model T 201 flowmeter (Transonic Systems, Inc.) simultaneously in conscious, unrestrained rats. Stroke volume was calculated as cardiac output divided by HR, cardiac index as cardiac output divided by body surface area, systemic vascular resistance as MAP divided by cardiac index.

**Infarct Size Measurements.** Infarct size was determined by morphometric analysis in the end of the experiment after blood collection. The right ventricular free wall was dissected from the left ventricle. The left ventricle was cut in four transverse slices from apex to base. Five-micrometer sections were cut and stained with Massons’ trichrome stain and mounted (Yang et al., 1995). The endocardial and epicardial circumferences of the infarcted and noninfarcted regions were determined with a planimeter digital image analyzer. The infarcted circumference and the total left ventricular circumference of all four slices for both endocardial and epicardial surfaces were summed and expressed as a percentage of infarcted circumference to total circumference for determination of infarct size.

**Blood Assay.** Plasma concentrations of recombinant human HGF were measured using a specific sandwich enzyme-linked immunosorbent assay (Roos et al., 1995). The antibody used for the assay was specific for human HGF. Serum biochemistry was measured on a Monarch model 761 microcentrifugal chemistry analyzer (Instrumentation Laboratories, Lexington, MA).

**Statistical Analysis.** Results are expressed as mean ± S.E.M. One-way analysis of variance was performed to assess differences in parameters between groups. Significant differences were then subjected to post hoc analysis using the Newman-Keuls method. P < 0.05 was considered significant.
Results

Myocardial Gene Expression of HGF and c-Met after Cardiac Ischemia/Reperfusion. Our results (Fig. 1) are consistent with previous findings that showed that HGF and c-Met were induced in myocardial tissue after MI. The peak in c-Met expression occurred 1 day after ischemia/reperfusion, whereas HGF gene expression peaked at 3 days. The peak mRNA levels were increased by 4- and 8.3-fold for HGF and c-Met, respectively, in the MI group, compared with the sham-operated controls. The relative mRNA abundance of c-Met returned to near normal levels by 1 week. Similarly, HGF gene expression was substantially reduced from the peak level by 1 week, but then gradually returned to baseline levels over 1 month.

Effects of HGF Observed at 1 Week. HGF was administered to rats with MI, commencing the day after ischemia/reperfusion and continuing for 6 days. The total amount of HGF given was equivalent to the dose previously shown to produce beneficial results toward acute cardiac ischemic injury (Nakamura et al., 2000). On day 7 after the initiation of HGF infusion, there was a 100-fold increase in plasma levels of HGF in the HGF-treated MI rats compared with those

Fig. 1. Myocardial mRNA levels of HGF and c-Met. Data expressed as mean ± S.E.M. The number in the parentheses is the animal number in each group. **, P < 0.01, comparing the MI group to the sham group at 8 h to 14 days for HGF, and at 3 h to 7 days for c-Met.
Effects of HGF at 1 week

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>MI + Veh (n = 4)</th>
<th>MI + HGF (n = 5)</th>
<th>Sham (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>297.4 ± 4.1</td>
<td>296.8 ± 7.0</td>
<td>309.2 ± 10.7</td>
</tr>
<tr>
<td>LV/BW (mg/g)</td>
<td>2.61 ± 0.07</td>
<td>2.79 ± 0.11*</td>
<td>2.49 ± 0.02</td>
</tr>
<tr>
<td>KW/BW (mg/g)</td>
<td>3.47 ± 0.13</td>
<td>3.56 ± 0.14</td>
<td>3.47 ± 0.13</td>
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<tr>
<td>SW/BW (mg/g)</td>
<td>2.28 ± 0.19</td>
<td>2.57 ± 0.31</td>
<td>2.33 ± 0.19</td>
</tr>
<tr>
<td>AP (U/l)</td>
<td>210 ± 17</td>
<td>159 ± 10*</td>
<td>217 ± 9</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>108.5 ± 25.5</td>
<td>89.0 ± 28.2</td>
<td>87.3 ± 11</td>
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<tr>
<td>GGT (U/l)</td>
<td>1.00 ± 0.41</td>
<td>1.25 ± 0.63</td>
<td>0.67 ± 0.33</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>5.45 ± 0.40</td>
<td>7.50 ± 0.27*</td>
<td>5.37 ± 0.24</td>
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<tr>
<td>ALB (g/dl)</td>
<td>2.40 ± 0.11</td>
<td>3.35 ± 0.15**</td>
<td>2.50 ± 0.15</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>12.0 ± 1.4</td>
<td>11.0 ± 0.71</td>
<td>11.7 ± 1.5</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.18 ± 0.03</td>
<td>0.20 ± 0.03</td>
<td>0.13 ± 0.03</td>
</tr>
</tbody>
</table>

ALB, albumin; ALT, alanine amino-transferase; AMY, amylase; AP, alkaline phosphatase; AST, aspartate amino-transferase; BUN, blood urea nitrogen; Cr, creatinine; GGT, \( \gamma \)-glutamyltranspeptidase; KW, kidney weight; LW, liver weight; SW, spleen weight; TP, total protein; Veh, vehicle; VW, ventricle weight.

* \( P < 0.05 \), ** \( P < 0.01 \), compared with the sham group.

Discussion

In the present study, vehicle-treated MI rats had depressed cardiac function that was characterized by reduced cardiac index and stroke volume index and increased systemic vascular resistance, indicating that heart failure developed in this animal model. Intravenous infusion of HGF, for 6 days beginning the day after ischemia/reperfusion, resulted in a significant improvement in cardiac performance, measured 8 weeks post-MI. Compared with rats with vehicle-
treated MI, animals receiving HGF had significantly higher cardiac index and stroke volume index and reduced systemic vascular resistance. These three parameters were improved to near normal levels. To our knowledge, this is the first demonstration that HGF improves cardiac function in conscious animals with heart failure induced by MI.

The premise for this work was based on the observations that myocardial HGF and c-Met were induced for several days after MI (Ono et al., 1997; Ueda et al., 2001) and that the peak levels occurred well past the acute phase of injury. Our hypothesis was that local HGF production may have beneficial effects on long-term outcome and that supplemental addition of HGF could enhance these effects.

HGF was previously shown to be cardioprotective toward acute cardiac ischemia-reperfusion injury. Ueda et al. (1999) showed that gene transfection of HGF into rat hearts attenuated subsequent ischemia injury. Nakamura et al. (2000) later found that neutralizing antibodies to HGF administered to rats before and after coronary artery ligation and reperfusion resulted in an increase in infarct size (Nakamura et al., 2000). They further showed that HGF administration, initiated immediately after a short period (20 min) of cardiac ischemia followed by reperfusion, decreased infarct size, and increased short-term cardiac performance, measured 48 h postsurgery. This effect was attributable to the ability of HGF to suppress cardiomyocyte apoptosis.

The purpose of this study was to determine the effects of HGF on the progression of MI to heart failure, thus a longer period of ischemia (2 h) was used and the onset of treatment was delayed until the day after coronary artery ligation. The mechanism by which HGF treatment improved cardiac performance in this model remains to be determined; however, the possibility of a reduction in apoptosis might be considered although infarct size was unaffected by the treatment. Myocyte cell death contributes not only to infarct size but also to myocardial remodeling after MI (Colucci, 1996; Fliss and Gattinger, 1996). Major pathophysiological events after acute MI include hypertrophic responses of cardiomyocytes in the surviving portion of the ventricle, followed by ventricular dilation characterized by a diminished cardiac function, and then heart failure. The underlying mechanism responsible for cardiac dilation has been linked to myocyte cell death in the surviving regions (Cheng et al., 1996; Ueda et al., 2001). As an antiapoptosis factor, HGF could maintain cardiac function by suppressing myocyte cell death (Ueda et al., 2001). In vitro studies have demonstrated that c-Met receptor expression is induced in cardiomyocytes and that HGF has cytoprotective effects on mature cardiac myocytes in a dose-dependent manner (Ueda et al., 2001). This indicates that HGF exerts a direct protective action on cardiac myocytes. The intracellular signals leading to antiapoptosis in
cardiac myocytes by HGF may be through the ERK pathway but not PI3-Akt pathway (Nakamura et al., 2000; Ueda et al., 2001). A recent clinical study suggests HGF may play an important role in human heart failure. It has been found that cardiac HGF secretion remains enhanced up to 4 weeks after infarction in patients with MI (Yasuda et al., 2000). The HGF secretion from infarct regions correlates inversely with the variables of heart function, i.e., the patients post-MI with enhanced cardiac HGF production in MI regions are associated with attenuated ventricular remodeling and improved cardiac function, indicating the HGF system may play a cardioprotective role during ventricular remodeling.

Because of the significant induction of endogenous cardiac HGF and c-Met post-MI, it is tempting to assume supplemental HGF exerted its effects directly on the heart. HGF is an important mitogen for hepatocytes, however, and we observed an approximate 50% increase in LW/BW in the MI + HGF group 1 week post-MI. The serum levels of total protein and albumin in HGF-treated rats were also 1.4 times that of rats with vehicle-treated MI. Thus, we cannot rule out the possibility that the results we observed on cardiac performance were secondary to HGF effects on liver growth and plasma protein levels. However, at the time, cardiac performance and hemodynamics were measured (8 weeks post-MI), there was no significant difference in LW/BW between the HGF-treated and vehicle-treated MI groups.

HGF production is influenced by the renin-angiotensin system (Nakamura et al., 1996; Yasuda et al., 1999). Angiotensin II plays an important role in the pathophysiology of heart failure, elevating vascular resistance, enhancing sympathetic activity, promoting cardiac hypertrophy and enlargement, and/or increasing water retention. More importantly, angiotensin II at the tissue level contributes to the modulation of heart and vessel remodeling (Yasuda et al., 1999). Clinical evidence indicates that blockade of the renin-angiotensin system can improve hemodynamics, cardiac remodeling, and performance, relieve symptoms, and reduce mortality in patients with congestive heart failure. Recent studies have shown that angiotensin II is a strong suppressor of HGF (Nakamura et al., 1996), and HGF production is significantly impaired in patients with chronic congestive heart failure post-MI (Yasuda et al., 1999). Treatment with ACE inhibitors (ACE-I) restores the impaired HGF production in these patients, suggesting that the restoration of HGF production may play a role in mediating the functional repair process of ACE-I (Yasuda et al., 1999). Our findings may provide direct evidence that supplemental addition of HGF significantly attenuates development of heart failure after moderate-large myocardial infarction.

Although the data presented herein are generally supportive of the use of systemically administered HGF for treatment of ischemic heart disease or heart failure, there are several potential problems that may be associated with this therapeutic approach. First, HGF administration can promote liver growth. It was previously shown that exogenous HGF caused a dose-dependent increase in liver weight in normal and partially hepatectomized animals (Fujitaka et al., 1993; Ishii et al., 1995; Roos et al., 1995). The mechanism for this effect is HGF-induced hepatocyte proliferation, although a trophic effect may also be involved (Roos et al., 1995). Our results showed that intravenous infusion of HGF for 1 week increased liver weigh by 57% in MI rats, but there was no difference in liver weight between vehicle- and HGF-treated MI rats 7 weeks after dosing, suggesting that liver overgrowth induced by HGF is reversible. In addition, the HGF-induced liver enlargement was associated with normal liver/kidney function. Furthermore, HGF has been shown to attenuate hepatic ischemia-reperfusion injury and liver dysfunction in rats (Sakakura et al., 2000; Oe et al., 2001).

Taken together, these data suggest that if the effects of HGF on the liver growth are minimized by dosing, they may be acceptable with caution.

A second issue to consider is the effects of HGF on serum biochemistry. Consistent with previous studies (Ishii et al., 1995; Roos et al., 1995), the present study showed exogenously administered HGF elevated serum total protein and albumin levels and reduced serum alkaline phosphatase. It is known that the liver is the major source of serum proteins. The detailed mechanism by which HGF increases the contents of serum proteins synthesized in the liver is unclear, but it is likely that HGF may stimulate albumin synthesis in hepatocytes through transcriptional activation (Ishii et al., 1995). HGF has also been reported to increase serum prothrombin and hepaplastin in addition to albumin (Ishii et al., 1995). These effects of HGF could result in a hypercoagulable state, and this is an area where further research is warranted. The mechanism for the HGF-induced reduction of alkaline phosphatase is unknown (Roos et al., 1995). Finally, because stimulation of c-Met by its ligand HGF can lead to angiogenesis, proliferation, enhanced cell motility, invasion, and eventual metastasis (Maulik et al., 2002), HGF should not be administered to patients with active neoplasms.

In summary, HGF treatment for 6 days beginning the day after myocardial ischemia/reperfusion enhanced cardiac function without altering infarct size, measured at 8 weeks post-MI. The data suggest that HGF exerts beneficial effects in experimental heart failure, however, the mechanism for the effect of HGF is unclear. Histological analysis showed no qualitative difference between HGF- and vehicle-treated groups with regard to vascularization in the infarcts and viable myocardia. This does not seem to be consistent with the hypothesis that myocardial angiogenesis contributes to the HGF-induced improvement in cardiac performance. Subsequent studies are needed to elucidate the direct protective mechanism of HGF on cardiomyocytes in ischemia cardiomyopathy and to determine whether systemic administration of HGF induces myocardial angiogenesis. In addition, because HGF production may be linked to benefits of treatment with ACE inhibition in heart failure, further studies are also warranted to test a possible cumulative effect of HGF and ACE inhibition.

References


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