Exercise-Induced Hepatocyte Growth Factor Production in Patients After Acute Myocardial Infarction — Its Relationship to Exercise Capacity and Brain Natriuretic Peptide Levels —

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**Background** The hepatocyte growth factor (HGF) is a multifunctional cytokine with cardioprotective properties and potent myogenic activity for vascular endothelium. In patients after acute myocardial infarction, exercise training has the beneficial effects on cardiovascular adaptations. We hypothesized that exercise induces HGF production in those patients. If this hypothesis is correct, HGF production may be associated with clinical parameters of cardiovascular function.

**Methods and Results** In 20 patients after acute myocardial infarction, HGF levels in the pulmonary artery (HGFPA) and aorta (HGF Ao) were determined at rest and during supine submaximal exercise, with cardiac output (CO) measured by catheterization. Exercise-induced HGF production was calculated by using the following equation: 

\[(HGFPA - HGF Ao) \times CO\] during exercise – \[(HGFPA - HGF Ao) \times CO\] at rest. On a separate day, peak oxygen uptake (VO2) was determined during a symptom-limited upright cardiopulmonary exercise test. Exercise increased HGF production (from 1.6±3.0 to 9.0±6.3 ng/ml, p<0.001). Exercise-induced HGF production was inversely related to peak VO2 (r=−0.664, p<0.01) and positively related to levels of brain natriuretic peptide (BNP), a biochemical marker for post-infarction ventricular remodeling (r=0.686, p<0.01).

**Conclusions** Exercise significantly increases HGF production. This phenomenon may play an important role in post-infarction patients, particularly with reduced exercise tolerance and elevated BNP levels. (Circ J 2004; 68: 304 – 307)

**Key Words:** Exercise; Growth substances; Myocardial infarction; Rehabilitation

Hepatocyte growth factor (HGF), originally identified and cloned as a potent mitogen for hepatocytes, has mitogenic, motogenic, morphogenic, and anti-apoptotic activities in a variety of cells through its receptor, c-Met.1,2 HGF is a unique growth factor to act protectively against endothelial dysfunction3–5 myocardial ischemia/infarction and remodeling6–8. Thus, the HGF system (HGF and its receptor c-Met) is attracting increasing attention in the field of cardiovascular pathophysiology.9

In patients with acute myocardial infarction (AMI), exercise training has the beneficial effects on cardiovascular systems10 Cardiac effects include attenuation of post-infarction ventricular remodeling11,12 for which brain natriuretic peptide (BNP) is a useful biochemical marker.13 Vascular effects include an increase in the density of skeletal-muscle capillaries14 and improvement in endothelial-dependent vasodilation15,16 which are important determinants for exercise tolerance and symptoms.

In the present study, we hypothesized that exercise induces HGF production, mediating the beneficial effects of exercise training in patients with AMI. If so, HGF production may be associated with clinical parameters of cardiovascular function.

**Methods**

**Study Patients**

The study group included 20 male patients (aged 61±12 years [mean±SD]) after AMI. The infarction site was anterior in 13 patients (65%), and inferior/lateral in 7 patients (35%). All patients underwent reperfusion therapy (percutaneous transluminal coronary angioplasty in 17 patients and intravenous administration of tissue-type plasminogen activator in 3 patients) on admission. The peak level of serum creatine kinase was 2,995±2,043 [mean±SD] U/L. The severity of heart failure ranged from New York Heart Association functional class I to II. The baseline patient characteristics are summarized in Table I. No patients had liver (elevated levels of aminotransferases), kidney (elevated levels of creatinine or urea), or lung dysfunction (restrictive or obstructive pattern in spirometry). No patients had prior myocardial infarction. Medications remained unchanged during the entire study.

The study was approved by the institutional review committee. The protocol was fully explained, and all patients gave their written informed consent to participate in the study.
Cardiac Catheterization and Supine Exercise Test

From the right brachial artery through a 6F sheath, chronic phase coronary angiography and left ventriculography were performed according to the conventional Judkins technique, 28±7 [mean ± SD] days after the onset of myocardial infarction. Heparin was initially administered at a dose of 5,000 IU into the distal brachial artery. For angiographic evaluation of left ventricular volumes, ventricular silhouettes in 30° right anterior oblique projections were digitized with an ANCHOR ventriculography analysis system (Siemens-Elema, Solna, Sweden). By the area-length method, the left ventricular end-systolic and end-diastolic volume indices and ejection fraction were calculated. Left ventricular pressure was measured with a 2F high-fidelity micromanometer catheter (model SPC-320; Miller Instruments, Houston, TX, USA). The pressure was read at 492 nm using a plate reader. The sensitivity of this HGF kit was 0.1 ng/ml. This assay system detects only HGF. HGF was measured by this assay system and those of rat hepatocytes in primary cultures. Previous studies have demonstrated that HGF levels measured by this assay system and those of rat hepatocytes in primary cultures.

Cardiopulmonary Exercise Test

On a separate day (3±1 [mean ± SD] days before the cardiac catheterization), patients underwent the symptom-limited cardiopulmonary exercise test (CPX), with determination of peak oxygen uptake (V˙O₂), workload and heart rate. The exercise test was performed on a calibrated, electronically braked bicycle in an upright position (Examiner, Lode B.V., Groningen, Netherlands). Ramp protocols began at a workload of 0 W for 1 min and increased in 15-W increments at 1-min intervals.Expired gas analysis was performed using a respiromonitor AE-280 (Minato Products, Tokyo, Japan). The V˙O₂ was measured on a breath-by-breath basis, and was averaged over contiguous 30-s intervals, except at peak exercise, when 18-s averaging was used.

Hepatocyte Growth Factor Measurements

Hepatocyte growth factor levels in the pulmonary artery (HGFPA) and aorta (HGFAo) were determined with specific enzyme-linked immunosorbent assay kits (Otsuka Assay Laboratories, Tokushima, Japan). Microtiter plates coated with an anti-HGF murine monoclonal antibody were incubated with standard HGF or serum samples, and an anti-HGF rabbit polyclonal antibody was added. After adding the anti-rabbit goat immunoglobulin G-peroxidase conjugate and the substrate, the absorbance was read at 492 nm using a plate reader. The sensitivity of the HGF kit was 0.1 ng/ml. This assay system detects only HGF. This assay system detects only HGF. Hepatocyte growth factor levels in the pulmonary artery (HGFPA) and aorta (HGFAo) were determined with specific enzyme-linked immunosorbent assay kits (Otsuka Assay Laboratories, Tokushima, Japan). Microtiter plates coated with an anti-HGF murine monoclonal antibody were incubated with standard HGF or serum samples, and an anti-HGF rabbit polyclonal antibody was added. After adding the anti-rabbit goat immunoglobulin G-peroxidase conjugate and the substrate, the absorbance was read at 492 nm using a plate reader.

Data Analysis

Exercise-induced HGF production (µg/min) was calculated by using the following equation:

\[ [(HGFPA - HGFAo) \times \text{CO at peak exercise}] - [(HGFPA - HGFAo) \times \text{CO at rest}] \]
The $\chi^2$ test was used for comparison of categorized variables. The Student’s t-test or Mann-Whitney U-test rank test was used for comparisons of mean values to determine significance of difference between the 2 groups. Linear regression curves and correlations were calculated according to the least squares method. All data are presented as mean ± SD. Differences were considered significant at $p<0.05$.

Results
Changes in Hemodynamics and HGF in Response to Supine Exercise
Table 2 shows the changes in hemodynamics and HGF levels, at baseline (= before exercise) and at peak exercise during the catheterization. At baseline, there were no significant differences in HGF levels between PA and Ao. The supine exercise (74±18 W in intensity, 10±2 min in duration) significantly increased heart rate, Ao pressure, PA pressure, and CO, whereas it decreased SV•O$_2$. Although the absolute HGF levels in PA and Ao appear unchanged, the difference in HGF levels between PA and Ao ($\Delta$HGF) significantly increased by approximately 3-fold after the exercise. When assessed based on the fold change compared with the baseline level, exercise increased the HGF$_{PA}$ to 1.02±0.11-fold (p<0.05), but did not change HGF$_{Ao}$ (0.96±0.09-fold). Finally, in the patients of the present study, exercise-induced HGF production ([HGF•CO at peak exercise]–[HGF•CO at baseline]) was calculated to be 7.4±6.3μg/min, on average.

Correlations With HGF Production
Peak VO$_2$, workload and heart rate determined during the symptom-limited upright cardiopulmonary exercise text (CPX) performed on a separate day were 23±7 ml/min per kg, 130±37 W, and 140±24 beats/min, respectively.

As shown in Fig 1, exercise-induced HGF production correlated inversely with peak VO$_2$ (r=-0.664, p<0.01). Eight patients with peak VO$_2$<20 ml/min per kg had greater exercise-induced HGF production (13.4±5.9 vs 3.9±2.4μg/min, p<0.05) and higher prevalence of angiographically significant stenosis in major coronary arteries (>60%) (50 vs 8%, p<0.05) in comparison with the remaining 12 patients with VO$_2$≥20 ml/min per kg.

Also, as shown in Fig 2, exercise-induced HGF production correlated positively with BNP levels at baseline (r=0.686, p<0.01). However, there were no significant relations with cardiac function at rest (left ventricular end-diastolic volume index and ejection fraction), percentage increase in heart rate, Ao pressure and PA pressure in response to the submaximal supine exercise (data not shown).

Discussion
The major finding of the present study is that HGF production is induced during exercise in accordance with the severity of exercise intolerance and the increase in BNP levels. In patients after AMI, exercise training is now emerging as an important component of the therapy. It attenuates post-infarction ventricular remodeling, which is associated with heart failure and increased mortality, and is accompanied by an elevated level of BNP. Regular exercise training also increases the density of skeletal muscle capillaries and induces repetitive increases in vascular blood flow and shear stress, thereby improving endothelium-dependent vasodilation. Both central (cardiac) and peripheral (skeletal muscle and vascular) effects of exercise training may consequently improve exercise tolerance and symptoms. From the data obtained in the present study, a causal relationship cannot be clearly determined. However, the several effects of exercise training are potentially mediated through HGF in patients after AMI.

As shown in Table 2, exercise increases the concentration gradients of HGF levels between PA and Ao, indicating exercise-induced HGF production. The vessel wall may be a potential source of circulatory HGF. Fig 1 shows that exercise-induced HGF production is associated with reduced peak VO$_2$. In particular, patients with peak VO$_2$<20 ml/min per kg were sensitive towards the HGF response to exercise. These patients with reduced exercise capacity had a higher prevalence of coronary artery stenosis. Myocardial ischemia appears to be one of the determinants for exercise capacity and is known to induce upregulation of non-cardiac HGF systems. HGF promotes angiogenesis as a potent growth factor of endothelial cells and promotes the functional recovery of nitric-oxide-mediated vasodila-
tion thus improving myocardial blood flow. Also, as shown in Fig 2, exercise-induced HGF production is associated with increased levels of BNP. The HGF may be systemically released during exercise in response to left ventricular dysfunction and may exert wound healing and systemic effects.

In a mouse myocardial infarction model, HGF appears to enhance myocardial blood flow. Also, as gene therapy attenuated left ventricular remodeling and dysfunction.

The present study provides a novel aspect of exercise training as cytokine-mobilization. HGF may have a therapeutic implication in patients after AMI.

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References