Dilated cardiomyopathy is one of the major causes of severe heart failure and is an indication for heart transplantation. Hamsters with inherited dilated cardiomyopathy are a well-known model of human dilated cardiomyopathy.\textsuperscript{1,2} Hepatocyte growth factor (HGF) is a mesenchyme-derived pleiotropic factor that has potent angiogenic and antifibrotic action.\textsuperscript{3-4} Further, administration of human recombinant HGF prevented fibrosis in liver and pulmonary injury models.\textsuperscript{5-7} HGF also has important roles in tumor growth and tumor angiogenesis.\textsuperscript{8}

Electroporation has been widely used to introduce DNA into various cell types in vitro. Gene transfer by in vivo electroporation has been effective for introducing DNA into animal tissues.\textsuperscript{10,11} Electroporation into skeletal muscle has been used for muscular disease\textsuperscript{12} and for the systemic delivery of bioactive proteins.\textsuperscript{13-16} Gene transfection into skeletal muscle has been used for systemic delivery of therapeutic proteins for liver\textsuperscript{6} and cardiac diseases.\textsuperscript{17-21} The goal of this study was to test the hypothesis that exogenous HGF protein might improve the deleterious changes in myocardial function and structure in the hamster with dilated cardiomyopathy.

Key Words: cardiomyopathy ■ genes ■ growth substances ■ hamsters ■ heart failure

Methods

Animals, Plasmid DNA, and Experimental Protocols

Male cardiomyopathic TO-2 hamsters and healthy F1b hamsters aged 10 weeks were obtained from BIO breeder, Inc (Watertown, Mass). Hamsters were handled according to animal experiment guidelines at our institute. Rat-HGF cDNA cloned by polymerase chain reaction was inserted into the unique Xho I site between the cytomegalovirus immediate early enhancer-chicken b-actin hybrid promoter and rabbit b-globin poly A site of the pCAGGS expression plasmid.\textsuperscript{9} The resulting plasmid, pCAGGS-HGF, was grown in Escherichia coli DH5a. The plasmid was purified with plasmid DNA kit (Quiagen). For electroporation of the DNA, we inserted needles into the bilateral anterior tibialis muscles and delivered electrical pulses \(6 \times \) each at 100 V and 50 ms with an electrical pulse generator (Electro Square Porator T820, BTX).

In a preliminary experiment of electroporation using 10 F1b hamsters, 6 of 10 hamsters had slight plasmid buffer leakage. In the inclusion experiment, we injected plasmid into 25 TO-2 hamsters aged 11 weeks. Four of 25 had buffer leakage. We measured plasma HGF level 3 days after the first electroporation of 800 μg of HGF plasmid (day 0) to determine the success of the procedure. Inclusion criterion was a plasma HGF level >5.0 ng/mL. Twelve TO-2 hamsters treated with HGF fulfilled the criterion and received the following electroporations and examinations.
Effect of Electroporation (Days 0, 7, 14) on Serum HGF Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Day −7</th>
<th>Day 7</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1b</td>
<td>12</td>
<td>0.08±0.05</td>
<td>0.09±0.05</td>
<td>0.09±0.06</td>
</tr>
<tr>
<td>Placebo</td>
<td>12</td>
<td>0.11±0.07</td>
<td>0.11±0.05</td>
<td>0.13±0.06</td>
</tr>
<tr>
<td>HGF</td>
<td>12</td>
<td>0.11±0.08</td>
<td>19.0±2.1†‡</td>
<td>20.2±2.2†‡</td>
</tr>
</tbody>
</table>

*Significantly (P<0.05) different from respective value in the control F1b group.
†Significantly (P<0.05) different from respective value in the placebo group.
‡Significantly (P<0.05) different from the value at day −7 in the HGF group.

We administered HGF plasmid (800 μg per animal) to TO-2 hamsters on days 0, 7, and 14 (HGF group, n = 12). We administered empty pCAGGS to control TO-2 at similar time points (placebo group, n = 12). We used F1b hamsters without any treatment as age-matched normal control (control group, n = 12). We determined the changes in serum HGF levels on days −7, 7, and 21 using an ELISA kit and conducted echocardiography for cardiac function (days −7 and 21) and pathology for cellular changes (days 21).

Echocardiography
Echocardiographic studies in each group under anesthesia were performed with leading-edge method on days −7 and 21. Left ventricular (LV) ejection fraction was calculated using the Pombo formula: (EDD3−ESD3)/EDD3, where EDD is end-diastolic dimension and ESD is end-systolic dimension. Cardiac output (CO) was calculated as CO=aortic velocity time integral×(π×[LV outflow tract/2]). Arterial pressure was measured with a polyethylene catheter inserted into the carotid artery after echo-Doppler studies on day 21. Mandibular wall stress σ was estimated as σ=arterial pressure×[ID(1+PWT/ID)], where ID is internal dimension, and PWT is posterior wall thickness. Systemic vascular resistance (SVR) was calculated as SVR=mean arterial pressure/CO.

Pathology and Tissue Biochemistry
Body weight and LV weight were measured on day 21. Transverse sections of the ventricle were stained with hematoxylin-eosin and Masson trichrome staining. Muscle fiber diameter was evaluated in cross sections that included a nuclear profile. A digital image analyzer (Mac SCOPE, Mitani Co.) was used to calculate percent fibrosis area. Capillary density was determined with anti-von Willebrand antibody staining. The hydroxyproline content of the myocardium was measured according to the method described by Green and Reagan. Matrix metalloprotease-1 (MMP-1) was evaluated by a collagenase type I activity test kit.

Statistics
Data are presented as mean±SD. Statistical analysis between the groups was performed by 1-way ANOVA followed by Bonferroni/Dunn method. Differences were considered significant at P<0.05.

Results

Serum Levels of HGF After Electroporation
Serum HGF levels on days 7 and 21 were significantly (P<0.05) higher in the HGF group than in the placebo and control groups (Table). Serum HGF levels did not differ when comparing the placebo and control groups.

Effect of HGF on Hemodynamics and Myocardial Parameters
There were no significant differences in EDD, LV ejection fraction, and PW thickening between the control, placebo, and HGF groups on day −7 (Figure 1). EDD tended to increase in the placebo group, and EDD was larger in the placebo group than in the HGF and control groups on day 21 (Figures 1 and 2). LV ejection fraction and PW thickening tended to decrease in the placebo and HGF groups, but were higher in the HGF group than in the placebo group on day 21. Thus, treatment by HGF seemed to prevent the development of systolic dysfunction in cardiomyopathy.

Doppler echocardiography revealed higher peak and steeper deceleration of the peak early diastolic filling velocity (E wave), that is, the restrictive pattern of mitral inflow in placebo hamster (Figure 3). In HGF hamsters, amplitudes of E and the peak filling velocity at atrial contraction (A wave) became similar, and E wave steepness became smaller (ie, pseudonormalization pattern of mitral inflow was observed). E/A ratio was higher in the placebo group than in the control and HGF groups (Figure 3). Isovolumic relaxation time was shorter in the placebo group than in the control and HGF groups. Deceleration time of E wave was shorter in the placebo group than in the control and HGF groups, and deceleration rate of E wave or E wave amplitude divided by deceleration time of E wave was greater in the placebo group than in the control and HGF groups. Thus, treatment by HGF seemed to reverse LV diastolic dysfunction in cardiomyopathy.

Mean arterial pressure ([in mm Hg] F1b 93.9±5.6, placebo 88.9±5.1, HGF 88.4±6.1) and heart rate ([in bpm] F1b 395±26, placebo 403±28, HGF 389±24) were similar among the 3 groups. LV wall stress was significantly higher in the placebo group than in the control and HGF groups (Figure 4). CO was lower in the placebo group than in the control and HGF groups. SVR was higher in the placebo group than in the control and HGF groups. Thus, hemodynamic parameters seemed to be preserved in the HGF group.
There were no differences in body weight among the 3 groups (Figure 5). LV weight/body weight ratio and LV weight/tibial length ratio were significantly higher in the placebo group than in the control group. Thus, the development of LV hypertrophy seemed to be prevented in the HGF group. However, myocardial diameter was similar among the 3 groups. Thus, this LV hypertrophy did not seem to be derived from myofibrillar hypertrophy.

Histological Analysis
Macroscopic imaging revealed LV cavity dilatation and fibrosis in a heart slice from placebo hamster compared with control F1b hamster (Figure 6). A heart slice image from HGF hamster showed smaller LV cavity and less fibrotic area compared with a placebo heart (LV internal diameter [in mm]: control F1b 4.4, placebo 6.3 mm, HGF 4.8 mm). Although fibrosis area was larger in the placebo and HGF groups than in the control group, the increase was attenuated in the HGF group (Figure 7). Tissue hydroxyproline content, an index of fibrosis, was higher in the placebo and HGF groups than in the control, but again, the increase was attenuated in the HGF group. Although myocardial capillary density was lower in the placebo and HGF groups than in the control, the density was higher in the HGF group than in the placebo group. MMP-1 activity was higher in the HGF group than in the control and placebo groups. There was a negative correlation between MMP-1 activity and % fibrosis area ($r = -0.62, r^2 = 0.39, P < 0.05$). There was a positive correlation between MMP-1 activity and capillary density ($r = 0.48, r^2 = 0.24, P < 0.05$).

Discussion
The present study demonstrated that (1) serum rat-HGF levels increased following in vivo electroporation of rat-HGF plasmid into the skeletal muscle of cardiomyopathic hamsters; (2) LV systolic and diastolic functional deterioration was attenuated in the HGF group; (3) LV hypertrophy was prevented in the HGF group; (4) LV cavity dilatation and fibrosis were attenuated in the HGF group; (5) myocardial capillary density was higher in the HGF group than in the control and placebo groups; and (6) tissue hydroxyproline content was higher in the placebo and HGF groups than in the control, but again, the increase was attenuated in the HGF group.
uated in cardiomyopathic hamsters treated with HGF; (3) the extent of cardiac hypertrophy and fibrosis was attenuated in cardiomyopathic hamsters treated with HGF; and (4) myocardial capillary density and MMP-1 activity were higher in the myocardium of the hamsters treated with HGF than in that of hamsters given placebo. HGF has been known to possess a remarkable potential for angiogenesis. Systemic HGF might have decreasing effects on afterload via the neoangiogenesis and vascular dilatation. However, in the present study, improvements in cardiac systolic and diastolic function were achieved without reduction in arterial pressure. Accordingly, antifibrosis effects of HGF might attenuate the progress of cardiomyopathy.

Cardiac dysfunction of cardiomyopathic hamster is an inherited condition caused by an autosomal recessive mutation in the gene for δ-sarcoglycan. Although the physiological consequences of the genetic defect remain unclear, investigators have demonstrated that these animals display calcium handling abnormalities, inhomogeneous capillary flow, and microvascular spasm. HGF might exert beneficial effects on the cardiovascular system via potentiation of angiogenesis and vasodilation against vascular spasm and antifibrosis action against fibrosis subsequent to calcium overload and ischemia.

In this study, elevated levels of HGF might result in prevention of the progress of LV systolic dysfunction, which was measured by echocardiography. Moreover, HGF might reverse the impaired relaxation measured by E/A ratio and isovolumic relaxation time and impaired LV filling measured by deceleration time of E wave and deceleration rate of E wave.

![Figure 4. Hemodynamic parameters on day 21 in each study group. Data are expressed as mean±SD. MAP indicates mean arterial pressure; \( \sigma \), stress. \(*P<0.05\) vs \( \text{F1b} \). \( \dagger P<0.05 \) vs placebo.](image1)

![Figure 5. Pathological parameters on day 21 in each group. Data are expressed as mean±SD. BW indicates body weight; LVW, left ventricular weight; TL, tibialis length. \(*P<0.05\) vs \( \text{F1b} \).](image2)
wave. Furthermore, HGF might reverse deteriorated hemodynamics. HGF might attenuate the increase in fibrosis and hydroxyproline and the decrease in capillary density in the myocardium of cardiomyopathy.

HGF is a potent activator of MMP-1, leading to collagen type I degradation and contributing to matrix restructuring before angiogenesis.33,34 In studies of liver fibrosis,35 increased HGF resulted in a 2-fold increase in interstitial MMP-1 activity and suppression of collagen deposition. These data were consistent with our results, which demonstrated a 2-fold increase in myocardial MMP-1 activity with increased HGF. The negative correlations between MMP-1 activity and fibrosis area and positive correlations between MMP-1 activity and capillary density suggest that increased HGF may exert its beneficial effects via stimulation of MMP-1.

The TO-2 cardiomyopathic hamster experiences rapid progression of heart failure with increasing age. Ryoke et al demonstrated that treatment of young TO-2 hamsters with growth hormone showed beneficial effects on cardiovascular function, whereas treatment of older TO-2 hamsters had little effect.2 Growth hormone decreases collagen type I in the failed heart.36 If this is the case, HGF might not have protective effects on the myocardium with advanced cardiomyopathy in older hamsters. Thus, the beneficial effects of exogenous HGF in our model might be dependent on a hamster’s younger age or milder status of cardiomyopathy.

HGF decreased arterial pressure in rats.32 SVR significantly decreased in the HGF-treated hamsters compared with the placebo hamsters in the present study. However, arterial pressure did not change at all. The capillary density in the transfected tibialis anterior muscles was greater in HGF hamsters than in placebo hamsters in the present study (1345±95 versus 1163±117 number/mm², P<0.05, n=5). However, the capillary density in other muscles such as gastrocnemius and quadriceps femoris muscles did not show differences. Differences in the density of HGF receptors...
between the heart and skeletal muscles or differences in response thresholds for systemic HGF protein might be attributable to the observed differences in the changes in capillary densities, and unchanged capillary density might explain the unchanged arterial pressure. Increment in cardiac output might be the primary cause of decrease in vascular resistance. The point of the present study was the improvement in cardiac systolic and diastolic function, and myocardial interstitial fibrosis without significant changes in blood pressure. Thus, antifibrotic action of HGF played an important role in the treatment of dilated cardiomyopathy.

Previous studies have demonstrated that the use of electroporation with a plasmid vector for interleukin (IL)-5 gene transfection resulted in a significantly elevated serum IL-5 level that persisted for at least 3 weeks. Further, electroporation of the IL-10 gene into skeletal muscle using this vector resulted in the attenuation of the progression of autoimmune myocarditis and a decrease in mortality. In the present study, we demonstrated that transfection of HGF gene into skeletal muscle resulted in systemic delivery of HGF protein and improved myocardial function and structure in animal model of idiopathic dilated cardiomyopathy.

**Perspectives**

Favorable effects of HGF on myocardial function and structure in the experimental cardiomyopathic heart should be used in the clinical situation. HGF gene and protein may be effective for the prevention of the progress of idiopathic dilated cardiomyopathy in humans and may decrease the number of candidates for heart transplantation. Systemic administration of HGF has risks for cancer proliferation or deterioration of diabetic retinopathy. Heart-specific local delivery of HGF is required for the future clinical application.

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