Gene transfection with human hepatocyte growth factor complementary DNA plasmids attenuates cardiac remodeling after acute myocardial infarction in goat hearts implanted with ventricular assist devices

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Background: Although a left ventricular assist device is often used to provide circulatory support until transplantation in severe heart failure, the mortality of long-term use of left ventricular assist devices remains high. We have shown that hepatocyte growth factor causes angiogenesis, antifibrosis, and antiapoptosis in the myocardium. Therefore, gene therapy with hepatocyte growth factor–complementary DNA plasmids may enhance the chance of “bridge to recovery.” In this study, we performed gene therapy with hepatocyte growth factor in the impaired goat heart with a left ventricular assist device.

Methods: Cardiac impairment was induced in 6 adult goats (56–65 kg) by ligation of the coronary artery, and ventricular assist devices were installed. The hepatocyte growth factor group (HGF; $n = 3$) was administered human hepatocyte growth factor–complementary DNA plasmid (2.0 mg) in the myocardium. The control group ($n = 3$) was similarly administered β -galactosidase plasmid. Four weeks after gene transfection, we attempted to wean all goats from the ventricular assist device.

Results: The myocardia transfected with human hepatocyte growth factor–complementary DNA contained human hepatocyte growth factor protein at levels as high as 1.0 ± 0.3 ng/g tissue 3 days after transfection. After weaning from the ventricular assist device, the HGF group showed good hemodynamics, whereas the control group showed deterioration. The percentage of fractional shortening was significantly higher in the HGF group than the control group (HGF vs control, $37.9\% \pm 1.7\%$ vs $26.4\% \pm 0.3\%$, respectively; $P < .01$). Left ventricular dilatation associated with myocyte hypertrophy and fibrotic changes was detected in the control group but not in the HGF group. Vascular density was markedly increased in the HGF group.

Conclusions: These results suggest that gene therapy with human hepatocyte growth factor may enhance the chance of bridge to recovery in the impaired heart supported with a ventricular assist device.

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Cardiac transplantation continues to be the destination therapy for patients with severe congestive heart failure (CHF). However, the overall applicability of cardiac transplantation is limited by a severe shortage of donors.¹ For many patients with severe CHF, pharmacologic therapy is insufficient, and revascularization or other surgical procedures are usually only palliative and do not

greatly reduce the overall ultimate mortality. Left ventricular assist devices (LVADs) are being used with greater frequency to provide circulatory support until transplantation can be achieved. Unfortunately, many patients are now being supported by these devices for several months and even years. Although improvements in LVADs have resulted in clinically meaningful survival benefits and an improved quality of life for patients with severe CHF, further improvements are needed.² There have been several recent reports of selected patients with end-stage CHF whose recovery of cardiac function with the LVAD was sufficient for the device to be explanted successfully.³⁻⁵ However, such patients constitute only a small percentage of patients with LVADs. The long-term outcome of recovery, the mechanism of recovery, and the identity of those patients who are capable of recovery remain unclear. Because the number of patients with severe CHF continues to increase, several efforts have been made to seek alternatives, such as regeneration therapy.

Hepatocyte growth factor (HGF) is a potent angiogenic agent with mitogenic, motogenic, and morphogenic effects through its own specific receptor, c-Met, in various types of cells, including myocytes.^{6,7} We have previously demonstrated that HGF exerts antifibrotic and antiapoptotic effects in the myocardium.⁸⁻¹⁰ Considering the pathogenic characteristics of severe heart failure, such as progression of fibrosis, progression of endothelial dysfunction, loss of functional capillaries, and apoptosis-related loss of contractile mass,^{11,12} HGF might have a beneficial effect in the impaired heart by attenuating these remodeling processes.^{13,14}

Therefore, gene transfection with the HGF gene may enable a “bridge to recovery” in the impaired heart that is supported with an LVAD. To investigate this possibility, we performed gene therapy with HGF in impaired goat hearts implanted with LVADs.

Materials and Methods

Preparation of Plasmid-Encoded Human HGF Complementary DNA

A human HGF (hHGF) was inserted into the *NotI* site of the pUC-SR α expression vector plasmid as described elsewhere.¹⁵ In this plasmid, expression of the hHGF complementary DNA (cDNA) is regulated under the control of the SR α promoter, which is composed of a simian virus 40 polyadenylation sequence. The purified plasmid containing 2000 μ g of hHGF-cDNA was reconstituted in sterile saline 2.0 mL and was directly injected into the myocardium at 10 points with a 2.5-mL syringe and a 30-gauge needle. The concentration of hHGF in the heart was determined by enzyme-linked immunosorbent assay (ELISA) with anti-hHGF antibody (Institute of Immunology, Tokyo, Japan). The antibody against hHGF reacts only with hHGF and not with goat HGF. The serum hHGF levels were also assessed with the same ELISA system at 1, 3, 5, 7, 14, and 28 days after cDNA injection.

Animal Model of Heart Impairment

Ten adult goats weighing 56 to 65 kg were used for this study. All animals were treated humanely in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (Publication No. 86-23, revised 1985).

With goats under general anesthesia with isoflurane and nitrous oxide, a thoracotomy was performed in the left fifth intercostal space. Polyethylene catheters were inserted into the thoracic aorta via the left carotid artery for measuring systemic blood pressure (BP) and into the left jugular vein for intravenous infusion. Fiber-optic pulmonary artery catheters (Oximetrix; Abbott Critical Care Systems, North Chicago, Ill) were placed in the pulmonary artery to allow mixed venous oxygen consumption (SvO₂) and pulmonary arterial pressure (PAP). Heart rate (HR) was continuously monitored by electrocardiography.

For measuring aortic blood flow and bypass flow, electromagnetic flowmeters (MF-2100; Nihon-Koden, Tokyo, Japan) were placed in the ascending aorta and LVAD outflow cannulas. Aortic blood flow was used as an index of native cardiac output, and the ventricular assist device (VAD) assist ratio was calculated as follows.

VAD assist ratio (%) = $\frac{\text{bypass flow (L/min)}}{\text{aortic blood flow [L/min]} + \text{bypass flow [L/min]}}$

The impaired heart was created by ligation of the left anterior descending (LAD) coronary artery distal to its first diagonal branch (Figure 1, A). After ligation, all goats underwent cardiogenic shock and developed severe arrhythmias, such as ventricular fibrillation and ventricular tachycardia. To maintain systemic circulation and unload the left ventricle (LV), an LVAD (Toyobo, Osaka, Japan) was installed extracorporeally between the left atrium and the descending aorta. A 1/2-inch vinyl chloride inflow cannula with multiple side holes was used for blood drainage, and a 1/2-inch vinyl chloride outflow cannula with a 12-mm woven graft (Meadox, Oakland, NJ) was used for blood return. This outflow cannula, sutured onto the descending aorta and inflow cannula, was inserted into the left atrium without cardiopulmonary bypass (Figure 1, B) after systemic heparinization (300 U/kg; intravenous injection). A right VAD (Toyobo) was installed extracorporeally between the right atrium and the pulmonary trunk in the same procedure (Figure 1, B). The goats were randomly divided into 2 groups. In the HGF group, hHGF-cDNA plasmid—2.0 mg of hHGF-cDNA in 2.0 mL of plasmid solution—was injected with 30-gauge needles into the myocardium at 10 points of the ischemic area of the LV wall (Figure 1, A). There were no changes in the hemodynamic conditions associated with the injection of hHGF-cDNA plasmid, and there were no obvious adverse effects, such as anaphylactic reaction, in the goats throughout this experiment. In the control group, an equivalent volume of β -galactosidase plasmid was injected in the same procedure. The chest was then closed, and goats were allowed to recover from anesthesia. To detect hHGF protein in the treated myocardium, 2 goats from each group were killed 3 days after the cDNA injection. In another 3 goats from each group, systemic circulation was subsequently maintained under biventricular assist devices (BVADs) for

Figure 1. Animal model of the impaired heart and experimental design. A, Creation of myocardial infarction in adult goat hearts by ligating the left anterior descending coronary artery provided direct administration (*crosses*) of plasmids encoding hHGF cDNA or β -galactosidase into the myocardium. B, Biventricular assist devices were installed in all goats with impaired hearts. C, Sites of ultrasonic crystals. Two ultrasonic crystals were implanted in the endocardium parallel to the short axis of the left ventricle at the level of papillary muscle at the time of operation.

4 weeks. Anticoagulation was performed 2 days after surgery. Warfarin sodium was administered with the target of the international normalized ratio, which ranged between 2.5 and 3.5. No platelet antiaggregation drugs were administered.

Assessment of Cardiac Function

We estimated the changes of cardiac function by means of 3-dimensional digital sonomicrometry (Sonometrics Corp, Ontario, Canada).¹⁶ Two ultrasonic crystals were implanted in the endocardium parallel to the short axis at the level of the papillary muscle at the time of operation (Figure 1, C). These crystals were placed in the anterior wall and its opposite site to assess myocardial contractility in the distribution of the LAD coronary artery. The LV dimension at end-diastole (LVDd) and end-systole (LVDs) were determined by simultaneously measured left ventricular pressure.

The LV percentage of fractional shortening (%FS) was calculated as follows:

$$\%FS = (LVDd - LVDs)/LVDd \times 100$$

Before and after ligation of the LAD coronary artery and at 1, 2, 3, and 4 weeks after gene transfection, we measured %FS and cardiac output with the BVAD turned off for short periods.

VAD Off Test

Four weeks after gene transfection, an attempt was made to wean all goats from BVAD. After systemic heparinization (300 U/kg; intravenous injection), the BVAD was turned off. At 5, 15, and 30 minutes after turning off the BVAD, we measured HR, BP, SvO_2 , PAP, cardiac output, and LVDd.

TABLE 1. Hemodynamic conditions under mechanical circulatory arrest

	Before MI	After MI	3 d	1 wk	2 wk	3 wk	4 wk
Heart rate (beats/min)							
HGF group	98 ± 2	59 ± 1	145 ± 5	146 ± 4	131 ± 9	121 ± 17	118 ± 17
Control group	86 ± 10	62 ± 10	140 ± 7	147 ± 4	134 ± 3	113 ± 28	90 ± 28
Mean AoP (mm Hg)							
HGF group	100 ± 10	59 ± 8	82 ± 3	82 ± 5	87 ± 5	94 ± 5	91 ± 5
Control group	96 ± 6	60 ± 10	80 ± 7	86 ± 9	97 ± 10	93 ± 10	96 ± 3

Data are expressed as mean ± SD. *MI*, Myocardial infarction; *HGF*, hepatocyte growth factor; *AoP*, aortic pressure.

Histologic Analysis

Four weeks after plasmid administration and after the VAD-off test, all goats were killed with an overdose of sodium pentobarbital, and the hearts were excised. The hearts were cut at the short axis into 5 pieces, and LV myocardium specimens were fixed with 10% buffered formalin and embedded in paraffin. A few serial sections from each specimen were cut into 5- μ m-thick slices and stained with hematoxylin and eosin for histologic examination and measurement of cardiomyocyte cell diameter or with azan-trichrome stain to assess the collagen content. The proportion of the fibrosis-occupying area at the border area neighboring the infarct area was measured on 10 randomly selected fields, and the result was expressed as the percentage of fibrosis.

To label vascular endothelial cells so that the blood vessels could be counted in the border area neighboring the infarct area, immunohistochemical staining of von Willebrand factor-related antigen was performed according to a modified protocol. We used enhanced polymer one-step staining (EPOS)-conjugated antibodies against von Willebrand factor-related antigen coupled with horseradish peroxidase (EPOS anti-human von Willebrand factor/horseradish peroxidase; DAKO, Carpinteria, Calif) as primary antibodies. The stained vascular endothelial cells were counted as vascular density under a light microscope at 200 $\times$ magnification by using at least 10 randomly selected fields per section. The result was expressed as the number of blood vessels per square millimeter.

Computer appraisals of pathology (cell diameter, percentage of fibrosis, and vascular density) were performed with a Macintosh computer (Apple Inc, Cupertino, Calif) by using a public domain image program developed at the US National Institutes of Health.

Statistical Analysis

All data are expressed as mean ± SD. Intergroup comparisons were made with analysis of variance and the unpaired Student *t* test. All analyses were performed with StatView (version 5.0; Abacus Concepts, Inc, Berkeley, Calif).

Results

In Vivo HGF Gene Transfection

Three days after transfecting hearts with hHGF-cDNA plasmid, we measured the hHGF protein content in the myocardial samples obtained from the cDNA-injected area by an ELISA assay. The myocardia transfected with the hHGF-cDNA contained hHGF protein at levels as high as 1.0 ± 0.3 ng/g tissue on the third day after transfection. In con-

trast, hHGF was not detected in the myocardia of the control group animals. The serum hHGF levels were not detected in either group throughout the experiment.

Animal Condition and Systemic Hemodynamic Data

Just after infarction, severe low-output syndrome and cardiogenic shock developed in all goats. Native cardiac outputs decreased approximately 20 or 30 mL · kg⁻¹ · min⁻¹. With BVAD support, all animals were maintained in good condition, and the HR and BP on unloaded conditions by VAD support did not differ between the 2 groups throughout this experiment (Table 1). The VAD assist ratio was maintained at approximately 70% throughout the experiment, and this ratio did not differ markedly between groups (Figure 2, A). However, at 4 weeks after the gene transfection, the cardiac output in the HGF group was significantly higher than that in the control group (85.0 ± 1.4 mL · kg⁻¹ · min⁻¹ in the HGF group vs 64.6 ± 6.0 mL · kg⁻¹ · min⁻¹ in the control group; *P* < .01; Figure 2, B).

Assessment of Cardiac Function

After infarction, the %FS was markedly decreased compared with the baseline values in both groups, and the degree of deterioration did not differ between groups. The %FS recovered gradually in the 2 groups after gene transfection. However, the %FS improvement in the HGF group was significantly larger than that in the control group. The %FS in the HGF group 4 weeks after gene transfection was significantly recovered compared with the control group (37.9% ± 1.7% in the HGF group vs 26.4% ± 0.3% in the control group; *P* < .01; Figure 3).

VAD Off Test

Four weeks after gene transfection, we performed a VAD-off test (Figure 4). HR was steadily increased and BP was steadily decreased in the control group on loaded condition after the BVAD was turned off, but in the HGF group, these parameters remained stable and did not deteriorate. The Svo₂ and the PAP of the control group also deteriorated relative to those of the HGF group. Cardiac output was significantly increased in the HGF group compared with the control group 30 minutes after weaning from VAD (80.1 ±

Figure 2. Comparison of VAD assist ratio (A) and native cardiac output (B) of the HGF group (diamonds) and the control group (circles) through this experiment. Data are presented as mean $\pm$ SD. * $P < .01$ versus the control group.

$6.2 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the HGF group vs $61.2 \pm 4.3 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the control group; $P < .05$; Figure 4). In the control group, LVDd was significantly increased relative to that in the HGF group 30 minutes after weaning from VAD ($35.8 \pm 1.6 \text{ mm}$ in the HGF group vs $46.9 \pm 0.1 \text{ mm}$ in the control group; $P < .05$; Figure 4).

Histologic Assessment

Macroscopic autopsy findings of expired hearts revealed that LV dilatation was markedly suppressed in the HGF group relative to that in the control group, although necrotic changes and scar formation of the anterior wall were recognized in both groups (Figure 5, A and B). Azan staining of the myocardium in the neighborhood of the infarcted area revealed that fibrous change was also suppressed in the

HGF group compared with that in the control group (Figure 5, C and D). The percentage of fibrosis was significantly reduced in the HGF group compared with the control group ($13.9\% \pm 1.7\%$ in the HGF group vs $22.3\% \pm 1.3\%$ in the control group; $P < .01$; Figure 6, A).

Hematoxylin and eosin staining of the border zone revealed hypertrophic change of cardiomyocytes in the control group, but not in the HGF group (Figure 5, E and F). The cell diameter was significantly smaller in the HGF group than in the control group ($39.6 \pm 0.5 \mu\text{m}$ in the HGF group vs $54.4 \pm 0.6 \mu\text{m}$ in the control group; $P < .01$; Figure 6, B).

Vascular density was examined in the border zone of the infarct area (Figure 5, G and H). Vascular density was significantly higher in the HGF group than in the control group (35.2 ± 2.1 vessels per field in the HGF group vs 24.5 ± 2.7 vessels per field in the control group; $P < .05$; Figure 6, C).

Figure 3. Comparison of wall contractile function as evaluated by the percentage of fractional shortening (%FS), which was calculated by sonomicrometry. The HGF group is indicated by diamonds and the control group by circles. Data are presented as mean $\pm$ SD. * $P < .01$ versus the control group.

Discussion

The major findings of this study were as follows: (1) LV unloading by VAD alone could not achieve sufficient suppression of cardiac remodeling after myocardial infarction, and (2) gene transfection with HGF-cDNA plasmids attenuated cardiac remodeling in the impaired heart under mechanical unloading with VAD and achieved markedly better improvement of cardiac function than that by VAD alone, thus suggesting its potential use as a bridge to recovery.

Recently, several studies of regenerative therapy with gene therapy or cell transplantation have reported the effect on protection of cardiomyocytes and improvement of cardiac function in the impaired heart.¹⁷⁻²⁰ However, such therapies require time to take effect and are not able to control heart failure immediately after treatment. VAD may not only support systemic circulation, but also provide an

Figure 4. Changes of hemodynamic conditions after weaning from VAD. Heart rate (A), systemic blood pressure (mean AoP) (B), mixed venous oxygen saturation (SvO₂) (C), pulmonary arterial pressure (mean PAP) (D), native cardiac output (E), and left ventricular end-diastolic dimension (LVDD) (F) were measured. Data are presented as mean $\pm$ SD. * $P < 0.05$ versus the control group.

optimal environment for myocardial recovery, along with ventricular unloading.^{2-5,21-23} We therefore propose a combination therapy consisting of gene therapy and VAD as a new strategy for the treatment of severe heart failure. VAD provides sufficient time and suitable circumstances for myocardial regeneration, and regenerative therapy promotes

myocardial recovery in the impaired heart, thus resulting in the increase of a bridge to recovery.

HGF is a potent angiogenic factor, and we have started its clinical application at Osaka University Hospital for patients with arteriosclerosis obliterans.²⁴ Furthermore, HGF not only is an angiogenic factor, but also shows

Figure 5. Histologic findings of the heart 4 weeks after gene transfection. **A and B,** Macroscopic findings of the short-axis area of the left ventricle. **C and D,** Azan-trichrome staining of the myocardium in the border zone of the infarcted and normal area (bar = 100 μ m; original magnification, 100 $\times$). **E and F,** Hematoxylin and eosin staining of the myocardium of the border zone (bar = 100 μ m; original magnification, 200 $\times$). **G and H,** Immunohistologic staining by von Willebrand antibody (bar = 100 μ m; original magnification, 200 $\times$). **Arrows** indicate von Willebrand antibody-positive endothelial cells. **A, C, E, and G** are from the HGF group; **B, D, F, and H** are from the control group.

various physiological activities, including antifibrotic and cardiotropic activities.^{6-9,25,26} Therefore, we believe that HGF has an advantage for promoting myocardial regeneration. In the chronic phase of myocardial infarction, the progression of cardiac remodeling with reduced cardiac function is responsible for interstitial fibrosis and for the

apoptosis of the cardiomyocytes. In particular, fibrosis remote from the infarcted area is considered to be the major cause of ventricular remodeling in ischemic cardiomyopathy. In this study, neoangiogenesis was induced and fibrosis was suppressed in the peri-infarcted area by HGF gene transfection. Some of the molecular contributors to fibrosis

Figure 6. Evaluation of histopathologic findings. A, Percentage fibrosis; B, cell diameter of myocytes in the border zone (mean $\pm$ SD). C, Vascular density: data are expressed as vessels per visual field (magnification, 100 $\times$).

during cardiac remodeling have been identified.²⁷ Transforming growth factor- β and angiotensin II are believed to play an important role in the pathogenesis of fibrosis.²⁸⁻³⁰ These molecules are the negative regulators of local HGF production in various cell types.⁷⁻⁹ In this study, increased local HGF expression may have prevented myocardial fibrosis, possibly by inhibiting the production of such molecules, as previously reported.⁶⁻¹⁰ Regarding delivery of HGF, we did not use any vector for gene therapy in this study: we have already reported that direct administration of the HGF-cDNA plasmid is enough for local and continuous intramural delivery of HGF to enhance angiogenesis and cardiac function in the infarct myocardium.^{6-8,24,25} It is speculated that native HGF also plays an important role as a cardioprotective factor, but native HGF was insufficient for attenuation of cardiac remodeling in this experiment. Gene transfection of hHGF plasmid also supported the cardioprotective role of native HGF, and thus less continuously expressed protein could be sufficient to induce angiogenesis and support the subsequent recovery of regional cardiac function.^{13,14} Moreover, HGF acts as a paracrine growth factor, and its production by the administration of

HGF-cDNA plasmid in the myocardium continues for approximately 14 days. Its local synthesis without viral vectors might safeguard against adverse effects while remaining undetected in the serum HGF level during gene therapy. Thus, our results offer promise for clinical applications.

To the best of our knowledge, this is the first report to demonstrate the effectiveness of regenerative therapy in the impaired heart with LVAD support, and the protocol of this study could be used as one of the new therapeutic strategies for severe heart failure. However, several limitations of this study must be considered before clinical application. First, because of limitations of the experimental protocol, we were not able to clarify the efficacy of this method with respect to scar thinning and expansion of the impaired myocardium in the chronic phase. When loss of the contractile mass is markedly increased, such as in patients with dilated cardiomyopathy, regeneration of cardiomyocytes is insufficient to increase the contractile function, even with HGF gene transfection. Thus, a method of supplementing the contractile mass, such as cellular cardiomyoplasty, may be needed to increase treatment efficacy. Second, because of the lack of techniques for measuring goat HGF, the changes and roles

of endogenous HGF in this experiment are not clear. Third, the long-term outcome of the effect of HGF is also unclear. In the setting of this experiment, after coronary ligation, severe arrhythmias such as ventricular tachycardia and fibrillation frequently occurred. We implanted right VADs to maintain the VAD flow and systemic circulation. To address these problems, further studies are needed.

In conclusion, we have demonstrated the possible therapeutic value of suppression of cardiac remodeling by hHGF gene transfection in the impaired heart under LVAD. Our results suggest that, in the setting of acute myocardial infarction causing cardiogenic shock, combined therapy with HGF gene therapy and LVAD can increase the chance of a bridge to recovery in the severely impaired heart under LVAD.

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