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Hepatocyte growth factor gene therapy reduces ventricular arrhythmia in animal models of myocardial ischemia.

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Abstract

It was recently reported that gene therapy using hepatocyte growth factor (HGF) has the potential to preserve cardiac function after myocardial ischemia. We speculated that this HGF gene therapy could also prevent ventricular arrhythmia. To investigate this possibility, we examined the antiarrhythmic effect of HGF gene therapy in rat acute and old myocardial infarction models. Myocardial ischemia was induced by ligation of the left descending coronary artery. Hemagglutinating virus of Japan (HVJ)-coated liposome containing HGF genes were injected directly into the myocardium fourteen days before programmed pacing. Ventricular fibrillation (VF) was induced by programmed pacing. The VF duration was reduced and the VF threshold increased after HGF gene therapy ($p < 0.01$). Histological analyses revealed that the number of vessels in the ischemic border zone was greatly increased after HGF gene injection. These findings revealed that HGF gene therapy has an anti-arrhythmic effect after myocardial ischemia.

KEYWORDS: ventricular arrhythmia, HGF (hepatocyte growth factor), ischemia, HVJ (hemagglutinating virus of Japan)

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Original Article

Hepatocyte Growth Factor Gene Therapy Reduces Ventricular Arrhythmia in Animal Models of Myocardial Ischemia

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It was recently reported that gene therapy using hepatocyte growth factor (HGF) has the potential to preserve cardiac function after myocardial ischemia. We speculated that this HGF gene therapy could also prevent ventricular arrhythmia. To investigate this possibility, we examined the anti-arrhythmic effect of HGF gene therapy in rat acute and old myocardial infarction models. Myocardial ischemia was induced by ligation of the left descending coronary artery. Hemagglutinating virus of Japan (HVJ)-coated liposome containing HGF genes were injected directly into the myocardium fourteen days before programmed pacing. Ventricular fibrillation (VF) was induced by programmed pacing. The VF duration was reduced and the VF threshold increased after HGF gene therapy ($P < 0.01$). Histological analyses revealed that the number of vessels in the ischemic border zone was greatly increased after HGF gene injection. These findings revealed that HGF gene therapy has an anti-arrhythmic effect after myocardial ischemia.

Key words: ventricular arrhythmia, HGF (hepatocyte growth factor), ischemia, HVJ (hemagglutinating virus of Japan)

The mortality rates of patients with coronary artery disease of new or recurrent myocardial infarction (MI) are high. Various strategies, including medical treatment and revascularization therapy to limit residual ischemia, have been used to try to reduce the clinical consequences of the loss of cardiac muscle mass.

Intramyocardial gene therapy using genes encoding angiogenic cytokines has been proposed as an alternative strategy for treating coronary artery disease of new or

recurrent MI [1, 2]. This strategy is designed to promote the development of supplemental collateral blood vessels that will constitute endogenous bypass conduits around occluded native arteries to prevent cardiac ischemia and to potentially restore myocardial function. It has also been reported that basic fibroblast growth factor (b-FGF) or vascular endothelial growth factor (VEGF) gene transfer may have a favorable effect on the recovery of myocardial ischemia in humans [3], and there are indications that this may be associated with an increase in myocardial performance [4, 5].

Hepatocyte growth factor (HGF) has also been postulated to be a potent angiogenic growth factor, since it is

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a mitogen for various endothelial cells [6, 7] and is also a potent inducer of tube formation. HGF gene therapy can induce angiogenesis and reduce the infarct size after myocardial ischemia [8, 9].

The existence of malignant ventricular arrhythmia (ventricular fibrillation or ventricular tachycardia) is an important factor in addition to left ventricular dysfunction for predicting prognosis after myocardial infarction [10–12]. However, the relationship between angiogenesis induced by HGF gene transfection and the inhibition of ventricular arrhythmia has not been elucidated. We hypothesized that angiogenesis induced by HGF gene transfection can reduce ventricular vulnerability in animals with acute myocardial infarction, and the ventricular fibrillation duration time in animals with old myocardial infarction.

The aim of this study, therefore, was to determine the effects of the direct injection of the HGF gene on the inhibition of ventricular arrhythmias in acute or chronic myocardial ischemia.

Materials and Methods

All animal experiments were carried out in accordance with the Guidelines for Animal Experiments at Okayama University Medical School, Japanese Government Animal Protection and Management Law (No. 105) and the Japanese Government Notification on the Feeding and Safekeeping of Animals (No. 6). All efforts were made to minimize animal suffering.

Preparation of HGF gene-containing HVJ-liposome. To produce a HGF expression vector, human HGF cDNA (2.2 kb) was inserted into the EcoRI and NotI sites of the pUC-SR α expression of the vector plasmid. In this plasmid, the transcription of HGF cDNA was under the control of the SR α promoter.

We previously reported a high efficacy of transfection with HVJ-coated liposomes [13]. Briefly, phosphatidylserine, phosphatidylcholine, and cholesterol were mixed at a weight ratio of 1:4.8:2 in tetrahydrofuran. The lipid mixture (10 mg) was deposited on the sides of a flask by removal of the solvent in a rotary evaporator. The high mobility group (HMG)-1 purified from calf thymus was mixed with plasmid DNA (300 μ g) in a 200 μ l balanced salt solution (BSS; 133 mM NaCl, 5.4 mM KCl, 10 mM Tris-HCl, pH 7.6) at 20 °C for 1 h, and then the mixture was added to the dried lipid. The liposome-DNA-HMG 1 complex suspension was mixed by vortex,

sonication for 3 sec, and shaking for 30 min. Purified HVJ (Z strain) was inactivated by UV irradiation (110 erg/mm²/s) for 3 min immediately before use. The liposome suspension (0.5 ml, containing 10 mg lipid) was mixed with HVJ (20000 hemagglutinating units) in a total volume of 4 ml BSS. The mixture was incubated for 10 min at 4 °C and then for 30 min with gentle shaking at 37 °C. Free HVJ was removed from the HVJ-liposome by sucrose density gradient centrifugation. The top layer of the sucrose gradient containing the HVJ-liposome-DNA complex was collected and used immediately.

The HVJ-liposome complex was injected into the rats' hearts using the method reported previously [13]. Briefly, HVJ-liposome complex containing the human HGF gene or a control vector (100 ng/10 μ l in each liposome) was injected directly into the apex of each rat's heart with a 27 G needle.

Rat Animal Models. The antiarrhythmic effects were evaluated by measurement of the ventricular fibrillation duration time in the old MI model and the ventricular fibrillation threshold (VFT) in an acute MI model.

(1) Old myocardial infarction (OMI) model.

Twenty male Wistar rats (weighing 200–300 g) were divided into an HGF vector transfected (MI + HGF) group (n = 10) and an empty vector transfected (MI + Cont) group (n = 10). The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The animals were intubated, and ventilation was performed with room air (1.2 ml/100 g, 65 bpm). The heart of each animal was quickly exteriorized through a left lateral thoracotomy. The left anterior descending artery (LAD) was ligated 3 mm from the aortic root. Immediately after ligation, 10 μ l HVJ-liposome complex was injected into the apex of the myocardium in a non-infarcted area using the method described previously [13]. Then the thorax was enclosed by the layer method, and a catheter was cannulated to prevent pneumothorax.

Fourteen days after surgery, the rats were anesthetized again with an intraperitoneal injection of urethane (1.1 mg/kg). A lead II surface electrocardiogram (ECG) was recorded. The rats were intubated and connected to a ventilator. After mediasternal thoracotomy, 2 needle electrodes were placed on the right ventricular (RV) epicardial surface outside the margin of the infarct area with a distance of 3 mm between the electrodes. The hearts were stimulated with rectangular pulses at a frequency of 25 Hz, impulse length of 8 ms and stimulation

duration of 500 ms with a current intensity of 10 mA. The duration of ventricular fibrillation (VF) was measured in an ECG.

(2) Acute myocardial infarction (AMI) model.

Thirty male Wistar rats (weighing 200–300 g) were divided into three groups: a non-infarcted myocardium transfected empty vector (Sham) group ($n = 10$), an infarcted myocardium transfected empty vector (Cont + MI) group ($n = 10$), and an infarcted myocardium transfected HGF vector (HGF + MI) group ($n = 10$). The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The animals were then intubated and connected to a ventilator. The heart of each animal was quickly exteriorized through a left lateral thoracotomy. Ten μl of HVJ-liposome complex was injected into the apex of the myocardium using a method described previously. The thorax was enclosed by the layer method, and a catheter was cannulated to prevent pneumothorax.

Fourteen days after the injection of the HVJ-liposome complex into the non-infarcted myocardium, the rats were anesthetized again with an intraperitoneal injection of urethane (1.1 g/kg). The rats were ventilated with room air using a respirator, and the ECG was recorded. In the HGF + MI and Cont + MI groups, LAD was ligated for 30 min after mediasternal thoracotomy, and reperfusion was performed for 3 h. In the Sham group, only

mediasternal thoracotomy was performed. Two needle electrodes were placed on the RV free wall outside the infarcted area. VF was induced by a.c. stimulation using a previously reported method [14] that was modified on the basis of the results of the preliminary experiments in the control and infarcted rats ($n = 30$). Briefly, the hearts were stimulated with rectangular pulses at a frequency of 25 Hz, impulse length of 8 ms, and stimulation duration of 500 ms. The current intensity was increased in increments of 0.1 mA until VF was attained. Two minutes after stabilization, another stimulus was given with the same current intensity. The ventricular fibrillation threshold (VFT) was determined as the lowest current intensity at which two consecutive stimuli precipitated VF.

Histological analysis. Hematoxylin and eosin (HE) staining was performed to assess the angiogenesis of the vessels in the Cont + HGF and MI + HGF groups. The hearts were arrested with potassium chloride, removed, and rinsed in saline solution. After dissection of the atria and great vessels, the heart weights were determined. Transverse sections of each heart at 2 mm intervals between the left ventricular base to the apex were obtained. The specimens were fixed in 10% formalin and embedded in paraffin. Transverse sections were subsequently stained with HE in a standard manner. Slides were observed with a Zeiss Axioskop 2 plus light microscope equipped with a polarized set and analyzed with Studio Lite software.

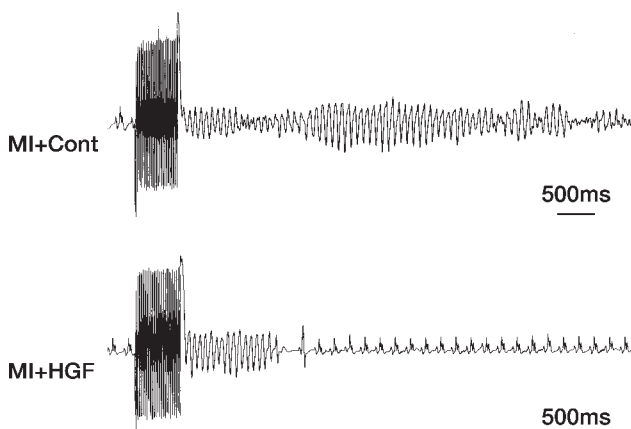


Fig. 1 Electrocardiogram of the old infarction model. MI + cont, empty vector transfected group. MI + HGF, HGF vector transfected group. The VF in a representative case in the MI + HGF group is shorter than that in the MI group.

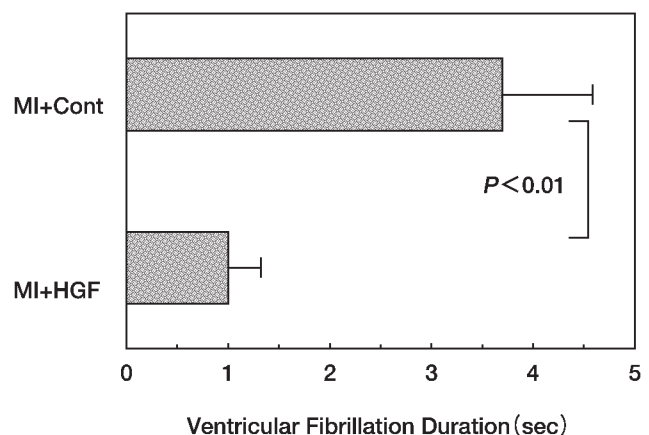


Fig. 2 Ventricular fibrillation duration in OMI model. VF duration was reduced when the HGF gene was induced. Data are given as means \pm S.E.M.

The tubular-shaped small vessels (diameter $< 100 \mu\text{m}$) were counted under light microscopy (magnification, $\times 100$) in a blind manner. The total number of vessels in each section was summed and expressed as the number of vessels per section. At least 8 sections for each heart were examined. Areas in which angiogenesis was quantified in the injected site and around the injected site were randomly selected.

Statistics. All values are expressed as means \pm SEM. Statistical analysis of variance with paired Student's *t* test was used to determine significant differences in the VF durations and numbers of vessels. Fisher's protected least significant difference test was used for comparison of the VFTs. Statistical significance was defined as $P < 0.05$.

Results

Ventricular fibrillation duration analysis.

To examine the arrhythmogenicity of HGF gene induction, we first measured ventricular fibrillation duration in the old myocardial infarction model. A current intensity of 10 mA was sufficient to induce ventricular arrhythmia. VF was inducible by any programmed stimulations, but was not sustained, and terminated spontaneously in all hearts. The VF duration was significantly reduced in the HGF vector transfected (MI + HGF) group (1.0 ± 0.27 sec) compared to that in the empty vector transfected (MI +

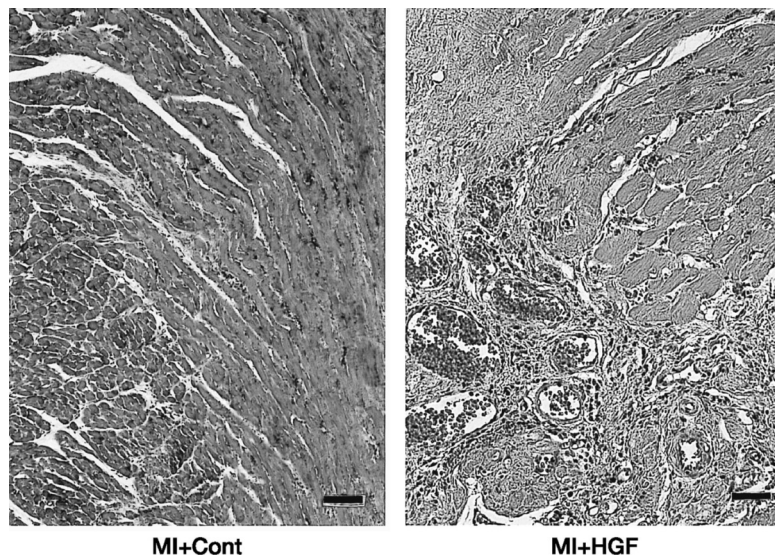


Fig. 4 Hematoxylin and eosin staining in the OMI model. Bar indicate $50 \mu\text{m}$.

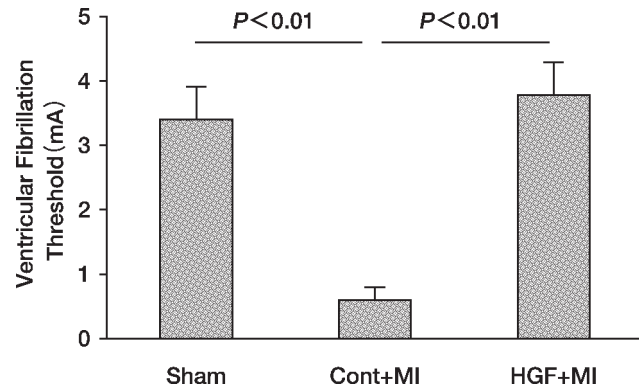


Fig. 3 Ventricular fibrillation threshold in the AMI model. VFT was decreased after coronary ligation when the HGF gene was not injected. In the MI + HGF group, VFT was significantly increased. Data are given as means \pm S.E.M.

Cont) group (3.7 ± 0.90 sec) ($P < 0.01$) (Fig. 1, Fig. 2).

Ventricular fibrillation threshold analysis.

The acute myocardial infarction model was used to evaluate VFT. In all rats, VF was precipitated by programmed stimulation until the current intensity reached 10 mA. VFT was lower in the MI group (0.63 ± 0.17 mA) than in the Sham group (3.4 ± 0.52 mA) ($P < 0.01$) and HGF + MI group (3.8 ± 0.49 mA) ($P < 0.01$) (Fig. 3).

Histological analysis. The angiogenesis was enhanced in the border zone around the infarcted myocardium in the MI + HGF group (Fig. 4). The number of

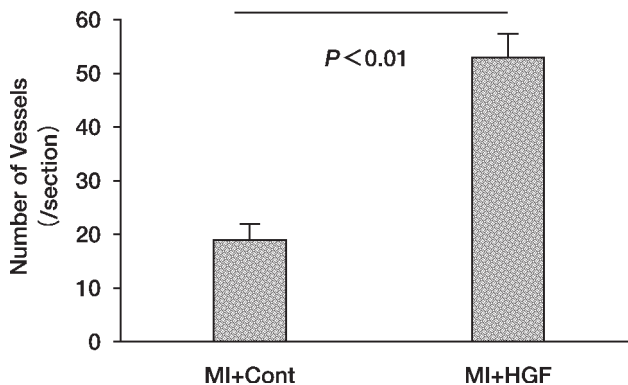


Fig. 5 Number of vessels. The number of small vessels increased when the HGF vector was transfected. Data are given as means \pm S.E.M.

vessels in the myocardium around the infarct area was greater in the rats in the MI + HGF (53 ± 4.8) group than in the rats in the MI + Cont group (19 ± 1.8) ($P < 0.01$) (Fig. 5).

Discussion

Recently, several new strategies, including gene therapy and cellular transplantation, have been successful in reducing the clinical consequences of the loss of cardiac muscle mass after myocardial ischemia [8]. However, the feasibility and safety of these new methods have not yet been determined. Some studies using autologous skeletal myoblast transplantation in patients with severe ischemic cardiomyopathy suggested an arrhythmogenic potential in such patients [15]. Actually, it has been reported that the existence of arrhythmic events, especially ventricular fibrillation/tachycardia associated with ventricular remodeling, is a major factor in determining the mortality after myocardial infarction [12]. Therefore, it is important to evaluate the arrhythmogenic potential before starting a human trial.

Intramyocardial gene delivery using genes encoding angiogenic cytokines has been proposed as a strategy for patients with ischemic heart disease to promote the development of supplemental collateral vessels around the occluded native arteries in order to prevent cardiac ischemia and to restore cardiac function [5, 8, 9]. We previously reported that HGF gene delivery using the HVJ-liposome method has a favorable effect on the recovery of myocardial ischemia in animal models of myocardial ischemia [13]. The present study was carried

out to determine whether this gene therapy prevents or increases arrhythmic events after myocardial ischemia.

We found that the duration of induced VF after chronic ischemia was significantly decreased in the MI + HGF group. Similar results were obtained using the VF threshold after acute ischemia in the HGF + MI group. The present study showed a marked fall in the VFT after coronary artery ligation, which agrees with the results of an earlier study [16]. Similar methods have been applied before [17, 18]. We cannot completely explain the mechanism of these results, but these favorable outcomes may lead to improvement in the survival rate after HGF gene therapy. It is known that infarcted myocardial volume can be reduced by means of HGF gene induction [19]. In the present study, the number of vessels was increased after HGF gene transfection. This angiogenesis effect might reduce the ischemic myocardial volume after myocardial infarction and lead to reduction of the arrhythmic property of the HGF gene-transfected myocardium.

We previously reported that HGF gene delivery using the HVJ-liposome method has a favorable effect on the recovery of myocardial ischemia in animal models of myocardial ischemia by means of pleiotropic effects on various cells [6]. Ueda *et al.* reported that the c-Met/HGF receptor is expressed in cardiomyocytes, and that HGF prevents myocyte death due to oxidative stress [20]. Nakamura *et al.* also reported that HGF gene transfection before myocardial ischemia attenuates reperfusion injury after releasing the coronary artery [9]. Taniyama *et al.* reported that local HGF expression is involved in the prevention of myocardial injury by angiotensin II blockade through its antifibrotic action [21]. In this study, we observed a large increase in the number of vessels in the border zone of ischemia. We speculate that this effect reduces fibrosis and is related to the negative remodeling after myocardial ischemia. It is known that infarct size can be reduced by means of HGF gene induction [19]. However, we did not measure the infarcted myocardial volume in the present study. Further investigation is needed to determine whether the infarcted myocardium volume after HGF gene transfection is associated with an arrhythmic response. It would also be interesting to include a Sham + HGF group in a VFT experiment in order to evaluate the arrhythmogenic response of HGF gene therapy.

In conclusion, HGF gene therapy has a favorable anti-arrhythmic effect after acute or chronic myocardial ischemia. Although further study is needed to determine

the mechanism by which this occurs, it has been shown that HGF gene therapy could be a safe treatment after myocardial ischemia.

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