MAP was performed in the usual fashion in all patients except one patient who had previously undergone MAP. This patient underwent only our Batista procedure. The rings used for MAP were Carpentier-Edwards Classic rings or Physio rings (Edwards Life Sciences, Irvine, CA, USA). The size used was 26 mm (n = 15) or 28 mm (n = 16).

The other additional subvalvular procedure used was chordal cutting. When preoperative echocardiography showed major secondary chordae contributing to the anterior leaflet tethering, an attempt was made to identify these chordae through the left ventriculotomy and then divide them. Care was taken not to cut any strut chordae, which may cause leaflet prolapse.

It has been reported that PMA through a left ventriculotomy per se reduces left ventricular volume to some degree [12]. However, the volume reduction effect of PMA alone may not be enough to achieve adequate ventricular volume reduction. In such cases, additional volume reduction techniques such as overlapping ventriculoplasty [6] or the SAVE procedure [10] were used in addition to PMA, particularly in patients with history of antero-septal myocardial infarction. The technical details of these procedures have been described elsewhere [6,10]. In brief, after a ventricular incision was extended proximally along the left anterior descending artery, the left marginal incision was directly sutured to the endocardium of the septal wall using continuous sutures (overlapping ventriculoplasty). Sometimes, an elliptical Dacron patch was sutured to the transitional zone to exclude the akinetic apical-septal area and restore the shape of the ventricle (SAVE procedure). The target left ventricular volume was estimated by the following formula:

$$\text{LVEDVI}\left(\text{ml}/\text{m}^2\right) = 1000 \times \text{Cardiac index}/\text{LVEF} \times \text{HR}$$

$$\left(\text{HR}: \text{ heart rate}\right)$$

For example, given the average LVEF of approximately 30% with heart rate at 90 beats per minute (paced immediately postoperatively), the calculated LVEDVI would be approximately 90-110 ml/m^{2}under target cardiac index at 2.5-3.0 L/min/m^2. The sizing device was also sometimes used to estimate the cavity volume. In terms of the selection of additional techniques, the decision was made based on the preexisting myocardial status and the left ventricular size and shape.

2.4. Data Collection and Statistical Analysis

Demographic and other patient-related data were obtained from medical records. All patients were contacted during a subsequent outpatient clinic visit for follow-up examination. Echocardiographic studies were routinely performed every 6-12 months at our institutions or the outpatient clinics of referring physicians. Results are expressed as mean ± standard deviation. For comparison of each variable between before and after operation, the Wilcoxon test was used and a $p < 0.05$ was considered statistically significant. The probability of survival was estimated by the Kaplan-Meier method. All the statistical analyses were performed using the JMP 8.0 software package (SAS, Carey, North Carolina, USA). This study was approved by the Institutional Review Board, and patients and families gave informed consent.

3. Results

In this study, surgical techniques used were posterior wall resection (the Batista procedure; n = 13:8 (47%) in DCM patients and 5 (33%) in ICM patients) or posterior wall plication (n = 19:9 (53%) in DCM patients and 10 (67%) in ICM patients). PMA was performed in majority of patients (n = 24:12 (71%) in DCM patients and 12 (80%) in ICM patients) during the posterior wall exclusion. Additional volume reduction techniques used were SAVE procedure (n = 12) and overlapping left ventriculoplasty (n = 5). Also, chordal cutting was added in some cases (n = 19). Preoperatively, all the patients except one were in New York Heart Association functional class III (n = 16) or IV (n = 15), six patients were inotrope dependent, three patients were in cardiogenic shock, and five patients required IABP support. Other preoperative co-morbidities included chronic renal dysfunction (serum creatinine ≥ 1.5 mg/dl, n = 9, 28%; and 2 patients were on hemodialysis) and liver dysfunction (serum total bilirubin ≥ 1.5 mg/dl, n = 8, 25%). Concomitant procedures included coronary artery bypass grafting (CABG) in 16, tricuspid annuloplasy in 10, maze procedure in 9 and aortic valve replacement in 2.

The mean aortic cross-clamp time and cardiopulmonary bypass time were 132 ± 33 minutes and 194 ± 38 minutes, respectively. A total of 15 patients required IABP support at the time of weaning from cardiopulmonary bypass. All the patients could be weaned off without ventricular assist devices. Two patients died within 30 days of surgery (6.3%), one from sepsis and the other from low output syndrome associated with refractory supraventricular tachyarrhythmia. Another 2 patients died during hospitalization; one from pneumonia, and the other from multiorgan failure due to congestive heart failure. The overall hospital mortality was 12.5%. As shown in the **Table 1**, there was no significant improvement in EF, however LVEDD decreased from 65 ± 8 mm to 61 ± 7 mm ($p < 0.001$), and LVEDVI decreased from 134 ± 35 ml/m^2 to 99 ± 28 ml/m^2 ($p < 0.001$). There was a significant reduction in degree of MR (from 3.1 ± 1.0 to 0.3 ± 0.8, $p < 0.001$). Moreover, the tethering height and papillary muscle distance in diastole decreased from 9.4 ± 2.1 mm to 2.9 ± 2.4 mm ($p < 0.01$), and 35 ± 7 mm to 19 ± 6 mm ($p < 0.01$), respectively. The restricted motion of the anterior leaflet was im-

Table 1. Echocardiographic data.

Variables	Preoperative (n = 32)	Postoperative	
		Early (n = 30)	Mid-term (n = 26)
Left ventricular ejection fraction (%)	28 ± 8	31 ± 8	31 ± 8
Left ventricular end-diastolic dimension (mm)	65 ± 8	61 ± 7*	60 ± 7*
Left ventricular end-systolic dimension (mm)	57 ± 10	51 ± 9*	53 ± 9¶
Left ventricular end-diastolic volume index (ml/m^2)	134 ± 35	99 ± 28*	101 ± 20*
Left ventricular end-systolic volume index (ml/m^2)	101 ± 33	72 ± 28*	80 ± 27¶
Left atrial dimension (mm)	51 ± 11	48 ± 8*	53 ± 8
Mitral regurgitation grade	3.1 ± 1.0	0.3 ± 0.8*	0.6 ± 0.8*
Tricuspid regurgitation grade	2.1 ± 0.9	1.2 ± 0.8§	1.5 ± 0.6
Estimated right ventricular pressure (mmHg)	51 ± 17	42 ± 14¶	45 ± 14
Tethering height (mm)	9.4 ± 2.1	2.9 ± 2.4¶	2.0 ± 1.0¶
Papillary muscle distance in diastole (mm)	35 ± 7	19 ± 6¶	20 ± 8¶

*$p < 0.001$ comparing with preoperative value; ¶$p < 0.01$ comparing with preoperative value; §$p < 0.05$ comparing with preoperative value.

proved in almost all the patients. One patient required reoperation due to recurrent severe MR 4 weeks after the operation. The cause of MR was found to be recurrent leaflet tethering presumably due to re-dilatation of the posterior wall. Subsequently, this patient successfully underwent mitral valve replacement and was discharged later.

The mean length of follow-up was 3.3 ± 2.1 years. Follow-up echocardiography were performed in 26 patients out of 28 who were discharged home. These studies demonstrated that EF, LVEDD and LVEDVI remained in the same range as when assessed immediate following surgery. However, three patients had developed moderate MR. There were no significant changes in the tethering height and papillary muscle distance at the mid-term follow-up (**Table 1**). There were 7 late deaths. The cause of late deaths included congestive heart failure in 2, sudden death in 2, sepsis in 2 and cancer in one. Of note, there were no known patients who died of congestive heart failure accelerated by the presence of MR. The cause of congestive heart failure was essentially pump failure due to either systolic or diastolic dysfunction, or combination of these. The 1-, 2- and 3-year survival rates after operation were 78%, 68% and 57%, respectively (**Figure 2**).

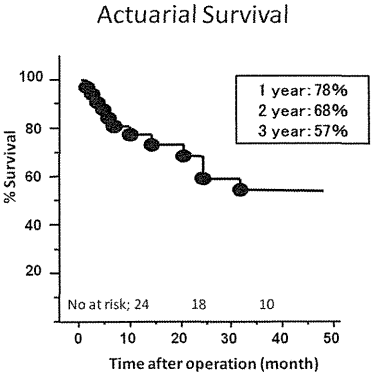

Figure 2. Probability of patient survival after operation.

4. Discussion

MR is one of the major complications following myocardial infarction, and the presence of MR is associated with excess mortality independent of baseline characteristics and degree of ventricular dysfunction [13]. The primary mechanism of FMR is mitral leaflet restriction with tethering due to left ventricular dilatation in addition to some degree of annular enlargement [1]. This is also true in patients with MR due to idiopathic DCM. Bolling *et al.* have demonstrated that MAP using undersized rings yields excellent early and mid-term outcomes in patients with severe FMR associated with DCM [14]. However, it has been reported that a significant number of patients developed recurrent MR in the early postoperative period [15]. If the leaflet tethering is too severe, the risk of recurrent MR becomes high [1]. In fact, it has been demonstrated that the risk factors for MAP failure include severely dilated left ventricle (LVEDD ≥ 65mm) [16] and severe leaflet tethering (tethering height ≥ 10 mm) [17]. In such cases, simple MAP is not sufficient to provide relief. In these circumstances, subvalvular proce-

dures become necessary to reduce the tethering. To date, several techniques have been proposed to treat leaflet tethering. Liel-Cohen *et al.* reported that posterior wall plication in an animal model may reduce the leaflet tethering and MR caused by geometric changes after posterior wall myocardial infarction [18]. This technique can be translated to the clinical setting where posterior wall resection (the Batista procedure) may reduce leaflet tethering and FMR as shown in the current study. In fact, Menicanti *et al.* have demonstrated that intraventricular papillary muscle imbrication at the time of SVR can reduce FMR without ring annuloplasty [19]. Also, Buckberg *et al.* published a preliminary report showing how papillary muscle dimension can be restored during SVR by PMA [7]. Although the mechanisms of FMR are multifactorial, elongation of papillary muscle distance associated with postero-lateral wall dilatation seems to be one of the most significant determinants. In other words, reducing the inter-papillary muscle distance can be the key component in ameliorating leaflet tethering [19].

Batista's original technique involved a volume reduction achieved by a partial left ventriculectomy in which the posterior wall between anterior and posterior papillary muscles was excised [20]. The essence of this approach was to reduce the wall stress and energy consumption of the myocardium by reducing the radius of the ventricle. A few initial reports showed some favorable outcomes, but a relatively high incidence of early failure and late recurrence of ventricular dilatation precluded its wide clinical application [20]. At the time of these early reports, the issues of MR reduction and leaflet tethering were not discussed at all. Interestingly, McCarthy *et al.* reported the results of their initial series of the Batista procedure in which repositioning one or both papillary muscles so as to sit side by side, along with MAP, virtually eliminated MR [20].

As an alternative to a left ventricular intervention, Kron *et al.* reported that direct relocation of the posterior papillary muscle may be an option for patients with minimally dilated left ventricles as a treatment of FMR [2]. Hvass *et al.* also reported on a technique in which the base of the papillary muscles are drawn together and secured with an intraventricular sling inserted through the left atrium [3]. In addition, it has been reported that cutting the secondary chords contributing to leaflet tethering may improve coaptation and reduce FMR [4]. These techniques may be useful to reduce leaflet tethering, but may not be sufficient to completely eliminate the tethering, particularly in severely dilated hearts.

It is important to evaluate the left ventricular wall characteristics in patients with DCM. The extent of left ventricle (LV) fibrosis and dilatation in DCM is not always homogeneous. One drawback to SVR is that it may compromise viable muscle tissue to restore the LV. The decision of which technique to use must be based on the findings of preoperative myocardial evaluations such as cardiac MRI, nuclear study and echocardiography in addition to direct vision and palpitation. Care should be taken to minimize an excision of viable myocardium to preserve cardiac function. However, as the postero- lateral wall is always impaired without exception in severely dilated hearts with severe FMR in our experiences, the presence of severe FMR due to leaflet tethering per se may be a clinical indicator for the posterior wall resection in SVR.

The ideal surgical treatment for the patients with end-stage heart failure may be heart transplantation. Both the survivorship and the quality of life after heart transplantation have been reported to be excellent. However, it is obvious that heart transplantation is not always an option because of strict indications and limited donor availability. There are a number of factors such as medical condition, psychosocial circumstance and financial issues that restrict transplant candidacy. In the current study, half of the patients were age of 65 or older, and a significant number of patients had had preoperative kidney dysfunction (28%), liver dysfunction (25%), inotrope dependent (19%) and in cardiogenic shock (10%). Given the severity of preoperative condition and advanced age, the majority of the patients did not meet the candidate criteria for heart transplantation. On the other hand, these patients may benefit from other surgical options such as left ventricular assist devices (LVAD). LVAD implantation using current rotary pumps as a destination therapy may be a good option, especially in elderly patients. According to the latest report from the registry [21], 1- and 2-year survivals after primary LVAD implantation were 74% and 55%, respectively. The majority of the registry patients could have been sicker than those in the current study, however, the survival after our SVR with MAP procedure in the current study was not inferior to this result from the LVAD registry.

5. Limitations

There are several limitations which need to be addressed. First, this is a non-randomized and retrospective study without a control group. Also, this cohort is relatively small and includes heterogeneous subsets of patients. Often, one patient underwent more than two procedures so that it may be difficult to discriminate which procedure contributed more to the reduction in mitral leaflet tethering. Moreover, the mean follow-up period was only 3.3 years. Therefore, further study is needed to determine more clearly the indications for this procedure.

6. Conclusion

In patients with severe MR due to leaflet tethering, the posterior wall exclusion procedure by either resection or

plication, paired with PMA as necessary, provides excellent reduction of leaflet tethering and MR. The reduction of tethering and MR was well maintained at the mid-term follow-up in this series. Thus, reduction of PM distance may be a key component in treating severe FMR.

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Effects of hepatocyte growth factor in myocarditis rats induced by immunization with porcine cardiac myosin

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Abstract

OBJECTIVES: Myocarditis is considered one of the major causes of dilated cardiomyopathy. Hepatocyte growth factor (HGF) has pleiotropic activities that promote tissue regeneration and facilitate functional improvement of injured tissue. We investigated whether the epicardial sustained-release of HGF, using gelatin hydrogel sheets, improves cardiac function in a chronic myocarditis rat model.

METHODS: Six weeks after Lewis rats were immunized with porcine cardiac myosin to establish autoimmune myocarditis, HGF- or normal saline (NS)-incorporated gelatin hydrogel sheets were applied to the epicardium (G-HGF and G-NS, respectively). At either 2 or 4 weeks after treatment, these were compared with the Control myocarditis group. Cardiac function was evaluated by echocardiography and cardiac catheterization. Development of fibrosis was determined by histological study and expression of transforming growth factor-β1 (TGF-β1). Bax and Bcl-2 levels were measured to evaluate apoptotic activity.

RESULTS: At both points, fractional shortening and end-systolic elastance were higher in the G-HGF group than in the Control and G-NS groups ($P < 0.01$). Fractional shortening at 2 weeks of each group were as follows: 31.0 ± 0.9%, 24.8 ± 2.7% and 48.6 ± 2.6% (Control, G-NS and G-HGF, respectively). The ratio of the fibrotic area of the myocardium was lower in the G-HGF group than in the Control and G-NS groups at 2 weeks (G-HGF, 8.8 ± 0.9%; Control, 17.5 ± 0.2%; G-NS, 15.6 ± 0.7%; $P < 0.01$). The ratio at 4 weeks was lower in the G-HGF group than in the G-NS group (10.9 ± 1.4% vs 18.5 ± 1.3%; $P < 0.01$). The mRNA expression of TGF-β1 in the G-HGF group was lower than in the Control group at 2 weeks (0.6 ± 0.1 vs 1.1 ± 0.2) and lower than that in the G-NS group at 4 weeks (0.7 ± 0.1 vs 1.3 ± 0.2). The Bax-to-Bcl-2 ratios at both points were lower in the G-HGF group than in the Control group.

CONCLUSIONS: Sustained-released HGF markedly improves cardiac function in chronic myocarditis rats. The antifibrotic and antiapoptotic actions of HGF may contribute to the improvement. HGF-incorporated gelatin hydrogel sheet can be a new therapeutic modality for myocarditis.

Keywords: Growth substances • Fibrosis • Myocarditis

INTRODUCTION

Although the aetiology of dilated cardiomyopathy (DCM) is not fully known, it is considered to be at least partly induced by autoimmune myocarditis, which is characterized by LV dilatation, systolic dysfunction, myocardial necrosis and collagen deposition [1, 2]. Despite this condition often being fatal, the number of treatments, such as heart transplantation and left ventricular assist devices (LVADs), remains limited. Moreover, because of donor shortages for heart transplantation, other treatments have long been necessary.

Hepatocyte growth factor (HGF) can be a therapeutic for myocarditis and DCM because of its cytoprotective and regenerative activities. While HGF was first found and purified from the plasma of a patient with hepatic failure, and was then molecularly cloned [3, 4], there have been several reports regarding the efficacy of HGF in cardiovascular diseases [5]. Nakamura *et al.* [6] reported that HGF administration improves cardiac function after ischaemia/reperfusion and that HGF exerts protective effects via its angiogenic and antiapoptotic actions. Furthermore, Taniyama *et al.* [7] reported that transfection of the HGF gene in the cardiomyopathic hamster model using the haemagglutinating virus of Japan (HVJ) liposome may facilitate angiogenesis and reduce fibrosis. These reports give us an idea that effective usage of HGF is a potential strategy to promote tissue regeneration and facilitate functional improvement of injured tissue in myocarditis.

As encouraging as this may seem, there are still several solutions to be found before HGF can be applied to human myocarditis

and DCM, whereas a phase I/II study of patients with fulminant hepatitis or late-onset hepatic failure has already been reported [8]. First, the half-life of HGF, in solution form, is too short to maintain its biological function *in situ*. Secondly, gene transfer necessitates the use of viral vectors, whose expression cannot be fully controlled after administration *in vivo*. There have also been concerns about inflammatory responses to these vectors [9]. Thirdly, it remains unclear whether sustained delivery of exogenous HGF to failing hearts improves cardiac function in rats with autoimmune myocarditis. To establish a system for delivery, we have therefore developed HGF-incorporated gelatin hydrogel sheets, which enable HGF to be gradually released *in situ* over 2 weeks [10]. In experimental studies, we demonstrated that application of gelatin hydrogel sheets can improve ventricular contractility and can attenuate fibrosis in both spontaneously hypertensive and myocardial infarction rat models [11, 12]. However, the effects of HGF gelatin hydrogel sheets on autoimmune myocarditis have not been explored in depth.

Our hypothesis was that epicardial sustained-release of HGF, using the gelatin hydrogel sheets, improves cardiac function in a rat autoimmune myocarditis model through its antifibrotic and antiapoptotic effects. Experimental autoimmune myocarditis was produced in the myocardium of Lewis rats by immunization with cardiac myosin [1, 2]. This model has demonstrated that active myocarditis subsides at 6 weeks after immunization, while post-myocarditic DCM develops in the chronic phase [1]. Previous studies have reported that about one-third of subjects die from extensive myocardial necrosis [2].

METHODS

Rat autoimmune myocarditis model

All experimental procedures were conducted in accordance with Kyoto University's guidelines for animal care and the 'Guide for the Care and Use of Laboratory Animals', published by the National Institutes of Health.

Five-week old male Lewis rats (weighing 120–160 g; Japan SLC, Inc., Hamamatsu, Japan) were used. The DCM model was produced by means of induction of autoimmune myocarditis [13]. In brief, 1 mg/0.1 ml of purified cardiac myosin from a porcine heart was mixed with an equal volume of Freund's complete adjuvant (Difco; BD Diagnostic Systems, Sparks, MD, USA) and injected into a footpad. Six weeks after immunization, these rats served as a model of heart failure owing to autoimmune myocarditis.

Echocardiography

Rats were anaesthetized with 1% isoflurane at 6 weeks after autoimmunization. Harvard-type ventilators were used for respiratory control. Left ventricular (LV) function was evaluated by a Vivid 7 echocardiography machine with an 11-MHz phased array transducer (GE Medical, Milwaukee, WI, USA). Echocardiographic measurements were performed as described previously [13, 14]. Briefly, after the rats were anaesthetized, their chests were shaved and they were placed in the supine position on a table. A two-dimensional targeted M-mode echocardiogram was obtained and averaged along the short-axis view of the LV at the level of the papillary muscles over three consecutive cardiac cycles according to the American Society of Echocardiography leading-edge method. The following parameters were measured three times and averaged by M-mode tracing: LV internal end-diastolic and end-systolic dimension (LVIDd, LVIDs) and diastolic and systolic wall thickness (LVWTd, LVWTs). Values were calculated using the following equation:

$$\text{Fractional shortening (\%)} = \frac{\text{LVIDd} - \text{LVIDs}}{\text{LVIDd}} \times 100$$

$$\text{Systolic thickening} = \frac{\text{LVWTs}}{\text{LVWTd}}$$

Preparation and application of gelatin hydrogel sheets

Gelatin was isolated from bovine bone collagen by an alkaline process using calcium hydroxide (gelatin; Nitta Gelatin Co, Osaka, Japan). Gelatin hydrogel sheets were prepared as described previously [10]. Briefly, after mixing 100 µl of 25 wt% glutaraldehyde aqueous solution with 50 ml of 5 wt% gelatin aqueous solution at 40°C, the mixture was cast into a polypropylene tray and left for 12 h at 4°C to perform chemical cross-linking of gelatin. The resulting hydrogel sheet was then punched out and immersed in 100 mM glycine aqueous solution at 37°C for 1 h. The cross-linked gelatin hydrogel sheet was twice washed with double-distilled water, freeze-dried and sterilized with ethylene oxide gas. Square sheets (20 × 20 mm) were impregnated with an aqueous solution containing 100 µg of human recombinant HGF (courtesy of Prof Tsubouchi, Kagoshima University). HGF-incorporated gelatin hydrogel sheets can gradually release HGF *in situ* over 2 weeks [10, 11].

Six weeks after immunization, fractional shortening was measured by echocardiography. The hearts of normal Lewis rats (age, 11 weeks) served as control to confirm the development of myocarditis. A small pericardial incision was made through a left-sided thoracotomy under general anaesthesia with 1% isoflurane. Without sutures, gelatin hydrogel sheets with saline or HGF were attached to the epicardium of the entire LV free wall. The pericardium was closed with interrupted polypropylene sutures. We confirmed that the sheet stayed on the LV epicardium during the entire study period by means of serial echocardiography.

Study groups

After echocardiographic examination, the rats in which myocarditis developed with an LV fractional shortening <40% were then randomly divided into the following three groups: (i) the Control group, which had the myocarditis heart without application of gelatin hydrogel sheets or HGF ($n = 14$), (ii) the gelatin-normal saline (G-NS) group, which had the myocarditis heart with application of gelatin hydrogel sheets and 100 µl of saline ($n = 14$) and (iii) the gelatin-HGF (G-HGF) group ($n = 14$), which had the myocarditis heart with application of gelatin hydrogel sheets and 100 µg of HGF. The concentration of HGF was determined based on our previous study, where 40 µg of HGF alone did not exert a favourable effect on cardiac function [12]. The hearts in each group were evaluated at either 2 or 4 weeks after treatment (establishing a 2-week set and a 4-week set; Fig. 1). The timing of the evaluation was chosen following our previous studies [11, 12] and based on the duration of the HGF release from the gelatin hydrogel sheets as described above.

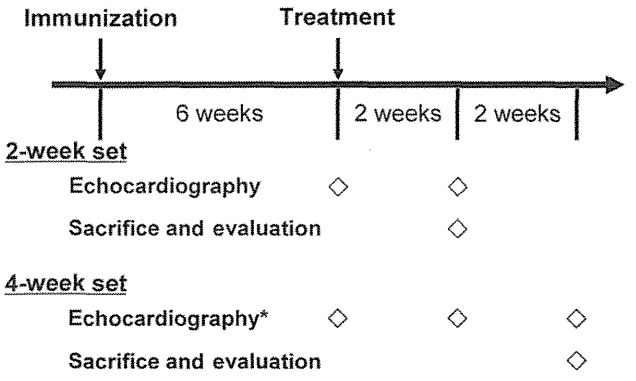

Figure 1: Protocol. *Echocardiography was serially performed only in the 4-week set (G-NS and G-HGF groups). G-HGF: gelatin-hepatocyte growth factor; G-NS: gelatin-normal saline.

Physiological studies

Echocardiography and cardiac catheterization were carried out under general anaesthesia with 1% isoflurane. LV pressure–volume loop analysis was performed as described elsewhere [15]. Following echocardiography, the right carotid artery was cannulated with a pressure–volume catheter (SPR-869, Millar Instruments, Houston, TX, USA) that was advanced into the aorta and then into the left ventricle. The inferior vena cava (IVC) was exposed via midline laparotomy. Following the measurement of the baseline pressure–volume loops, a series of loops was recorded after LV preload was reduced by directly compressing IVC. End-systolic elastance was determined from these pressure–volume loops (>5 loops) using the Integral 3 system (Unique Medical, Tokyo, Japan). Time constant (τ) was measured from baseline loops.

In order to follow the time course of LV geometric changes in the rats from the 4-week set, echocardiographic evaluation was performed both 2 and 4 weeks after treatment in the G-NS and G-HGF groups (7 rats in each group; Fig. 1).

Histology

Following catheterization, each heart was removed after animals were sacrificed with carbon dioxide; the LV myocardium was transversely sliced into sections (2 mm in diameter) at the base of the papillary muscle and then fixed in 10% buffered formalin. The remaining LV myocardium was frozen at −80°C until analysis. Transverse sections of the LV myocardium were stained with Sirius-red reagents thus determining the fibrotic area. The Sirius-red-stained myocardium was serially photographed with high-power field, and a whole section was reconstructed from the serial images using a microscope (BIOREVO BZ-9000; Keyence Corp., Osaka, Japan). Red-stained fibrotic area was automatically calculated with an automated image analysis system (BIOREVO BZ-9000). The stained area was calculated as a percentage of the total area excluding the left and right ventricular cavities.

Analysis of messenger RNA expression

Total messenger RNA (mRNA) was prepared from the frozen LV pieces where gelatin hydrogel sheets were applied ($n = 7$, in each group) with TRIzol reagent (Life Technologies Corporation, Carlsbad, CA, USA), and reverse transcribed with the SuperScript III first-strand synthesis system (Invitrogen). Quantitative reverse-transcription polymerase chain reaction was performed using a TaqMan Gene Expression Assay (Applied Biosystems, Foster City, CA, USA) and amplified with the StepOnePlus system (Applied Biosystems). Polymerase chain reaction conditions included 40 cycles of denaturing at 94°C for 20 s and primer annealing/extension at 62°C for 60 s. The polymerase chain reaction sequence of transforming growth factor-β1 (TGF-β1) was reported in our previous research [16]. The TaqMan rodent glyceraldehyde-3-phosphate dehydrogenase control reagent was used to detect rat glyceraldehyde-3-phosphate dehydrogenase as the internal standard. In each sample, the expression level of the target gene was normalized against glyceraldehyde-3-phosphate dehydrogenase levels.

Measurement of apoptosis-related proteins

Bax and Bcl-2 levels in tissues where gelatin hydrogel sheets were applied ($n = 7$ per group) were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Uscn Life Science, Inc., Wuhan, China). ELISA procedures were carried out according to the manufacturer's protocol. Results are reported as a Bax-to-Bcl-2 ratio.

Statistical analysis

All data are presented as means ± standard error of the mean. Comparisons between two groups were made using unpaired or paired *t*-tests. Differences among three groups were evaluated using a one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. All statistical analyses were performed with IBM SPSS Statistics version 19 (IBM, Armonk, NY, USA). Statistical significance was set at the level of $P < 0.05$.

RESULTS

Development of autoimmune myocarditis

Autoimmune myocarditis developed in 42 of the 85 rats at 6 weeks after autoimmunization. Twenty-four rats died before treatment, while the remaining 19 rats with fractional shortening of >40 were excluded from the study. No significant differences were observed in the baseline echocardiographic parameters among the study groups (Table 1), although fractional shortening in the study groups was significantly lower than that in normal rats.

Effects of HGF on cardiac function

No rats died or showed a pyrogenic response after surgery. Echocardiography and cardiac catheterization showed that, at both 2 and 4 weeks after surgery, and when compared with the G-HGF group, the other two groups had significant deterioration of LV systolic functionality characterized by enlargement of the LV cavity and both had decreased LV fractional shortening and end-systolic elastance (Fig. 2A–C). However, there were no significant differences, regarding these variables, between the Control and the G-NS groups. Among the three groups, no significant differences were observed in the other echocardiography and cardiac catheterization parameters (Table 2).

Figure 2D depicts the time course of LV geometric changes in the rats from the 4-week set. Although LVIDd and LVIDs in the

Table 1: Baseline echocardiographic data

	Control	G-NS	G-HGF	*P*-value**	Normal rats
Body weight (g)	304.6 ± 4.9*	296.4 ± 4.3*	306.1 ± 4.2*	0.27	339.6 ± 3.0
LVIDd (mm)	7.94 ± 0.13*	8.26 ± 0.21*	7.87 ± 0.12*	0.19	5.26 ± 0.19
LVIDs (mm)	5.23 ± 0.16*	5.76 ± 0.27*	5.30 ± 0.20*	0.17	2.41 ± 0.16
Systolic thickening	1.58 ± 0.08	1.49 ± 0.09	1.48 ± 0.07	0.65	1.45 ± 0.04
Fractional shortening (%)	34.2 ± 1.7*	30.6 ± 1.7*	32.9 ± 1.5*	0.30	54.6 ± 1.9

*$P < 0.01$: difference compared with the normal Lewis rats of the same age. **P-values: differences between the Control, G-NS and G-HGF groups by one-way ANOVA.
G-HGF: gelatin-hepatocyte growth factor; G-NS: gelatin-normal saline; LVIDd: left ventricular internal end-diastolic dimension; LVIDs: left ventricular internal end-systolic dimension.

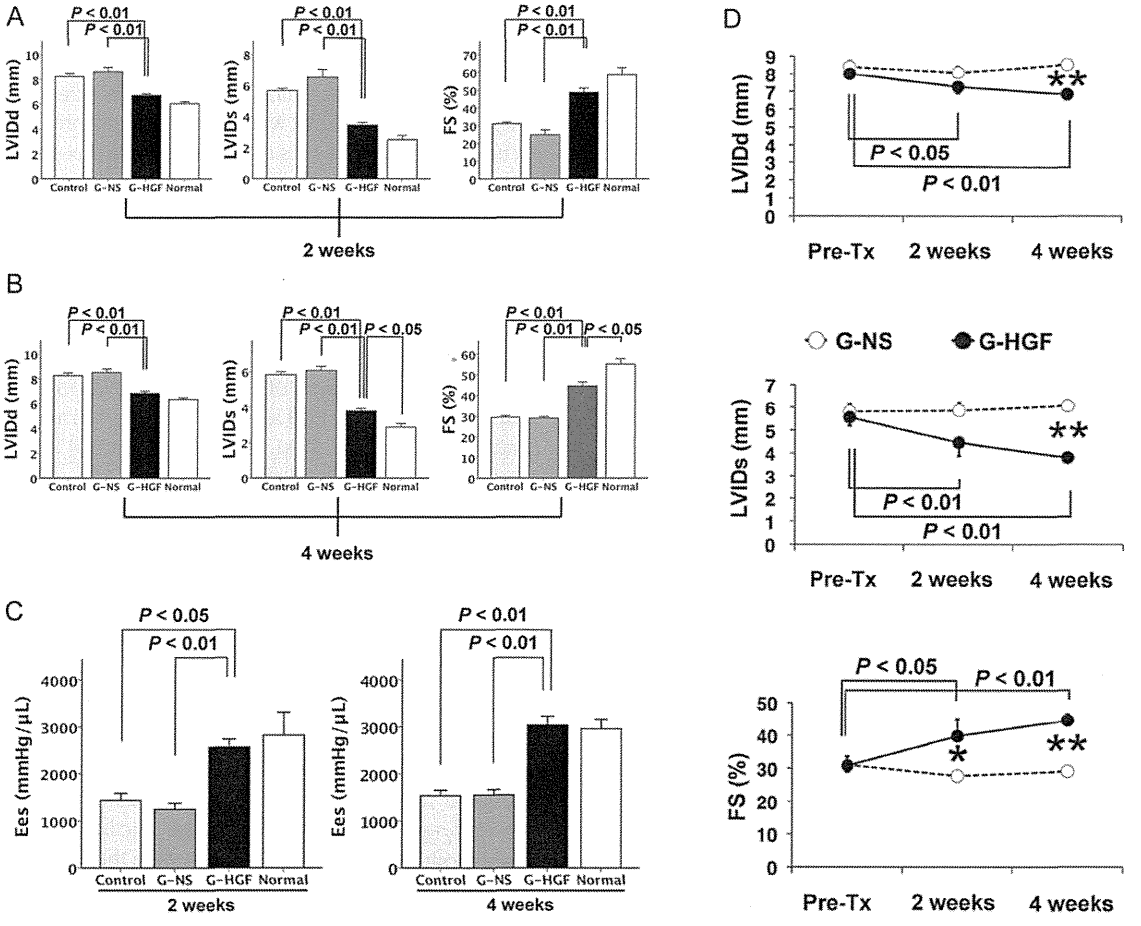

Figure 2: (**A**) Echocardiographic studies of the 2-week set. (**B**) Echocardiographic studies of the 4-week set. (**C**) End-systolic elastance (Ees) analysis at 2 and 4 weeks after treatment. All values are represented as means ± SEM. (**D**) The time course of left ventricular geometric changes in the 4-week set (G-NS and G-HGF groups). *$P < 0.05$, **$P < 0.01$: difference between G-NS (open circles) and G-HGF (filled circles). FS: fractional shortening; G-HGF: gelatin-hepatocyte growth factor; G-NS: gelatin-normal saline; LVIDd: left ventricular internal end-diastolic dimension; LVIDs: left ventricular internal end-systolic dimension; Pre-Tx: pretreatment.

Table 2: Physiological studies

	Two weeks				Four weeks			
	Control	G-NS	G-HGF	Normal[a]	Control	G-NS	G-HGF	Normal[a]
Systolic thickening	1.5 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
Tau (ms)	9.3 ± 0.8	10.4 ± 0.5	11.2 ± 1.2	9.6 ± 1.2	9.5 ± 0.3	9.5 ± 0.5	10.7 ± 0.5	11.8 ± 1.8

[a]Normal: normal Lewis rats of the same age.
G-HGF: gelatin-hepatocyte growth factor; G-NS: gelatin-normal saline.

G-NS group were similar during the 4-week period, those in the G-HGF group had decreased significantly. Accordingly, LV fractional shortening at 2 and 4 weeks after surgery was significantly higher in the HGF group than in the G-NS group ($P < 0.05$, $P < 0.01$ in unpaired *t*-tests, respectively). However, in the G-HGF group, no significant differences in LVIDd, LVIDs and fractional shortening was observed between 2 and 4 weeks after treatment ($P = 0.29$, $P = 0.27$, $P = 0.37$ in paired *t*-tests, respectively).

Histology

When we assessed myocardial fibrosis using Sirius-red-stained sections (Fig. 3), we found significantly greater fibrosis in the Control and G-NS groups than in the G-HGF group 2 weeks after treatment (17.5 ± 0.2%, 15.6 ± 0.7%, 8.8 ± 0.9%, respectively; $P < 0.01$, $P < 0.01$, respectively). At 4 weeks after surgery, the fibrotic area of the G-HGF group was significantly lower than that of the G-NS group (10.9 ± 1.4%, 18.5 ± 1.3%, respectively; $P < 0.01$). There were no significant differences, regarding myocardial fibrosis, between the Control and the G-NS groups at both time points.

Analysis of mRNA expression

The mRNA expression of TGF-β1 was higher in the Control group than in the G-HGF group 2 weeks after treatment ($P = 0.01$; Fig. 4). Similarly, the mRNA expression of TGF-β1 was higher in the G-NS group than in the G-HGF group 4 weeks after treatment ($P < 0.01$; Fig. 4).

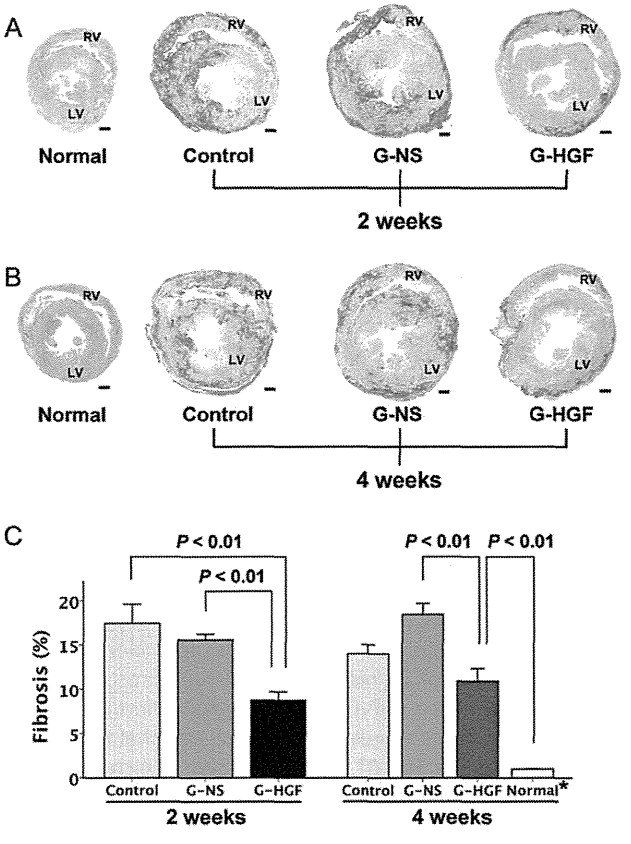

Figure 3: (**A**) Sirius-red-stained sections at 2 weeks after treatment. (**B**) Sirius-red-stained sections at 4 weeks after treatment. (**C**) Quantitative analysis of fibrotic area as a percentage. Bars = 1 mm. All values are expressed as means ± SEM. G-HGF: gelatin-hepatocyte growth factor; G-NS: gelatin-normal saline; LV: left ventricle; RV: right ventricle.

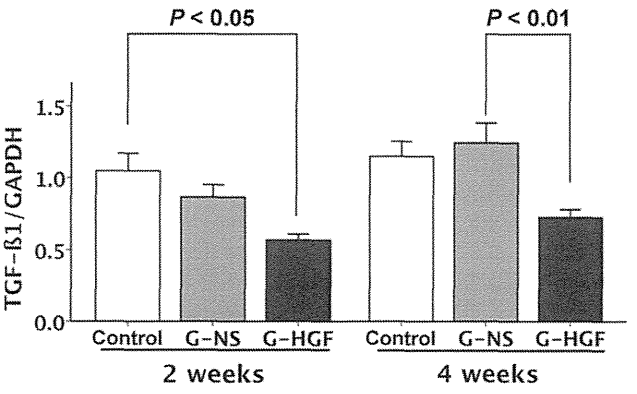

Figure 4: mRNA expression of TGF-β1. G-HGF: gelatin-hepatocyte growth factor; G-NS: gelatin-normal saline; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; TGF-β1: transforming growth factor-β1.

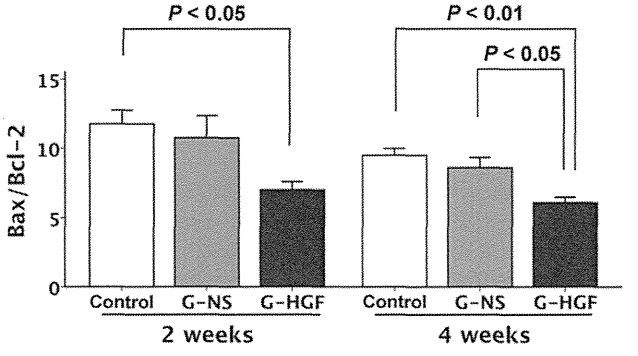

Figure 5: Apoptosis-related proteins; Bax-to-Bcl-2 ratios. G-HGF: gelatin-hepatocyte growth factor; G-NS: gelatin-normal saline.

Measurement of apoptosis-related proteins

In order to test whether HGF modulates apoptosis, ELISA for Bax and Bcl-2 was performed. The Bax-to-Bcl-2 ratios at both 2 and 4 weeks after treatment were lower in the G-HGF group than in the Control group ($P = 0.04$ and < 0.01, respectively; Fig. 5). The ratio at 4 weeks after surgery was lower in the G-HGF group than in the G-NS group ($P = 0.03$; Fig. 5).

DISCUSSION

Main findings

This study demonstrated that sustained-release of HGF using gelatin hydrogel sheets improves myocardial contractility in a rat myocarditis model. There were no significant differences in LV dimensions or systolic function between the Control and the G-NS groups. Therefore, placement of the saline-incorporated hydrogel sheet did not affect cardiac function when compared with the Control group. Histological study revealed that HGF administration attenuated the ratios of the fibrotic area. Evaluation of TGF-β1 mRNA expression was consistent with this result. The proapoptotic-to-antiapoptotic protein ratios were lower after the

application of HGF gelatin hydrogel sheets than in the Control and the G-NS groups.

Delivery of HGF

In order to address the problem of the short HGF half-life in solution form, investigators either administered daily subcutaneous injections of HGF for 3 weeks [17] or used the transfection of HGF genes by using adenovirus or HVJ [18, 19]. However, there have been concerns about possible adverse effects of HGF associated with systemic administration of HGF, and inflammatory responses to these vectors [9].

In order to establish a delivery system, we have developed HGF-incorporated gelatin hydrogel sheets, which enable HGF to be gradually released *in situ* over 2 weeks. We previously followed tissue concentrations of HGF up to 4 weeks after epicardial application of an HGF-incorporating gelatin hydrogel sheet to using ^{125}I-labeled HGF as described elsewhere [10, 11]. In brief, HGF levels remaining in the myocardium were 101 ± 8 and 21 ± 2 ng/g at 1 week and 2 weeks, respectively, but were below detectable levels thereafter. The present study provides breakthrough evidence that the HGF gelatin hydrogel sheet preserves LV systolic function in a rat myocarditis model.

Although gene transfer with viral vectors showed good results [18], gene expression cannot be fully controlled after transfection. In connection to this, there have been concerns about inflammatory responses to these vectors [9]. In contrast, our delivery system can easily control the release of HGF by changing the water content of the gelatin hydrogel [10]. Gelatin of protein is degraded by proteolysis. The higher the glutaraldehyde concentration used for hydrogel preparation, the higher the crosslinking extent of hydrogels. Higher extent of crosslinking may result in less susceptibility to proteolysis. Moreover, the gelatin hydrogel sheet is fully degraded in the body, thus circumventing inflammatory or pharmacological responses *in vivo* [11]. Considering that our method necessitates a single application of the HGF gelatin hydrogel sheet, it is more feasible than multiple injection of HGF from a clinical point of view [17, 20]. When HGF is administered systemically in its solution form *in vivo*, it rapidly diffuses into the circulation and disappears 1 day after the injection [10, 11]. Frequent injection of HGF solution at physiologically excessive doses may be necessary to induce the biological effects to be expected. We have to consider that persistent or prolonged circulation of HGF could be even harmful (e.g. neoplasms). In contrast, we previously confirmed that the blood HGF level stayed under the limit of detection for 2 weeks after the first day when applied with the gelatin hydrogel sheets [11].

One of the limitations is that this technique requires a major surgical intervention (i.e. thoracotomy). However, we believe that it might be clinically applicable to DCM or myocarditis patients in conjunction with other surgical interventions (e.g. LV volume reduction surgery) or LVAD. Considering clinical utility of the gelatin hydrogel sheets, minimally invasive surgical procedures (e.g. video-assisted thoracotomy) may also be useful.

DCM model

The DCM model in this study was induced by autoimmune myocarditis and is characterized by LV dilatation, systolic dysfunction, myocardial necrosis and collagen deposition [1, 2]. Lewis rats that were immunized with porcine cardiac myosin develop acute myocarditis that starts ~2 weeks from the induction [2]. The inflammation and necrosis subsides within 6 weeks, while fibrotic area gradually increases between the 3rd and 6th weeks. This model has been demonstrated to progress into the state similar to DCM in the chronic phase [1, 2, 13]. There have been several animal models simulating human DCM: Coxsackievirus-infected mice [20], cardiomyopathic Syrian hamster (BIO TO-2) [7, 17, 19], doxorubicin-injected mice [18] and stroke-prone spontaneously hypertensive rats [11]. We adopted the autoimmune myocarditis model because it has been suggested that it closely resembles the fulminant form of human myocarditis and the DCM from autoimmune myocarditis at its chronic phase. As given in Table 1 and Fig. 3A and B, LV dimensions increased and fractional shortening decreased when compared with those of normal Lewis rats of the same age.

Myocardial contractility

We evaluated end-systolic elastance as an indicator of myocardial contractility by means of a conductance- and pressure-measuring catheter. This methodology has some advantages over other approaches. First, it is unaffected by loading conditions and heart rate. Secondly, end-systolic elastance reflects not only contractility but also chamber end-systolic stiffness: fibrosis [15].

Cardiac function was evaluated at both 2 and 4 weeks in the rats in the 4-week set that were sacrificed at 4 weeks after surgery. Serial echocardiographic study showed that cardiac contractility had already improved at 2 weeks after treatment, when compared with pretreatment. No differences were observed between 2 and 4 weeks for LVIDd, LVIDs or fractional shortening. We previously reported HGF levels in the myocardium after implantation of the HGF gelatin hydrogel sheet [11]. Levels gradually decreased over time thereafter. These two findings suggest that 2 weeks may be long enough for this method of HGF delivery to exert its effects. However, further studies are required to test how long the contractile recovery lasts after application of the HGF gelatin hydrogel sheet.

Fibrotic area and TGF-β1

This study showed that the fibrotic area in the G-HGF group was lower than in the Control and G-NS groups. It is possible that HGF exerts its beneficial effects on cardiac function, at least in part, through antifibrotic action. Myocardial fibrosis is related to the number of abnormal clinical features and the extent of LV systolic dysfunction in DCM. This may also represent an alternative prognostic marker for a functional decline. Therefore, HGF is expected to be a potential therapeutic option for clinical myocarditis and DCM.

TGF-β1 is known to be a key factor for promotion of tissue fibrosis. Taniyama *et al.* [7] reported that tissue fibrosis is regulated by a balance between TGF-β1 and HGF production. In this study, analysis of the mRNA expression demonstrated that HGF application suppressed the TGF-β1 gene expression, which in turn supports the results of the histological study. Given the chronic phase of autoimmune myocarditis, HGF may exert its antifibrotic actions not only by inhibition of collagen synthesis through suppression of TGF-β1 gene expression but degradation of collagen through activation of matrix metalloproteinase-1 [7]. Nakamura *et al.* [17] showed suppression of TGF-β1, type I collagen and ANP

expression by exogenous HGF, which was consistent with the improvement of established myocardial fibrosis and hypertrophy in the late stage of the cardiomyopathic hamster model. In addition, Futamatsu *et al.* [21] demonstrated that HGF gene therapy was effective in attenuating established inflammation through its effects on T-cell mediated immunity. They also revealed that HGF gene transfer on the same day as the immunization inhibits the development of autoimmune myocarditis. In combination with our results, HGF therapy might be effective in both early and chronic phases of the autoimmune myocarditis. Reduced fibrosis may lead to increased blood flow in the myocardium, which contributes to the improvement of global systolic function.

This method of HGF administration can be an adjunct therapy in human DCM or myocarditis cases. We previously reported that the ratio of the fibrotic area and apoptosis increases with time in rat hearts with DCM, under mechanical unloading after heterotopic transplantation, although myocardial contractility was preserved [22]. HGF gelatin hydrogel sheets may thus offset the drawbacks of mechanical unloading.

Apoptosis

Nakamura *et al.* [6, 17] reported that subcutaneous administration of HGF decreased the number of terminal dUTP nick end-labeling (TUNEL)-positive cardiomyocytes in the ischaemia/reperfusion injury rat model and the cardiomyopathic hamster model. Futamatsu *et al.* [21] also reported that the HFG gene therapy resulted in a reduction in the incidence of apoptotic cardiomyocytes in the same experimental autoimmune myocarditis model as ours. On the other hand, it was reported that TUNEL-positive cells were only rarely detected in the doxorubicin-induced cardiomyopathy model [18]. We analysed whether HGF would alter the balance of apoptosis-related proteins by using ELISA. Bax is known to be proapoptotic, whereas Bcl-2 has an antiapoptotic effect [23, 24]. ELISA analysis showed that the Bax-to-Bcl-2 ratios at both 2 and 4 weeks after treatment were lower in the treatment group (Fig. 5). The balance of Bax and Bcl-2 was found to be antiapoptotic along with the suppressed gene expression of TGF-β1, which induces hypertrophy and apoptotic cell death in cardiomyocytes [17]. Based on this observation, attenuated apoptosis may be another mechanism by which the HGF gelatin hydrogel sheet preserved cardiac function in the DCM rat model. However, further evaluations using other examination methods for apoptotic parameters (e.g. TUNEL and caspase) are necessary.

Limitations

The present study has several limitations. First, DCM model in this study was produced by inducing autoimmune myocarditis. This animal model may not exactly correspond with idiopathic cardiomyopathy; however, it has been used and presented elsewhere [13, 22]. Secondly, although the amount of 100 μg was sufficient for HGF to exert its positive effects on cardiac function in the myocarditis rat model, the optimal or safe amount of HGF and its release profile have not been determined. Thirdly, because human myocardium is thicker than that in rats, it is unclear whether tissue HGF concentrations in the myocardium obtained by the same delivery system are sufficiently high for HGF to exert its activities. Fourthly, we did not evaluate the adverse effects of HGF. All rats in the G-NS and G-HGF groups survived during the study period. In addition, tissue HGF levels in the myocardium, blood, lungs and liver were evaluated, and no side-effects were observed in our previous study using HGF gelatin hydrogel sheets in a hypertensive rat model [11]. Other investigators have also reported no side-effects in their lung model [25]. Nevertheless, longer observation periods are necessary to rule out neoplastic changes after HGF application. Finally, although the present findings regarding attenuation of fibrosis and suppressed gene expression of TGF-β1 explain, at least in part, therapeutic effects of HGF, other biological activities of HGF may explain additional mechanisms (e.g. angiogenesis and anti-inflammation) responsible for improvement of deteriorated cardiac function.

CONCLUSIONS

Sustained-release of HGF, using gelatin hydrogel sheets, improves LV systolic function in a rat myocarditis model. The beneficial effects are attributable to the antifibrotic action of HGF. HGF favourably alters expression of fibrosis-related mRNA *in vivo*. It is also possible that the antiapoptotic action of HGF may contribute the preservation of myocardial contractility. HGF-incorporated gelatin hydrogel sheet can be a new therapeutic modality for chronic myocarditis.

ACKNOWLEDGEMENTS

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Conflict of interest: none declared.

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Early angiographic evaluation after off-pump coronary artery bypass grafting

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Objectives: One of the potential drawbacks of off-pump coronary artery bypass is reduced patency compared with conventional coronary artery bypass. This study examined the systematic angiographic evaluation after off-pump coronary artery bypass.

Methods: Of the 1604 consecutive patients who underwent off-pump coronary artery bypass over 6 years, 1422 (89%) who underwent postoperative angiography were analyzed. Generalized estimating equations logistic analyses were used to investigate potential predictors of graft failure (FitzGibbon B or O).

Results: Bilateral internal thoracic arteries were used in 78% of the patients. The mean number of distal anastomoses was 3.7 ± 1.2. The in-hospital mortality rate was 0.4%. Recipient coronary diameter less than 1.5 mm (odds ratio [OR], 1.62; 95% confidence interval [CI], 1.24-2.11) was an independent predictor of graft failure, whereas percent stenosis diameter greater than 75% (OR, 0.71; 95% CI, 0.53-0.93), sequential graft (OR, 0.69; 95% CI, 0.51-0.94), and left main disease (OR, 0.72; 95% CI, 0.53-0.96) were protective factors. In the subanalyses for each conduit, percent stenosis diameter was protective against left internal thoracic artery failure (OR, 0.61), whereas smaller recipient coronary diameter was associated with right gastroepiploic artery and saphenous vein graft failure (OR, 2.37 and 2.36, respectively). Left circumflex artery was associated with gastroepiploic artery graft failure, whereas sequential graft was again protective for the gastroepiploic artery (OR, 4.39 and 0.33, respectively).

Conclusions: Smaller coronary diameter would be a predictor of graft failure, whereas percent stenosis diameter greater than 75%, sequential graft, and left main disease would be protective factors for off-pump bypass grafts. (J Thorac Cardiovasc Surg 2013;146:1119-25)

Considerable knowledge has been accumulated since off-pump coronary artery bypass (OPCAB) gained resurgent interest among cardiac surgeons. However, contrary to surgeons' expectations, major outcomes have not been demonstrated to be better with OPCAB than with conventional coronary artery bypass (CCAB).[1,2] Several studies[3] showed that patients undergoing OPCAB experienced reintervention more frequently at follow-up than those undergoing CCAB. Reduced patency in OPCAB may, in part, account for the higher rate of reintervention at follow-up, because OPCAB is a more technically demanding procedure than CCAB.[4] Although some investigators[1] reported equivalent patency rates for OPCAB and CCAB, 2 randomized controlled studies[2,5] have shown that OPCAB was associated with an increased risk of graft failure than CCAB. Shroyer and colleagues[5] demonstrated that the patency rate of the OPCAB arm was lower than that of the on-pump arm on 12-month angiography, and the 1-year composite adverse outcome rate (death from any cause, nonfatal myocardial infarction, and any reintervention procedure) was higher for OPCAB than for CCAB. Several meta-analyses concerning graft patency also showed similar results.[4]

Graft failure is one of the major determinants of clinical prognosis.[6] However, the optimal graft choice for OPCAB has not been determined, and, at present, it is derived from extrapolation of the previous findings in CCAB.[7] High patency rates of arterial conduits at long-term follow-up have been well described in the literature, and attrition seems to be limited to within a few months after the operation.[8] Thus, the early patency of off-pump bypass grafts needs to be evaluated. This study was conducted to examine the findings of the systematic angiographic evaluation done after OPCAB surgery to identify predictors of graft failure.

MATERIALS AND METHODS

Study Design

This was a database study based on Kokura Memorial Hospital patients' medical records. The primary objective of this study was to identify the independent predictors of graft failure on the basis of the postoperative

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Abbreviations and Acronyms

BITA = bilateral internal thoracic artery
CCAB = conventional coronary artery bypass
CI = confidence interval
LAD = left anterior descending
LCx = left circumflex
LITA = left internal thoracic artery
GEA = gastroepiploic artery
GEE = generalized estimating equation
MDCT = multidetector computed tomography
OPCAB = off-pump coronary artery bypass
OR = odds ratio
RCA = right coronary artery
SVG = saphenous vein graft

angiography. In the subanalyses, risk factors for failure of each conduit were explored. This study was approved by the Kokura Memorial Hospital Institutional Review Board, with patient consent waived.

Patients

From January 2000 to December 2005 inclusive, 1604 consecutive patients underwent isolated OPCAB by a single surgeon at Kokura Memorial Hospital. Of these, 1521 patients (95%) underwent systematic angiographic evaluation by means of catheter-based angiography (1422 patients, 89%) or multidetector computed tomography (MDCT; SOMATOM Sensation 16; Siemens AG, Munich, Germany; 99 patients, 6%) before discharge. Because 16-detector row CT has some limitations (eg, lower spatial and temporal resolution), patients who underwent catheter-based angiography comprised the study cohort. Perioperative data were collected prospectively and entered into the institutional database. The definitions of data concerning baseline characteristics conformed to those reported in the European System for Cardiac Operative Risk Evaluation.[9] The definitions of perioperative factors were delineated previously.[10]

Target territories were defined as the left anterior descending (LAD) artery, left circumflex (LCx) artery, or right coronary artery (RCA) on the basis of their anatomy. Percent stenosis diameter was qualitatively assessed by the operating surgeon and dichotomized as greater than 75% or 75% or less in this study.[11] Recipient vessel diameter was determined from an assessment at surgery by means of 1.0-, 1.5-, or 2.0-mm probe insertion.

Surgical Technique

OPCAB was the intended procedure, except for patients with acute myocardial infarction who were in a hemodynamically unstable state even with an intra-aortic balloon pump. There were 77 patients who underwent intended CCAB during the study period. Approximately half of them were in a hemodynamically unstable condition, even with an intra-aortic balloon pump; the remaining patients had previous cardiac surgery and required cardiopulmonary bypass for resternotomy and exposure. All OPCAB procedures were performed under general anesthesia with pulmonary artery pressure monitoring. The heart was approached via median sternotomy. Heparin (100 KIU/kg and an additional dose) was administered to achieve and maintain the activated clotting time at more than 250 seconds. Graft selection was based on the following strategies: (1) Patients with significantly stenosed (≥75%) multivessel disease involving the left coronary arteries received bilateral internal thoracic arteries (BITAs); (2) for the left coronary arteries with less than 75% stenoses, saphenous vein grafts (SVG) or radial arteries were used as additional conduits; (3) for less than 75% and 75% or more stenosed RCAs, SVG and right gastroepiploic arteries (GEAs) were used, respectively. Internal thoracic arteries and GEAs were harvested in a skeletonized fashion using an ultrasonic scalpel (Harmonic Scalpel; Ethicon Endo-Surgery, CVG, Cincinnati, OH) by trained surgeons. Diluted papaverine hydrochloride (1:20) was injected into the arterial conduits from the distal end. The conduits were wrapped in a papaverine-soaked gauze until anastomoses. SVG and radial arteries were dissected using the conventional open harvest technique. SVG was gently dilated by injection of heparin-added blood.

The left internal thoracic artery (LITA) was usually anastomosed to the LAD region, and the right internal thoracic artery (RITA) was anastomosed to the LCx via the transverse sinus. When the RITA was not long enough to reach the LCx system, (1) the proximal portion of the RITA was cut and anastomosed to the side of the LITA graft in a Y-shaped fashion as a composite graft (30% of RITA anastomoses) or (2) the pedicled RITA was directed to the LAD system, whereas the LITA was anastomosed to the LCx system (14% of RITA anastomoses). The choice of BITA configurations was based on the surgeon's preference. Heart positioning was achieved with the help of deep pericardial sutures, suction-type devices (Octopus system, Medtronic, Inc, Minneapolis, Minn; and Acrobat SUV system, Guidant Corporation-Cardiac Surgery, Santa Clara, Calif), and table tilting. Of the sequential grafts, the distal anastomosis was done in end-to-side fashion. Side-to-side anastomoses were made in a diamond shape for SVGs and in a parallel fashion for the arterial grafts. Endarterectomy was performed if a coronary artery had long, calcified plaque. Proximal anastomoses of SVGs and radial arteries were made under a single tangential clamp. When the ascending aorta was not eligible for clamping, proximal anastomotic devices were applied (Symmetry Aortic Connector System, St Jude Medical, Inc, St Paul, Minn; and PAS-Port System, Cardica, Redwood City, Calif).[12] Grafts were intraoperatively evaluated with flowmetry (CardioMeds; Medi-Stim, Oslo, Norway) to detect bypass dysfunction, although the data were not recorded in our database. Intraoperative decisions (eg, re-anastomosis) were made on the basis of the results of the flowmetry. Heparin was reversed with a half-reversal dose of protamine sulfate after completion of all anastomoses. Subcutaneous heparin at a dose of 100 KIU/kg was given twice per day on postoperative days 1 to 4 in every patient to prevent postoperative stroke.[12] Oral aspirin (100 mg) was started on postoperative day 1 and continued thereafter. Other antiplatelets (eg, clopidogrel) were not used.

Angiographic Evaluation

Catheter-based angiography was performed before discharge in the patients, all of whom provided their written informed consent. Postoperative angiography was performed as routine evaluation and is standard of care in Japan. Patients with cerebrovascular disease, renal dysfunction, or respiratory failure were excluded for clinical reasons (83 patients). Most patients underwent angiography within 2 weeks after surgery. The experienced interventional cardiologists who performed angiography reviewed the results. Both native coronary arteries and conduits were selectively visualized, and in at least 2 orthogonal views, the conduit was reviewed and scored on the worst appearance of the proximal anastomosis, body of the conduit, and distal anastomosis according to the FitzGibbon classification.[6] When grafts could not be selectively intubated, an aortogram or a subclavian arteriogram was obtained. Each anastomosis was analyzed separately. A string sign was recorded as FitzGibbon grade B. A patent graft was defined as a graft without 50% or greater stenosis (ie, FitzGibbon A).

Statistical Analysis

Continuous data are expressed as means ±1 standard deviation or medians (interquartile range), and categoric variables are expressed as numbers (proportions). Because the graft patency of multiple anastomoses within a patient cannot be assumed to be independent,[13] generalized estimating equation (GEE) logistic analyses were used to evaluate the influence of the potential predictors (Main Analysis).[14] An event was defined as FitzGibbon grade B or O. The working correlation matrix was set to

exchangeable, and the robust variance estimators were adopted for constructing confidence intervals (CIs) of the odds ratios (ORs). Model variables used in the multivariable analyses were selected a priori on the basis of the previous reports.[15,16] Grafting techniques also were included in the model on the presumption that these were clinically relevant. The following 20 factors were included in the analysis: age (>75 years), sex (female), New York Heart Association classification (≥3), recent myocardial infarction (within 90 days), left ventricular ejection fraction (<50%), chronic renal failure (creatinine >200 μmol/L), hypertension, hypercholesterolemia, diabetes mellitus, left main disease (≥50%), extracardiac arteriopathy, previous cardiac surgery, emergency, operator's experience (first 100 consecutive cases), percent stenosis diameter (>75%), recipient coronary diameter (<1.5 mm), number of anastomoses (continuous), conduits (LITA, RITA, GEA, SVG, and radial artery), territories (LAD, LCx, and RCA), and grafting techniques (sequential, composite, and endarterectomy). In the subanalyses for each conduit, cases with exceptional anastomoses (eg, GEA-LAD anastomosis) were excluded from the GEE models. Subanalysis for the radial artery graft was not performed because there were only 76 radial artery anastomoses (1.4% of all anastomoses). All analyses were performed using SAS version 9.2 (SAS Institute, Inc, Cary, NC). All *P* values quoted are 2-sided.

RESULTS

Patients' Characteristics and Operative Results

The patients' baseline and perioperative characteristics are shown in Tables 1 and 2. Univariate analyses for graft occlusion revealed estimates of naïve ORs by the GEEs. The patients' average age was 68 ± 9 years; 20% of the patients were aged more than 75 years (75% were male, 25% were female). Left main disease 50% or greater was present in 44% of patients. The mean number of distal anastomoses was 3.7 ± 1.2. BITA grafts were used in 78% of patients, and SVGs were used in 41% of patients. Sequential grafting was performed in 76% of patients. Of the 5262 anastomoses, 63% had proximal stenoses greater than 75%. The recipient coronary diameter was less than 1.5 mm in 35% of anastomotic sites. Postoperative morbidity included perioperative myocardial infarction in 33 patients (new Q-wave and creatine kinase-myocardial band >50 IU/L), low output syndrome in 7 patients, new-onset atrial fibrillation in 370 patients, stroke in 8 patients, renal failure necessitating dialysis in 18 patients, and prolonged ventilation (>48 hours) in 42 patients. Operative (within 30 days) and in-hospital mortality were 0.1% and 0.4%, respectively.

Angiographic Evaluation: Main Analysis

Table 3 shows the distribution and patency rate for each graft (FitzGibbon A). The patency rates of the LITA and RITA grafts were comparable (95.6% and 95.5%, respectively), whereas those of the SVG in the LCx and RCA systems were slightly lower (92.4% and 92.9%, respectively). The patency rate of the GEA grafts when used in the LCx system was markedly low (86.7%); there were only 60 anastomoses with this configuration.

By using the GEE logistic model, 4 variables were found to be independent predictors of overall graft fate (ie, failure or patency; Table 4). Recipient coronary diameter less than 1.5 mm was a risk factor for graft failure (OR, 1.62; 95% CI, 1.24-2.11), whereas percent stenosis diameter greater than 75% (OR, 0.71; 95% CI, 0.53-0.93), sequential graft (OR, 0.69; 95% CI, 0.51-0.94), and left main disease (OR, 0.72; 95% CI, 0.53-0.96) protected against graft failure. Other baseline variables, such as LVEF and diabetes mellitus, or operative factors such as type of conduit and target territory, were not associated with graft failure in the multivariable analysis. Although the patency rate of those patients with coronary endarterectomy was relatively low (82.1%), this technique was not associated with graft failure.

TABLE 1. Preoperative characteristics

	Total, N = 1422	Univariate analysis*		
Variable	No. (%)	OR	95% CI	*P* value
Age (y)	68 ± 9			
Age > 75 y	290 (20)	1.39	1.02-1.90	.039
Female sex	379 (25)	1.45	1.08-1.95	.014
NYHA class ≥ 3	265 (19)	0.88	0.62-1.25	.468
Unstable angina	318 (22)	0.81	0.57-1.16	.256
Emergency	109 (8)	0.86	0.50-1.48	.582
Previous MI	614 (43)	0.99	0.75-1.30	.924
Recent MI (<90 d)	140 (10)	1.21	0.76-1.82	.371
LV dysfunction				
Fair (LVEF > 50%)	1093 (77)	Reference		
Moderate (LVEF 30%-50%)	299 (21)	0.75	0.52-1.07	.113
Poor (LVEF < 30%)	30 (2)	0.93	0.38-2.25	.865
Diseased vessels				
1- or 2-vessel disease	450 (32)	Reference		
3-vessel disease	972 (68)	1.20	0.58-2.48	.518
Left main disease	619 (44)	0.69	0.51-0.91	.010
Previous PCI	619 (44)	0.97	0.73-1.28	.815
Previous cardiac surgery	33 (2)	1.64	0.81-3.35	.172
CRF (creatinine > 200 μmol/L)	94 (7)	0.61	0.31-1.19	.145
ESRF on dialysis	70 (5)			
Diabetes mellitus	659 (46)	1.04	0.79-1.38	.771
Taking insulin	163 (11)			
Hypertension	951 (67)	0.93	0.70-1.23	.601
Hypercholesterolemia	793 (56)	1.07	0.81-1.42	.635
COPD	36 (3)	1.16	0.60-2.26	.658
Extracardiac arteriopathy	296 (21)	0.85	0.61-1.19	.350
CVA	240 (17)	1.04	0.71-1.51	.837

OR, Odds ratio; *CI,* confidence interval; *NYHA,* New York Heart Association; *MI,* myocardial infarction; *LV,* left ventricle; *LVEF,* left ventricular ejection fraction; *PCI,* percutaneous coronary intervention; *CRF,* chronic renal failure; *ESRF,* end-stage renal failure; *COPD,* chronic obstructive pulmonary disease; *CVA,* cerebrovascular accident. *Univariate analysis for graft occlusion. Estimates of naïve ORs by the GEEs.

Angiographic Evaluation: Subanalysis for Each Conduit

For LITA grafts, percent stenosis diameter greater than 75% was the only predictor of patency (OR, 0.61; 95% CI, 0.40-0.94; Table 5). None of the variables were

TABLE 2. Perioperative characteristics

Variable	Total, N = 1422 No. (%)	Univariate analysis* OR	95% CI	P value
First 100 cases	90 (6)	0.92	0.53-1.60	.765
No. of anastomoses	3.7 ± 1.2	1.01	0.88-1.16	.853
Incomplete revascularization	187 (13)	0.89	0.57-1.39	.607
Conversion to CCAB	4 (0.3)	—	—	—
Operation time (min)	288 ± 72	1.13	1.01-1.27	.032
Conduit				
LITA	1365 (96)	Reference		
RITA	1083 (76)	1.04	0.73-1.48	.836
GEA	476 (33)	1.27	0.82-1.95	.285
SVG	586 (41)	1.68	1.17-2.43	.005
Radial artery	47 (3)	0.95	0.29-3.11	.936
Territory				
LAD	1400 (98)	Reference		
LCx	1237 (87)	1.20	0.87-1.65	.273
RCA	962 (68)	1.39	0.99-1.94	.056
Grafting technique				
Sequential graft	1076 (76)	0.80	0.61-1.04	.099
Composite graft	345 (24)	1.36	0.91-2.04	.128
Endarterectomy	31 (2)	2.23	0.65-7.68	.202
Proximal anastomotic devices	83 (5)	0.46	0.20-1.06	.068
Recipient coronary (N = 5262)				
Stenosis >75%	3293 (63)	0.73	0.56-0.95	.019
Diameter <1.5 mm	1860 (35)	1.61	1.25-2.09	<.001
<1.0 mm	21 (0.4)			
1.0 mm	2462 (47)			
1.5 mm	2613 (50)			
>1.5 mm	166 (3)			
Max CK-MB IU/L (N = 1402)	13 (8.6-24)†	1.02	1.00-1.03	.026
CK-MB > 50 IU/L	148 (10)	1.66	1.12-2.47	.012
Mortality				
Operative (within 30 d)	1 (0.1)			
In-hospital	5 (0.4)			

OR, Odds ratio; *CI,* confidence interval; *CCAB,* conventional coronary artery bypass; *LITA,* left internal thoracic artery; *RITA,* right internal thoracic artery; *GEA,* gastroepiploic artery; *SVG,* saphenous vein graft; *LAD,* left anterior descending; *LCx,* left circumflex; *RCA,* right coronary artery; *CK-MB,* creatine kinase-myocardial band isozyme. *Univariate analysis for graft occlusion. Estimates of naïve ORs by the GEEs. †Median (interquartile range).

TABLE 3. Graft distribution and patency rate

Conduit	Territory	Total no.	FitzGibbon A	FitzGibbon B*	FitzGibbon O	Patency rate†
LITA	LAD	1925	1841	70 (13)	14	95.6%
	LCx	227	218	7 (1)	2	96.0%
	RCA	4	2	0	2	50.0%
	Total	2156	2061	77 (14)	18	95.6%
RITA	LAD	231	220	10 (1)	1	95.2%
	LCx	1145	1093	31 (3)	21	95.5%
	RCA	37	36	1	0	97.3%
	Total	1413	1349	42 (4)	22	95.5%
GEA	LAD	7	7	0	0	100%
	LCx	60	52	2	6	86.7%
	RCA	585	557	16 (3)	12	95.2%
	Total	652	616	18 (3)	18	94.5%
SVG	LAD	88	84	3	1	95.5%
	LCx	288	266	4	18	92.4%
	RCA	588	546	16 (1)	26	92.9%
	Total	964	896	23 (1)	45	92.9%
Radial artery	LAD	4	4	0	0	100%
	LCx	49	48	1	0	98.0%
	RCA	23	21	1	1	91.3%
	Total	76	73	2	1	96.1%

LITA, Left internal thoracic artery; *LAD,* left anterior descending; *LCx,* left circumflex; *RCA,* right coronary artery; *RITA,* right internal thoracic artery; *GEA,* gastroepiploic artery; *SVG,* saphenous vein graft. *Numbers in parentheses represent string sign. †FitzGibbon A.

associated with RITA graft failure. Smaller recipient coronary diameter (<1.5 mm) showed a trend to correlate with GEA graft and SVG failure (OR, 2.37 and 2.36; 95% CI, 1.08-5.20 and 1.32-4.19, respectively). Of note, the LCx system was a predictor of GEA graft failure (OR, 4.39; 95% CI, 1.66-11.61), whereas sequential graft was a protective factor for GEA graft patency (OR, 0.33; 95% CI, 0.15-0.70).

Patients Who Did Not Undergo Angiography

Among the patients who did not undergo postoperative angiography, all but 1 had no sign of ischemia during the hospital stay. One death occurred in a 69-year-old woman. Vasospasm of the radial artery graft was suspected to be the cause.

Postangiography Outcomes

Eight patients (0.6%) experienced complications of postoperative angiography: limb thrombosis in 2 patients, ventricular arrhythmia in 1 patient, dissection of the aorta in 1 patient, transient ischemic attack in 2 patients, and cerebral infarction in 2 patients. The 2 patients with cerebral infarction showed neurologic dysfunction, but all 8 patients were discharged.

Of the 216 patients with at least 1 suboptimal graft (FitzGibbon B or O), 25 underwent repeated interventions as staged procedures (24 percutaneous coronary interventions and 1 surgery) after scintigraphy.

DISCUSSION

One of the possible drawbacks of OPCAB surgery is reduced patency compared with CCAB.[2,4,5] Graft failure is relevant to the clinical prognosis of patients undergoing CCAB,[6] and it is logical to think that this association may fit the OPCAB cohort. Many studies have been published concerning graft patency in CCAB. However, few reports have specifically dealt with this issue in OPCAB surgery.[17] In addition, the results from these studies should be interpreted cautiously, because few of them took clustering into account,[1,17] and the findings of most studies were derived from symptom-driven angiography.

TABLE 4. Multivariable analysis: Predictors of graft occlusion (FitzGibbon B or O)

Variable	OR	95% CI	P value
Age > 75 y	1.37	0.98-1.90	.064
Female sex	1.24	0.91-1.68	.183
NYHA class ≥ 3	0.89	0.60-1.35	.592
Recent MI (<90 d)	1.25	0.82-1.91	.301
LVEF < 50%	0.79	0.56-1.12	.181
CRF	0.67	0.34-1.33	.253
Hypertension	0.93	0.69-1.25	.627
Hypercholesterolemia	1.07	0.79-1.44	.668
Diabetes mellitus	1.06	0.79-1.40	.712
Left main disease	0.72	0.53-0.96	.028
Extracardiac arteriopathy	0.86	0.61-1.22	.401
Previous cardiac surgery	1.25	0.57-2.75	.580
Emergency	0.88	0.48-1.61	.683
First 100 cases	1.00	0.58-1.73	.995
Recipient coronary			
Stenosis > 75%	0.71	0.53-0.93	.013
Diameter < 1.5 mm	1.62	1.24-2.11	<.001
No. of anastomoses	1.04	0.89-1.21	.618
Conduit			
LITA	Reference		
RITA	0.75	0.45-1.26	.275
GEA	1.16	0.57-2.38	.684
SVG	1.37	0.78-2.40	.275
Radial artery	0.70	0.20-2.48	.579
Territory			
LAD	Reference		
LCx	1.06	0.69-1.61	.805
RCA	0.88	0.48-1.60	.668
Grafting technique			
Sequential graft	0.69	0.51-0.94	.018
Composite graft	1.61	0.98-2.62	.058
Endarterectomy	1.96	0.62-6.24	.254

OR, Odds ratio; *CI*, confidence interval; *NYHA*, New York Heart Association; *MI*, myocardial infarction; *LVEF*, left ventricular ejection fraction; *CRF*, chronic renal failure; *LITA*, left internal thoracic artery; *RITA*, right internal thoracic artery; *GEA*, gastroepiploic artery; *SVG*, saphenous vein graft; *LAD*, left anterior descending; *LCx*, left circumflex; *RCA*, right coronary artery.

In 1604 consecutive patients who underwent OPCAB, 89% received systematic catheter-based angiography before discharge. GEE logistic analysis was performed to identify the independent predictors of graft failure, and percent stenosis diameter (>75%), recipient coronary diameter (<1.5 mm), sequential graft, and left main disease were found to be predictors.

Percent Stenosis Diameter

When arterial grafts are used to bypass a low-grade stenosis, competitive flow may increase from the native coronary vessel.[18] Several authors also noted an association between decreased severity of stenosis and frequency of the string sign.[11] Miwa and colleagues[19] reported that the string sign of patent radial arteries was a consequence of competitive flow. The current authors and Desai and colleagues[15] have speculated that arterial remodeling to maintain shear stress against the endothelium in the setting of low flow may lead to graft failure.

Recipient Coronary Diameter

Desai and colleagues[15] noted that the distal run-off was strongly correlated with the size of the distal target vessel. Similar to percent stenosis diameter, recipient coronary diameter is deemed to influence the flow through the bypass conduits. Grafting small target vessels may be more technically demanding, especially on the beating heart. On the basis of the results of the subanalysis of each conduit (Table 5), an internal thoracic artery conduit could be expected to have excellent patency for the left coronary arteries even with small target-vessel size.

Sequential Graft

Sequential graft was revealed to be a determinant of graft patency. The primary advantages of this technique include a higher blood flow through the sequential graft than through the individual graft.[20] Intraoperative velocity studies showed a higher velocity of blood flow in the sequential graft.[21] Although a sequential graft may be technically difficult in OPCAB surgery, several authors have reported excellent results.[22]

Left Main Disease

The reason why left main disease served as a protective factor for graft patency was not identified from this retrospective study. When the study cohort was divided as to the sides of the coronary arteries grafted (left or right), left main disease was associated with graft patency in the left coronary arteries, not in the RCAs (data not shown). This result may imply that left main disease, in addition to percent stenosis diameter, decreased the risk of competitive flow. It is also conceivable that patients who have significant left main disease are good candidates for not only CCAB[23] but also OPCAB surgery. However, noncritical, isolated left main stenosis may cause competitive flow. Relationships between graft patency in OPCAB surgery and left main disease (eg, ostial, mid-shaft, and noncritical lesions) need to be evaluated in the future.

Subanalysis: Left Internal Thoracic Artery

In our subanalyses, less severe percent stenosis diameter was associated with LITA graft failure. Doppler studies suggested that severity of stenosis is associated with flow and size of the LITA graft.[18] However, adaptive shrinkage of the arterial conduits may be reversible as the native coronary stenosis progresses.

Subanalysis: Gastroepiploic Artery

Recipient coronary diameter influenced the patency of the GEA. The GEA may be more susceptible to having

TABLE 5. Subanalysis of each conduit

Variable	LITA			RITA			GEA			SVG		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Recipient coronary												
Stenosis > 75%	0.61	0.40-0.94	.025	0.77	0.45-1.31	.331	0.91	0.93-2.11	.826	0.96	0.53-1.73	.878
Diameter < 1.5 mm	1.14	0.71-1.82	.591	1.65	0.98-2.80	.061	2.37	1.08-5.20	.032	2.36	1.32-4.19	.004
Territory												
LAD	Reference			1.22	0.66-2.25	.531	—	—	—	—	—	—
LCx	0.85	0.37-1.97	.706	Reference			4.39	1.66-11.61	.003	1.25	0.70-2.26	.449
RCA	—	—	—	—	—	—	Reference			Reference		
Grafting technique												
Sequential graft	0.99	0.59-1.67	.968	0.94	0.53-1.69	.845	0.33	0.15-0.70	.004	0.76	0.41-1.40	.375
Composite graft	—	—	—	0.86	0.44-1.72	.676	—	—	—	1.46	0.64-3.30	.369
Endarterectomy	—	—	—	—	—	—	—	—	—	3.45	0.75-15.88	.111

LITA, Left internal thoracic artery; *RITA*, right internal thoracic artery; *GEA*, gastroepiploic artery; *SVG*, saphenous vein graft; *OR*, odds ratio; *CI*, confidence interval; *LAD*, left anterior descending; *LCx*, left circumflex; *RCA*, right coronary artery.

a contractile response in the low-flow situation than the LITA because of their different histologic characteristics.[24] We preferentially used the GEA grafts for the RCA system with severe stenosis (>75%). This may partly explain the reason why recipient coronary diameter, not percent stenosis diameter, influenced GEA patency. On the other hand, a sequential graft seems advantageous with GEA grafts, as shown in the subanalysis (Table 5). This technique may help increase the graft flow.

The LCx system was associated with graft failure. In our experience, by harvesting in a skeletonized fashion, the GEA grafts could reach the LCx in some cases. The higher failure rate of this configuration may be attributed to spasm of the distal portion of the GEA and tension on the graft. We have abandoned this configuration and adopted the SVG and the RITA for mild and severe lesions of the LCx, respectively.

Subanalysis: Saphenous Vein Graft

SVG failure was associated with smaller recipient coronary diameter. Goldman and colleagues[16] reported that the smaller diameter of the recipient vessel was a determinant in both early and long-term vein graft patency. Schwartz and colleagues[25] noted that the SVG was a risk factor for graft occlusion in the Bypass Angioplasty Revascularization Investigation study, whereas the type of conduit was not associated with graft patency in our series. This discordance may be, in part, ascribed to routine heparin use.[12] Presumably, an increase in coagulability may account for the early graft occlusion. Therefore, we believe that high-risk patients may require aggressive anticoagulation in addition to antiplatelet therapy after OPCAB.

In 83 cases, proximal anastomotic devices were applied. We previously reported that the early patency rate of these SVGs was 100%.[12] As a result, these devices did not adversely influence the graft patency in this study.

Study Limitations

First, percent stenosis diameter was derived from qualitative assessment by one observer. Other investigators drew conclusions similar to ours in that small target vessel size adversely affected graft patency, although the size of the target vessel was derived from single-observer visual assessment at the time of patient enrollment and not quantitative angiography.[15] Second, all procedures were performed by a single surgeon, and the results of the angiography were derived from the OPCAB surgery on the basis of our grafting strategy, limiting the generalizability of these results. Third, catheter-based angiography was performed in only 89% of the consecutive patients, because MDCT took the place of catheter-based angiography after 2 patients experienced cerebral infarction. Because 16-detector row CT has some limitations (eg, lower spatial and temporal resolution), patients who underwent MDCT were omitted from the study cohort. In addition, the angiography results were read by several cardiologists. Fourth, we did not evaluate long-term patency rates. However, we agree with Goldman and colleagues[16] in that initial patency at 1 week after surgery is an important predictor of long-term graft patency. Fifth, we could not compare the angiographic results between OPCAB and on-pump coronary artery bypass grafting, because only 77 patients underwent on-pump coronary artery bypass grafting in Kokura Memorial Hospital (Kokura, Japan) during the study period. Sixth, we cannot reach any conclusions regarding the radial artery graft because of the small sample size. Finally, the quality of the recipient coronary arteries, the size of the conduits, and the flow data were not recorded in our database. Despite these limitations, the findings of this study were derived from the largest angiographic study to date of OPCAB surgery. In addition, the analyses were based on rigorous statistical methodology.

CONCLUSIONS

Severe percent stenosis diameter and small recipient coronary diameter were associated with graft patency in

OPCAB surgery. A better patency rate would be expected when anastomosing the LITA to a coronary artery with severe stenosis. Sequential grafting technique could be expected to improve the patency rates of off-pump bypass grafts, especially for the GEA. Patients with significant left main disease may be good candidates for OPCAB surgery.

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