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CASE REPORT

Implantation of a Jarvik 2000 left ventricular assist device as a bridge to eligibility for refractory heart failure with renal dysfunction

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Abstract A 55-year-old man, who previously underwent surgical ventricular restoration and mitral valve surgery, was referred to our department for management of refractory heart and multiple organ failure. At the time of admission to our hospital, he could not be registered as a candidate for heart transplantation because of severe renal failure with a serum creatinine level of 4.6 mg/dl. We considered that he was a marginal candidate for heart transplantation; thus, it was essential to understand the etiology of renal failure and estimate whether it was reversible. Cardiac catheterization revealed poor hemodynamic function with a systemic pressure of 107/60 mmHg, cardiac index of 2.5 l/min/m^2, and pulmonary artery pressure of 63/27 mmHg, despite intense medical treatment. Contrary to biochemical examination findings of blood, renal biopsy findings showed no significant glomerular abnormality. Furthermore, the severity of tubular atrophy and interstitial fibrosis in the cortex was mild. These pathological findings suggested that the renal dysfunction in this case was possibly attributable to a hemodynamic factor. His symptoms gradually deteriorated despite an increasing dose of inotropic support; thus, we planned implantation of a Jarvik 2000 axial-flow pump (Jarvik Heart Inc., New York, NY, USA) as a bridge to eligibility, and informed consent was obtained. Because of a tight adhesion on the anterior wall, we placed the device on the lateral wall of the left ventricle, making sure not to direct the pump at the septum. Postoperatively, the implantable left ventricular assist device provided relief from heart failure symptoms as well as recovery of renal function, with serum the creatinine level at 1.2 mg/dl, which allowed the patient to become an appropriate candidate for heart transplantation. At an 18-month follow-up examination, his status was uneventful, and he is now at home awaiting heart transplantation.

Keywords Jarvik 2000 · Left ventricular assist device · Renal dysfunction · Heart transplantation · Cardiomyopathy

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Introduction

Although the gold standard therapy for end-stage heart failure remains heart transplantation, this is not an option for patients with irreversible renal dysfunction, which is often complicated with advanced heart failure [1]. Herein, we report a patient complicated with renal dysfunction who underwent successful implantation of a Jarvik 2000 left ventricular assist device (LVAD) (Jarvik Heart Inc., New York, NY, USA) as a bridge to eligibility for refractory heart failure.

Case report

A 55-year-old man, who was diagnosed with idiopathic dilated cardiomyopathy on the basis of myocardial biopsy

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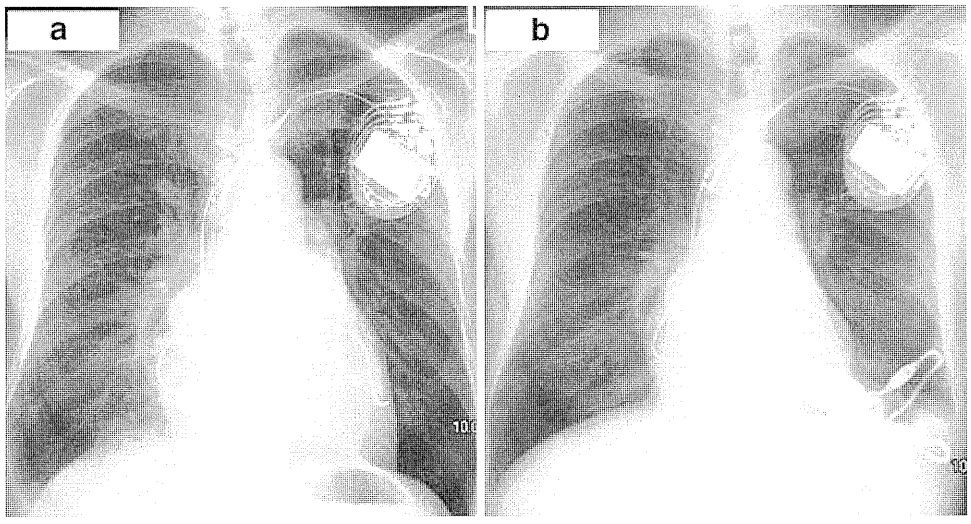

Fig. 1 **a** Chest X-ray image obtained before surgery showing pulmonary congestion and cardiomegaly with a cardio-thoracic ratio of 55%. **b** Chest X-ray image obtained at 1 month after surgery showing the Jarvik 2000 pump in an appropriate position, as well as relief of pulmonary congestion and absence of cardiomegaly

findings in 1995, underwent septal anterior ventricular exclusion (SAVE) and papillary muscle approximation for advanced left ventricular (LV) remodeling (end-systolic volume index 90 ml/m^2) in 2005. He also underwent mitral valve repair for severe functional mitral regurgitation at the time of the SAVE operation, followed by mitral valve replacement with a mechanical valve for recurrent mitral regurgitation in 2008, which did not improve the heart failure symptoms. The patient was referred to our institution on March 2009 for further management of sustained heart failure refractory to maximum pharmacological treatment and cardiac resynchronization therapy. On admission, he exhibited biventricular heart failure symptoms with pitting edema and peripheral coldness. Chest X-ray findings revealed pulmonary congestion and cardiomegaly with a cardio-thoracic ratio of 55% (Fig. 1a), while echocardiographic findings showed advanced left ventricular remodeling with an LV end-diastolic dimension of 75 mm and ejection fraction of 15%. A biochemical examination of blood showed multiple organ failure with serum creatinine at 4.6 mg/dl, blood urea nitrogen at 106 mg/dl, total bilirubin at 4.4 mg/dl, and brain natriuretic peptide at 637 pg/ml. A test of renal function also revealed a low endogenous creatinine clearance of 11.3 ml/min and high urinary fractional excretion of sodium of 3.0%, while neither urinary protein nor urinary occult blood were seen in urinalysis. The patient could not be registered as a candidate for heart transplantation at the time of admission, because of severe renal dysfunction. As the patient had a strong desire to spend his time at home and clearly refused to undergo implantation of a para-corporeal Toyobo LVAD (Nipro, Tokyo, Japan), we in consultation with cardiologists initiated administration of inotropic agents to improve cardiac function and determine whether hemodynamic improvements would result in improvement of the multiple organ failure.

Total bilirubin decreased from 4.4 to 1.1 mg/dl after beginning administration of the inotropic agents, while serum creatinine slightly decreased from 4.6 to 2.9 mg/dl and did not show signs of further improvement. Cardiac catheterization revealed poor hemodynamic function with a systemic pressure of 107/60 mmHg, cardiac index of 2.5 l/min/m^2, and pulmonary artery pressure of 63/27 mmHg, despite intense medical treatment. Renal sonography showed that both kidneys were of normal size and normal renal cortex echogenicity. It also showed no evidence of hydronephrosis, suggesting the absence of postrenal renal dysfunction. Microscopic findings from the renal biopsy revealed no significant glomerular change (Fig. 2a). Furthermore, the severity of tubular atrophy and interstitial fibrosis in the cortex was mild (Fig. 2b), which was not compatible with the findings of biochemical examination findings of blood. In other words, these pathological findings suggested that the renal dysfunction in this case was possibly attributable to a hemodynamic factor. His symptoms gradually deteriorated despite increasing the dose of inotropic support; thus, we planned to implant an implantable LVAD (Jarvik 2000) via a left thoracotomy. We explained to the patient and his family that he was a marginal candidate for heart transplantation, and they agreed to treatment with LVAD implantation as a bridge to eligibility, that is, possibly for transplantation or as destination therapy.

Under routine hemodynamic monitoring and double-lumen endotracheal intubation, a left thoracotomy was made through the seventh intercostal space, and the heart and distal part of the descending thoracic aorta were exposed. The anterior part of the heart was firmly adhered to the chest wall because of previous open heart surgery procedures. As the apex of the left ventricle could not be used as the coring site, because of the tight adhesion, we planned to place the Jarvik 2000 pump on the lateral wall

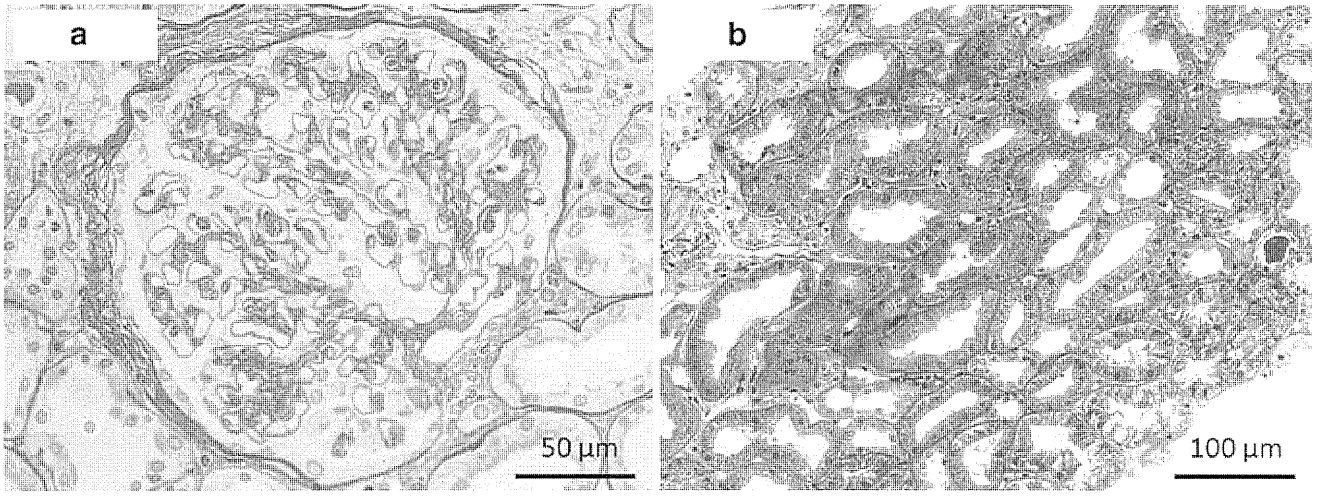

Fig. 2 **a** Periodic acid-Schiff staining results (at ×400 magnification) showing no evidence of significant glomerular change. **b** Elastica-Masson staining results (at ×200 magnification) showing mild tubular atrophy and interstitial fibrosis in the cortex

of the left ventricle. The pericardium was opened between the felt strip of the previous surgical ventricular restoration and left phrenic nerve. The heart was severely dilated, and there was sufficient area for LV coring in the lateral wall of the left ventricle. Following systemic heparinization, a partial cardiopulmonary bypass was established through a femoro-femoral bypass. The aorta was partially occluded, while the outflow graft was anastomosed to the descending aorta in an end-to-side fashion. The anterior part of the LV lateral wall was cored with a coring knife, with no thrombus found within the left ventricle. The Hemashield patch placed during the previous SAVE operation, and reconstructed anterior and posterior papillary muscles could be identified. A sewing cuff was sewn to the lateral wall of the left ventricle, and then the device was introduced into the left ventricular cavity through the sewing cuff, making sure not to interfere with the Hemashield patch or papillary muscles. As the cardiopulmonary bypass was gradually discontinued, the Jarvik 2000 pump was turned on. Hemodynamics were stabilized with an inotropic agent and pulmonary vasodilators.

Postoperatively, the implantable LVAD provided relief of heart failure symptoms, and inotropic agents were promptly discontinued 1 week after surgery. The endogenous creatinine clearance and serum creatinine level improved from 33.0 to 67.5 ml/min and 2.9 to 1.2 mg/dl, respectively, along with increased urine output. Chest X-ray findings at 1 month after surgery revealed that the Jarvik 2000 pump was in an appropriate position, as well as relief of pulmonary congestion and absence of cardiomegaly (Fig. 1b). Postoperative echocardiography findings showed remarkable unloading of the left ventricle (LV end-diastolic dimension 64 mm) as well as normal function of the mitral mechanical valve. At an 18-month follow-up examination, the status of the patient was uneventful, and he is at home awaiting heart transplantation at the time of writing.

Discussion

Axial flow pumps are generally smaller devices than the pulsatile pumps and may be associated with a lower incidence of complications [2–5]. Because of its small size, the Jarvik 2000 pump can be placed within the left ventricle, avoiding the need for an inflow cannula, while the outflow graft can be connected to either the ascending or descending aorta. Frazier et al. [6] reported their initial clinical experience with the Jarvik 2000 LVAD for patients with advanced heart failure, and found that it was safe and satisfactory to provide significant unloading of the left ventricle, resulting in improvements of hemodynamic parameters and functional status. In the present case, we selected this device with particular consideration given to the small body size of our patient (body mass index 17.8 kg/m^2) as well as his clinical history of open heart surgery procedures, both via a median sternotomy. Application of a left thoracotomy enabled us to avoid dissection of the anterior part of the heart and safely place the Jarvik 2000 pump on the lateral wall of the left ventricle. Implantation of a Jarvik 2000 pump through the lateral wall of the left ventricle might be suitable for patients with a history of surgical ventricular reconstruction. However, considerable attention must be paid to not directing the pump toward the septum, which may cause unstable blood removal and inadequate systemic perfusion.

Although the gold standard therapy for end-stage heart failure remains transplantation, this is not an option for patients with irreversible renal dysfunction, which often accompanies advanced heart failure [1]. If the

contraindications for cardiac transplantation are reversible (e.g., hepatic failure, renal failure), an LVAD can be very beneficial for stabilizing these patients and may serve as a bridge to eligibility for transplantation. The most important point of this report may be the expectation of reversibility of renal function by LVAD use in a marginal candidate for heart transplantation. This issue is quite important, especially in Japan where an implantable LVAD can be used only for approved heart transplantation candidates. In our case, the Jarvik 2000 LVAD provided significant improvement of renal function, which allowed the patient to become an appropriate candidate for heart transplantation. We speculated that several of the preoperative findings support the notion that some improvement in renal function could be anticipated after LVAD implantation. First, cardiac catheterization revealed advanced heart failure accompanied by low output syndrome, which indicated an etiology of secondary prerenal failure due to decreased renal perfusion. Possible explanations for improved renal function with LVAD support include improved cardiac output along with increased perfusion of the kidneys, as well as correction of neurohormonal dysregulation associated with congestive heart failure [7]. Second, the present urinalysis demonstrated no evidence of proteinuria, which is recognized as an important risk factor for progression of chronic kidney disease [8, 9]. Third, our patient had no history of diabetes mellitus or hypertension, which several authors have reported to be predictors of improvement of renal function following LVAD implantation. Sandner et al. [10] reported that absence of diabetes was the most important predictor of improvement of renal function following continuous LVAD implantation. Similarly, Butler and colleagues [11] showed that absence of diabetes, lower cardiac output prior to implantation, and lower body mass index, which were also observed in our patient, each had an association with improved renal function after pulsatile LVAD implantation. Additionally, another study showed a strong graded relationship between blood pressure and progression to end-stage renal disease [12]. These findings suggest that patients without structural kidney disease secondary to diabetic nephropathy or nephrosclerosis may be more likely to gain improved renal function after LVAD implantation. Indeed, the renal biopsy results showed no findings of obvious diabetic nephropathy, nephrosclerosis, or glomerulonephritis. We recommend that renal biopsy is useful to clarify the etiology of renal dysfunction. However, other studies are mandatory to confirm the usefulness of renal biopsy to determine the outcome of renal function following LVAD implantation in patients with these conditions.

In summary, we report successful implantation of a Jarvik 2000 LVAD as a bridge to eligibility for a patient with refractory heart failure complicated with renal dysfunction. Close collaboration with well-trained cardiologists or nephrologists is mandatory to evaluate the patient's organ function accurately and make more careful patient selection.

Acknowledgments This study is supported by the Health and Labour Sciences Research Grants, Research on intractable diseases.

Conflict of interest There are no conflicts of interest to report.

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CASE REPORT

Exchange of DuraHeart left ventricular assist device via a subcostal approach

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Abstract We report a successful case of a DuraHeart left ventricular assist device (LVAD) exchange via a subcostal approach. A 35-year-old woman was implanted with a DuraHeart LVAD due to dilated cardiomyopathy. Approximately 8 months after the implantation, the magnetic levitation system failed. The DuraHeart LVAD was exchanged emergently. The pump was freely dissected via a subcostal approach, avoiding redo sternotomy. De-airing of the new pump and the left ventricle was carefully performed. When the systemic flow was transferred from the cardiopulmonary bypass to the DuraHeart LVAD, an adequate flow was not initally obtained. Positional correction of the inflow conduit was needed to obtain full systemic flow. The postoperative course was uneventful. She was successfully discharged and is waiting at home for a heart donation.

Keywords DuraHeart · Device exchange · Subcostal approach

Introduction

A continuous-flow ventricular assist device (VAD) has recently been developed for the treatment of advanced heart failure. Effective hemodynamic support and excellent long-term results have been reported upon the application of the device [1, 2]. The DuraHeart left ventricular assist device (LVAD) (Terumo Heart, Inc., Ann Arbor, MI, USA) is the world's first magnetically levitated centrifugal blood pump. The European experience suggests that the DuraHeart LVAD can provide safe and reliable long-term circulatory support with both improved survival and acceptable adverse event rates in patients with advanced heart failure [3]. The excellent reliability and durability of the DuraHeart LVAD are beneficial for Japanese patients with severe heart failure, because the average waiting time for heart transplantation in Japan is more than 800 days. The potential for device malfunction increases with prolonged use of the LVAD. Prompt and appropriate management of device failure is essential to prevent lethal complications. We herein report our experience with the failure of a DuraHeart pump exchange.

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Clinical summary

The patient was a 35-year-old woman with a diagnosis of dilated cardiomyopathy. She suffered from heart failure and was treated with β blocker and ACE inhibitor. However, her condition suddenly deteriorated due to exaggerated heart failure, and her hemodynamics was supported by extracorporeal devices. A Toyobo paracorporeal LVAD (Toyobo-Nipro, Osaka, Japan) was implanted emergently, and the patient was listed as a potential heart transplant recipient.

During the postoperative course, an ischemic stroke occurred. Fortunately, the patient recovered without any significant neurologic sequelae. In addition, mobile pump thrombi were often detected, and several paracorporeal pump exchanges were required during a short postoperative

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period. Due to the long waiting time for heart donation, conversion to a DuraHeart LVAD was performed. After the DuraHeart implantation, her postoperative recovery was uneventful. The patient was discharged and waited at home for a heart donation.

However, approximately 8 months following the DuraHeart LVAD implantation, the "Mag-Lev Failure" alarm was activated, indicating levitation position errors leading to the failure of the magnetic levitation system. The pump continued to provide the patient with full and stable hemodynamic support by automatically converting to the hydrodynamic bearing rotation mode during this period. The malfunction could not be resolved by exchanging the controllers. We immediately consulted with Terumo Heart, Inc., and exchange of the DuraHeart LVAD was indicated by an analysis of the data downloaded from the controllers.

An emergent operation was arranged. An abdominal midline incision was made along the prior operative incision and extended to the subcostal area. The pump—including the connector nuts of both the outflow and inflow conduits—was freely dissected, and we determined that the exchange could be accomplished without redo sternotomy. Further, a cardiopulmonary bypass (CPB) was established by cannulating the right femoral artery and the right common femoral vein. Immediately after the DuraHeart LVAD was turned off and the outflow conduit was clamped, CPB was initiated. Systemic CPB flow was maintained with a high flow rate and a high perfusion pressure to prevent air embolism. The patient was kept in the Trendelenburg position during the CPB. The operative field was flooded with carbon dioxide. After that, the outflow and the inflow conduits were sequentially disconnected. To control bleeding from the inflow conduit, a suction machine was directly inserted into the conduit and a bloodless field was achieved (Fig. 1). The malfunctioning DuraHeart LVAD, including the driveline, was removed. A new DuraHeart pump was placed, and the outflow conduit was declamped after adequately refilling both the pump and the left ventricle. However, when the systemic flow was transferred from the CPB to the DuraHeart LVAD, adequate flow could not be obtained by the DuraHeart pump. Malposition of the inflow conduit was suspected. While checking the pump flow on the DuraHeart console, the malposition of the inflow conduit was corrected. The inflow conduit was held firmly with a clamp during reconnection to the device in order to prevent another positional change. After that, full systemic flow was obtained and the transfer from the CPB to DuraHeart LVAD was successful. De-airing from the outflow conduit was maintained using a 18G needle after declamping. We continuously monitored the aortic root with transesophageal echocardiography during the procedure, and no air entraining was detected. The patient was successfully discharged and had an unremarkable outpatient course.

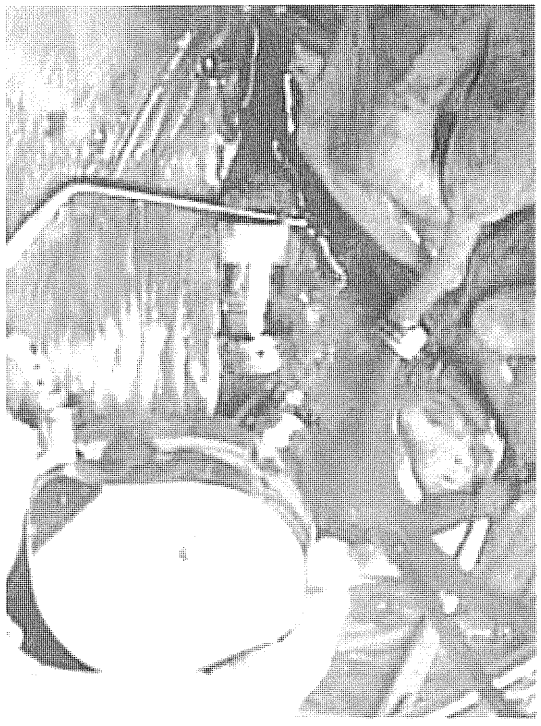

Fig. 1 The DuraHeart pump, including the connector nuts of both the outflow and inflow conduits, was freely dissected using a solely subcostal approach without redo sternotomy

An investigation of the pump and percutaneous cable by Terumo Heart, Inc. identified a fracture on a position sensor wire in the percutaneous cable.

Discussion

We have presented a case of successful DuraHeart device exchange via a subcostal approach without redo sternotomy. In the operative findings, small segments of the outflow conduit were freely dissected to clamp it and good exposure of both the inflow and outflow connector nuts was needed to disconnect from or reconnect to the device using the wrench. To achieve a bloodless field after the pump was disconnected, a suction machine was inserted directly into the inflow conduit. To prevent air embolism, changes in the patient's position, CPB management, and the de-airing procedure were all carefully performed. Positional correction of the inflow cannula was required to obtain adequate systemic flow when transferring the flow from the CPB to the new DuraHeart LVAD.

Technical considerations associated with LVAD exchange have been previously described [4, 5], and the surgical approach will vary depending on the part, type, and initial technique for implanting the LVAD. A previous

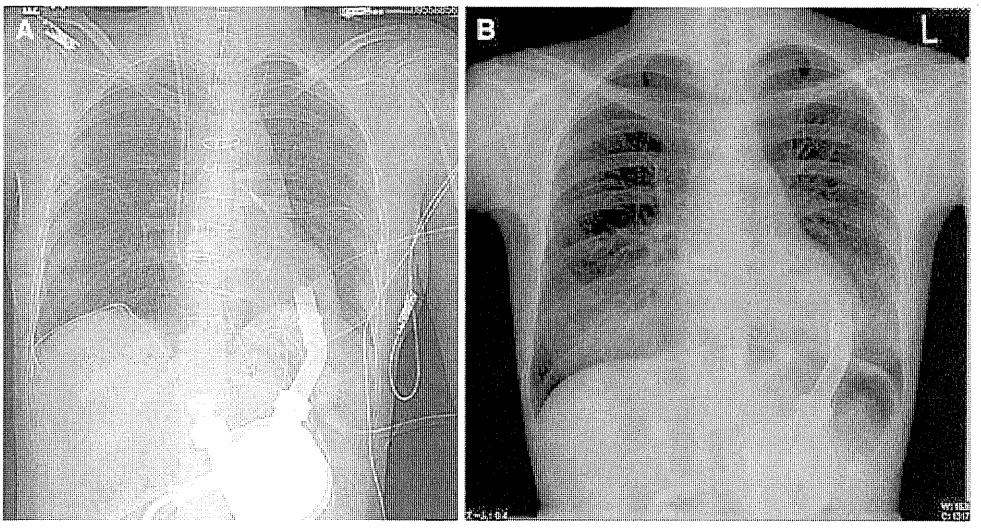

Fig. 2 A change in the position of the inflow cannula in relation to the left ventricular wall was observed in the present patient by chest radiography. **a** Just after DuraHeart implantation, **b** just before DuraHeart exchange

study indicated that replacing a HeartMate XVE with a HeartMate II can be accomplished with relatively low mortality and morbidity through a subcostal approach, as compared with sternotomy [6]. Based on our present experience, the feasibility of performing DuraHeart LVAD exchange solely by a subcostal approach should be assessed.

The risk of air embolization is a major concern in an LVAD exchange through a subcostal approach. Woo and Acker [7] described a novel continuous intravascular ascending aortic air removal technique that is particularly useful when employing a nonsternotomy approach. A number 6 French pigtail catheter was placed in the ascending aorta through the common femoral artery, exactly above the entry site of the LVAD aortic graft anastomosis. The catheter was connected to the CPB and used as an intra-aortic venting cannula. This simple de-airing method would be useful and reliable when using a nonsternotomy approach for LVAD exchange.

Chest radiography revealed reverse ventricular remodeling and a change in the position of the inflow cannula in relation to the left ventricular wall in the present patient (Fig. 2), which were not accompanied by low cardiac output symptoms. We found that correcting the position of the inflow cannula was essential in order to obtain adequate DuraHeart LVAD flow during the operation. The position or direction of the inflow conduit could not be accurately evaluated during the operation, because we could only see the connector nut of the inflow conduit through the operative field (Fig. 1). The pump flow on the DuraHeart console was our sole aid during intraoperative position correction. Therefore, the position of the inflow conduit should be evaluated preoperatively. Raman et al. [8] stated that cardiac computed tomography (CCT) is a useful modality for patients with LVADs, and CCT can detect inflow cannula malpositioning in patients with low cardiac output symptoms. In the current case, CCT could have provided some supporting information about the inflow cannula before the operation.

In conclusion, we have reported a case of successful DuraHeart LVAD exchange via a subcostal approach. The feasibility of performing this procedure solely through a subcostal approach should be assessed.

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Induced Adipocyte Cell-Sheet Ameliorates Cardiac Dysfunction in a Mouse Myocardial Infarction Model : A Novel Drug Delivery System for Heart Failure

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Induced Adipocyte Cell-Sheet Ameliorates Cardiac Dysfunction in a Mouse Myocardial Infarction Model

A Novel Drug Delivery System for Heart Failure

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Background—A drug delivery system that constitutively and effectively retains cardioprotective reagents in the targeted myocardium has long been sought to treat acute myocardial infarction. We hypothesized that a scaffold-free induced adipocyte cell-sheet (iACS), transplanted on the surface of the heart, might intramyocardially secrete multiple cardioprotective factors including adiponectin (APN), consequently attenuating functional deterioration after acute myocardial infarction.

Methods and Results—Induced ACS were generated from adipose tissue–derived cells of wild-type (WT) mice (C57BL/6J), which secreted abundant APN, hepatocyte growth factor, and vascular endothelial growth factor in vitro. Transplanted iACS secreted APN into the myocardium of APN-knockout (KO) mice at 4 weeks. APN was also detected in the plasma of iACS-transplanted APN-KO mice at 3 months (245±113 pg/mL). After left anterior descending artery ligation, iACS, generated from either WT (n=40) or APN-KO (n=40) mice, were grafted onto the surface of the anterior left ventricular wall of WT mice, or only left anterior descending artery ligation was performed (n=43). Two days later, inflammation and infarct size were significantly diminished only in the WT-iACS treated mice. One month later, cardiomyocyte diameter and percent fibrosis were smaller, whereas ejection fraction and survival were greater in the WT-iACS treated mice compared with the KO-iACS–treated or nontreated mice.

Conclusions—Cardioprotective factors including APN, hepatocyte growth factor, and vascular endothelial growth factor were secreted from iACS. Transplantation of iACS onto the acute myocardial infarction heart attenuated infarct size, inflammation, and left ventricular remodeling, mediated by intramyocardially secreted APN in a constitutive manner. This method might be a novel drug delivery system to treat heart disease. **(*Circulation*. 2011;124[suppl 1]:S10–S17.)**

Key Words: acute myocardial infarction ■ adiponectin ■ cell therapy ■ drug delivery system ■ tissue engineering

Despite recent progress in medical and surgical treatments for heart failure, acute myocardial infarction (AMI) and the subsequent deterioration of cardiac performance is still a major cause of death, worldwide. An array of cardioprotective reagents have been identified to be effective in ameliorating AMI by administrating into the infarcted myocardium in experimental models.[1] However, these reagents have failed to show consistent therapeutic efficacy in several clinical trials, probably due to poor retention or rapid inactivation of reagents in the injured myocardial tissues.[1] Therefore, a drug delivery system (DDS) that retains cardioprotective reagents in the targeted myocardial area has long been sought.

Intramyocardially transplanted autologous stem cells secrete various cardioprotective cytokines and growth factors, enhance angiogenesis, reduce fibrosis, attenuate apoptosis, and suppress myocyte hypertrophy, consequently ameliorating AMI in a paracrine manner.[2,3] However, cell transplantation for AMI has shown only modest therapeutic efficacy in large-scale clinical studies. It appears to result from insufficient paracrine effects whose magnitude and figure are largely affected by the cell delivery method or transplanted cell source.[4] To enhance the survival and functions of the transplanted cells, we developed a cell-sheet–based delivery method in which isolated cells, cultivated in vitro as a sheet without a scaffold, are simply placed on the surface of the myocardium. This treatment enhances the paracrine effects, resulting in better therapeutic efficacy.[5,6]

Adipocytes differentiated from adipose tissue–derived stromal-vascular fraction (SVF) cells are a promising cell

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source for treating AMI because as they secrete hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and, importantly, adiponectin (APN).[7,8] APN is a circulating secretory protein that has multiple cardioprotective effects, including the attenuation of inflammation, fibrosis, and myocyte hypertrophy.[8,9] However, the clinical use of APN for treating AMI has been hampered by the lack of effective systems for delivering APN to the heart. We hypothesized that using cell-sheet technology to deliver adipocytes that secrete multiple cardioprotective factors, including APN, might attenuate the functional deterioration after AMI.

Methods

Animal care complied with the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 85 to 23, revised 1996). Experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Osaka University Graduate School of Medicine.

Generation and Assessment of Adipocyte Cell-Sheet

The SVF cells of adipose tissue were isolated from wild-type (WT; male C57BL/6J) or APN-knockout (KO) mice,[10] as described previously.[11] The isolated SVF cells were cultured until they become confluent on Upcell dishes (CellSeed Inc, Tokyo, Japan). Differentiation into adipocytes was induced by insulin, dexamethasone, isobutylmethylxanthine, and pioglitazone (Sigma-Aldrich, MO). Incubation at 20°C induced the cells to detach from the culture dishes, yielding a scaffold-free cell-sheet, which we call an "induced adipocyte cell-sheet" (iACS). The secretion of HGF, VEGF, leptin, interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-10 into the culture supernatant was assessed by ELISA. Before transplantation, WT mouse–derived iACS (WT-iACS) and APN-KO mouse–derived iACS (KO-iACS) were labeled with the use of a PKH26 kit (Sigma-Aldrich).

Generation of AMI Model and Cell-Sheet Transplantation

An AMI model was generated by permanent ligation of the left anterior descending artery (LAD) in male C57BL/6J mice, 10 to 15 weeks old.[12] The mice were anesthetized by isoflurane inhalation (Mylan Inc). Five minutes after the LAD ligation, WT-iACS (W group, n=40) or KO-iACS (K group, n=40) was grafted onto the surface of the anterior left ventricular (LV) wall, or a sham operation was performed (C group, n=43). The mice were euthanized at 2 and 28 days after LAD ligation and cell-sheet transplantation.

Assessment of Cardiac Function and Survival

Cardiac function was assessed with the use of an echocardiography system equipped with a 12-MHz transducer (GE Healthcare) at 4 weeks. The LV dimensions were measured, and LV ejection fraction was calculated as $(LVDd^3-LVDs^3)/LVDd^3\times100$, where LVDd and LVDs are the LV end-diastolic and end-systolic dimensions, respectively.[12] The mice were housed in a temperature-controlled incubator for 50 days after treatment to determine their survival.

Histological Analysis

Freshly excised hearts were stained with 1% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich). The red-stained infarct area was quantified by computerized planimetry, using MetaMorph Software (Molecular Devices). Frozen sections (8 μm) of hearts and cell-sheets were stained with antibodies against APN (Otsuka Pharmaceutical, Tokushima, Japan) or CD11b (Abcam, Cambridge, UK). The secondary antibody was Alexa 488 goat anti-rabbit (Life Technologies). Counterstaining was performed with 6-diamidino-2-phenylindole (Life Technologies). To analyze the myocardial collagen accumulation, heart sections were stained with Masson trichrome. The collagen volume fraction was calculated in the peri-infarct area. To assess cardiomyocyte diameter, heart sections were stained with periodic acid-Schiff. MetaMorph Software was used for the quantitative morphometric analysis.

Cytokine Antibody Array

Proteins were isolated from whole-heart samples and analyzed using a Milliplex Mouse Cytokine/Chemokine Panel Premixed 32Plex, according to the manufacturer's instructions (Millipore).

Quantitative Real-Time PCR

Total RNA was isolated from the peri-infarct area by use of the RNeasy Mini Kit and reverse-transcribed, using Omniscript Reverse transcriptase (Qiagen, Hilden, Germany). Quantitative PCR was performed with the PCR System (Life Technologies). The expression of each mRNA was normalized to that of glyceraldehyde-3-phosphate dehydrogenase.

Statistical Analysis

Data are expressed as the mean±SEM. The data distributions were checked for normality with the Shapiro-Wilk test and for equality of variances with the Bartlett test. Comparisons between 2 groups were made using the unpaired t test or the Wilcoxon-Mann-Whitney U test, as appropriate. For comparisons among 3 groups, we used 1-way ANOVA, followed by Fisher protected least-significance difference test or the Kruskal-Wallis test, followed by the post hoc pairwise Wilcoxon-Mann-Whitney U test, as appropriate. The survival curves were prepared by using the Kaplan-Meier method and were compared using the overall log-rank test, followed by the post hoc pairwise log-rank test. The multiplicity in pairwise comparisons was corrected by the Benjamin-Hochberg procedure. All probability values are 2-sided, and values of $P<0.05$ were considered to indicate statistical significance. Statistical analysis was performed with the StatView 5.0 Program (Abacus Concepts, Berkeley, CA) and the R program.[13]

An expanded Methods section can be found in the online-only Data Supplement.

Results

Characterization of the Adipocyte Cell-Sheets

Most SVF cells differentiated into mature adipocytes bearing oil droplets by 7 days after differentiation induction. Induced ACS or undifferentiated SVF cell-sheets were then generated by lowering the temperature (Figure 1A). Each iACS was approximately 7 mm in diameter (Figure 1B) and 100 μm thick (Figure 1C). WT-iACS expressed abundant APN in the cytoplasm and extracellular matrix around the oil-droplet–rich adipocytes, as assessed by immunohistochemistry (Figure 1D) and ELISA (Figure 1E). In contrast, neither the SVF cell-sheets of either origin nor the KO-iACS expressed APN (Figure 1D and 1E). The ELISA showed abundant HGF expression in WT-iACS and KO-iACS, which was downregulated compared with the WT SVF cells (Figure 1F). The secretion of VEGF and leptin was remarkably enhanced by the SVF cell differentiation into adipocytes. IL-6 and IL-10 were secreted by the WT-iACS and WT-SVF cells at similar levels, which were lower than the levels secreted by KO-iACS. The secretion of TNF-α was not evident in any group because the cell-free culture medium also contained 2.29 pg/mL TNF-α.

Transplanted Induced ACS Supplied APN to the Myocardium

WT-iACS were transplanted onto the heart of intact APN-KO mice to evaluate behavior of the WT-iACS, including APN

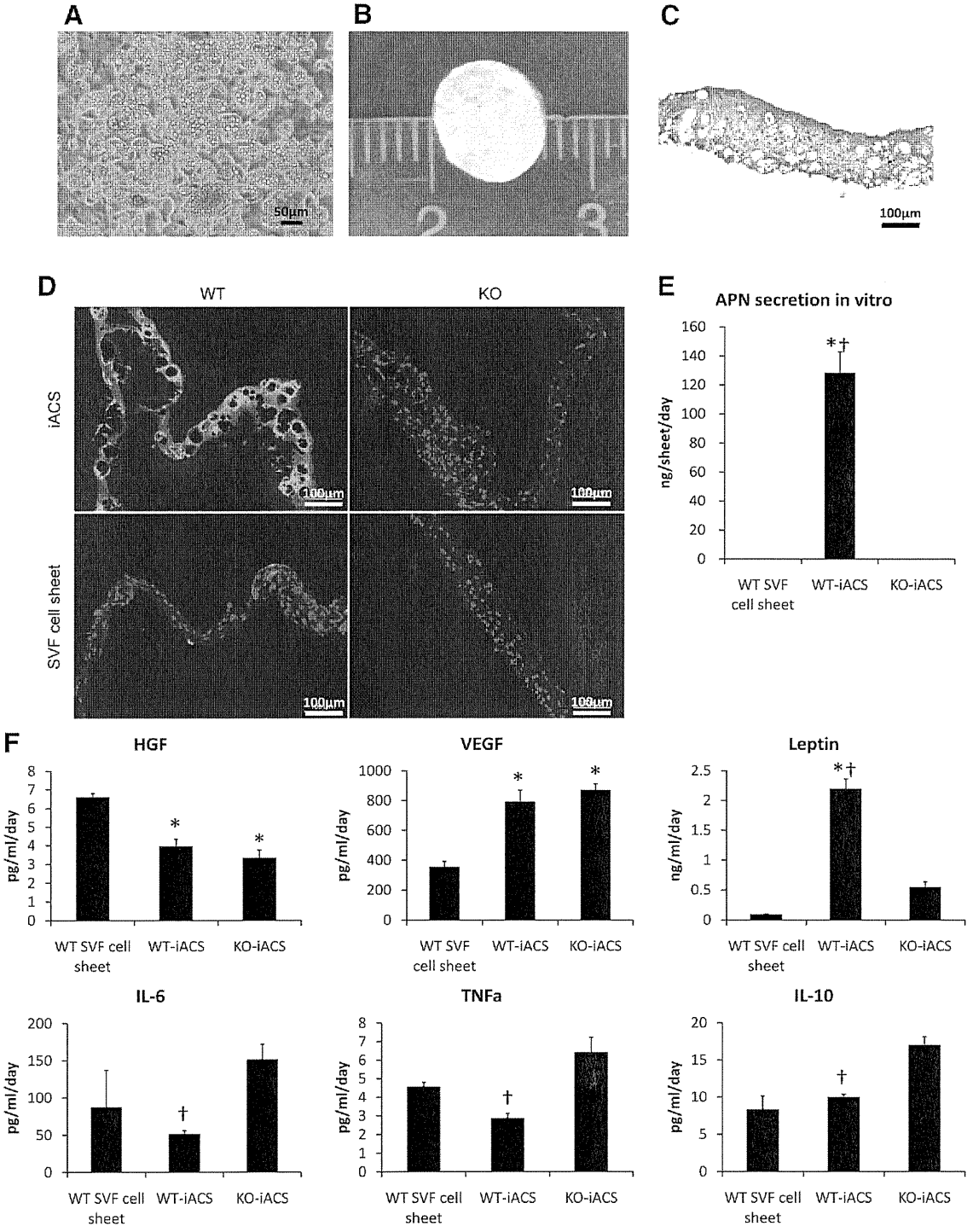

Figure 1. Characterization of induced adipocyte cell-sheet (iACS) in vitro. **A**, Histological analysis showing mature adipocytes with oil droplets in the cytosol. **B**, Induced ACS detached from the temperature-responsive culture dish. **C**, Cross-sectional view of hematoxylin and eosin–stained iACS. **D**, Representative pictures of adiponectin (APN)-stained cell-sheets. Wild-type (WT)-iACS showed strong labeling for APN. The WT stromal-vascular fraction (SVF) cell-sheet, knockout (KO)-iACS, and KO SVF cell-sheet were negative for APN. Green indicates APN; blue, nuclei. **E**, APN secretion into the WT-iACS culture supernatant determined by ELISA (WT SVF cell sheet, n=2; WT-iACS, n=5; KO-iACS, n=8; $P<0.05$, Kruskal-Wallis test). *$P<0.05$ versus WT SVF cell-sheet, †$P<0.05$ versus KO-iACS, post hoc Wilcoxon-Mann-Whitney *U* test. **F**, Hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), leptin, interleukin (IL)-6, IL-10, and tumor necrosis factor (TNF)-α secretion into the culture supernatant, measured by ELISA. WT-iACS secreted HGF, VEGF, leptin, IL-6, and IL-10 but not TNF-α (WT SVF cell sheet, n=2; WT-iACS, n=8 to 12; KO-iACS, n=6 to 9). HGF, VEGF, and leptin ($P<0.05$, ANOVA); *$P<0.05$ versus WT SVF cell-sheet, †$P<0.05$ versus KO-iACS, post hoc Fisher protected least-significance difference test. TNF-α, IL-6, and IL-10 ($P<0.05$, Kruskal-Wallis test); *$P<0.05$ versus WT SVF cell-sheet, †$P<0.05$ versus KO-iACS, post hoc Wilcoxon-Mann-Whitney *U* test.