Combined Procedure of Surgical Repair and Cell Transplantation for Left Ventricular Aneurysm: An Experimental Study

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Background—This study was designed to investigate the efficacy of the combined procedure of left ventricular (LV) repair and fetal cardiomyocyte transplantation (CM-TX) in a rat myocardial infarction model.

Methods and Results—A moderate-sized LV aneurysm was created by proximal ligation of the left coronary artery in 47 Lewis rats. Four weeks later, they were underwent another operation and received culture medium injection (n=10; group I), fetal CM-TX (n=10; group II), purse-string LV repair with culture medium injection (n=14; group III), or LV repair with fetal CM-TX (n=13; group IV). They were echocardiographically followed-up during the subsequent 4 weeks, and cardiac catheterization was performed in the final week. In the late period, LV dimension in group IV was smaller than that in group III (end-diastolic dimension, 0.92±0.02 versus 1.01±0.03 cm, P=0.0090; end-systolic dimension, 0.62±0.02 versus 0.74±0.04 cm, P=0.0093; at the fourth week), although they initially showed similar decreases in both groups. At the final week, end-systolic elastance was higher in group IV than in groups I, II, or III (0.61±0.10 versus 0.19±0.03, 0.30±0.09, 0.33±0.07 mm Hg/μL, P=0.0002, 0.0037, and 0.0042, respectively).

Conclusions—Fetal CM-TX exerted preventive effects against late LV dilation and dysfunction after LV repair in the rat model. The results suggest that repair surgery combined with fetal CM-TX may enhance the surgical benefits for patients with LV aneurysm in the long term. (Circulation. 2002;106[suppl I]:I-193-I-197.)

Key Words: aneurysm ■ cells ■ myocardial infarction ■ surgery ■ transplantation

Recently, the efficacy of geometric surgical repair for left ventricular (LV) aneurysm has been established, at least in light of its early postoperative results. Because endovascular circular patch plasty (EVCPP) was developed,1 Dor and colleagues2–4 have reported a number of clinical studies that showed satisfactory early and midterm outcome after EVCPP with LV dimension decreased and its ejection fraction elevated as a result of restoration of LV geometry and decrease in LV wall tension. However, extensive and longer follow-up data have not been to our knowledge, published, and long-term efficacy of LV repair is not clear. In fact, postoperative LV dilation after repair surgery has been reported, along with some possible explanations.3 In addition, our recent study using a rat model also showed that the initial favorable effects of LV repair lasted only less than 4 weeks (equivalent to a few years in human beings in view of their lifespan) after surgery when untreated medically.5 Therefore, there is an apprehension that the salutary effects of LV repair may be attenuated in the long term.

Cell transplantation has recently emerged as a potential strategy for end-stage heart failure, including ischemic cardiomyopathy.6–19 Presently, promising candidates may include fetal cardiomyocytes, skeletal myoblasts, smooth muscle cells, and bone marrow cells. Recent studies have demonstrated that fetal cardiomyocyte transplantation (CM-TX) improved cardiac function both in vitro9,14 and in vivo11,17–19 after myocardial infarction in rat hearts. However, there are no studies that describe the efficacy of cell transplantation performed concomitantly with other procedures such as surgical repair for infarct scar.

Thus, the purpose of this study was to investigate the efficacy of combined procedure of repair surgery and fetal CM-TX for LV aneurysm in a rat myocardial infarction model.

Methods

Male syngeneic Lewis rats were used as recipients in this study. All experimental procedures were performed by experienced surgeons (Y.S., K.T., and F.L) in accordance with the guidelines for Animal Experiments of Kyoto University, which conforms to the law of “Guide for the Care and Use of Laboratory Animals” in Japan.

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show endoventricular circumferences of the left ventricles. The heart just after repair surgery. The dotted circles in A and B ventricular repair, respectively. F, Intraoperative view of the end-diastolic and end-systolic phase just after purse-string left cardiographical views in the end-diastolic and end-systolic 4-week period after this operation.5,19,20 Images were recorded using before the second operation and followed-up every week during the By echocardiography, LV dimension and function were assessed just immediately followed by fetal cardiomyocyte transplantation as in procedures in the Second Operation group III.

Experimental Groups

Myocardial Infarction Model

Experimental Groups

Procedures in the Second Operation

Echocardiography

Cardiac Catheterization

Histologic Study

Data Analysis

Results

Figure 1. A, The second operation in group III and IV. B, Echocardiographical views in the end-diastolic and end-systolic phase before surgery, respectively. C, Intraoperative view of the heart before surgery. D and E, Echocardiographical views in an end-diastolic and end-systolic phase just after purse-string left ventricular repair, respectively. F, Intraoperative view of the heart just after repair surgery. The dotted circles in A and B show endoventricular circumferences of the left ventricles. The arrows in C point to the infarct area.

Fetal Cardiomyocyte Isolation and Culture

Ventricular cardiomyocytes were isolated from fetal Lewis rat hearts and cultured as previously described.8,10 In brief, 20-day-old embryo hearts were digested in phosphate-buffered saline solution containing trypsin (0.35%), collagenase (0.07%), and glucose (0.02%) for 15 minutes. The cells were cultured in 2 to 3 days after preplating. All cells were labeled with a fluorescent dye using PKH26 (PKH26 Red Fluorescent Cell Linker Mini Kit, Sigma Chemical Co, St. Louis, Mo).

Myocardial Infarction Model

Ventricular cardiomyocytes were isolated from fetal Lewis rat hearts and cultured as previously described.8,10 In brief, 20-day-old embryo hearts were digested in phosphate-buffered saline solution containing trypsin (0.35%), collagenase (0.07%), and glucose (0.02%) for 15 minutes. The cells were cultured in 2 to 3 days after preplating. All cells were labeled with a fluorescent dye using PKH26 (PKH26 Red Fluorescent Cell Linker Mini Kit, Sigma Chemical Co, St. Louis, Mo).

Myocardial infarction was created in rats weighing 250 to 290 g by proximal ligation of the left coronary artery, as described in our previous report.5,19,20 After ligation, an ST elevation on electrocardiography was performed in the survivors. Among them, 10 had no or a small myocardial infarction (infarct area considered statistically significant.

Coronary artery ligation was performed totally in 80 rats, among which 17 died within a week. Four weeks later, the rats with an akinetic LV aneurysm of moderate size targeted M-mode tracings obtained along the short-axis view of the left ventricle at the papillary muscle level.21 Fractional area change (FAC) and the percentage of akinetic endocardial length to the whole LV endocardial circumference (AL)21 were estimated from the same short-axis view as in the measurement of EDD and ESD using the cine-loop feature for retrospective visualization of fast heart beatings. All measurements were performed in a blind fashion according to the American Society for Echocardiography, and averaged over 3 consecutive cardiac cycles.

After the final echocardiography, the rats underwent cardiac catheterization for more precise assessment of global LV function.5 Under general anesthesia, a 2-F micromanometer-tipped catheter (Millar Instruments Inc, Houston, Tx) was inserted via the right carotid artery into the left ventricle, and a 3-F occlusion balloon catheter through the right femoral vein into the inferior vena cava. LV pressure and its first time-derivative were continuously monitored using a multiple recording system. M-mode echocardiograms were obtained as described in the above section to calculate LV systolic volume from ESD by the cube formula. During inferior vena cava occlusion with the balloon, LV pressure waveforms and M-mode tracings were simultaneously recorded on the same monitor. The end-systolic pressure-volume points obtained from echocardiography and cardiac catheterization were subjected to least squared linear regression, and Emax was calculated as the slope of the fitted line. r was calculated from the continuous pressure monitoring assuming a zero-pressure asymptote. LV end-diastolic pressure (LVEDP) was also measured. All data were acquired under stable conditions.

Histologic Study

After all measurements were finished, the rats were sacrificed for histologic study. Half of the heart specimens in groups II and IV were cryopreserved for staining with PKH26. Then they were microscopically examined with the use of fluorescence microscopy for PKH26 dye.

In addition to the histologic assessment for the hearts from the study groups, Masson’s trichrome staining was performed in sections from the heart 1 day after repair surgery to demonstrate the condition of the heart just after LV repair surgery in comparison with the unrepaired heart.

Data Analysis

All data are expressed as the mean±SEM. Comparisons of echocardiographic data among the groups were performed by 2-way repeated measures analysis of variance (ANOVA) including time, group, and group-by-time interaction terms. Comparisons of cardiac catheterization data among the groups were conducted by one-way factorial ANOVA. If significance was found for group effect, post hoc comparisons among groups were made, and if significance was found for group-by-time interaction, post hoc comparisons among groups at each time point were performed to explore the tendency, when appropriate, using Fisher’s protected least significant difference method. Statistical analyses were performed with Statview for Windows version 5.0 (SAS Institute Inc., Cary, NC). A P<0.05 was considered statistically significant.

Coronary artery ligation was performed totally in 80 rats, among which 17 died within a week. Four weeks later, echocardiography was performed in the survivors. Among them, 10 had no or a small myocardial infarction (infarct size:21 <20%), and 6 had a large one (infarct size:21 >40%). Then, the rats with an akinetic LV aneurysm of moderate size (infarct size:21 20–40%) in the anterior LV wall (n=47) underwent the second surgery, in which there was no intraoperative or postoperative death. Although exclusion of the infarct area was almost accomplished in LV repair, the
marginal area was not completely excluded in some cases (Fig. 2B). The purse-string stitches were put on marginal area of myocardial infarction.

**Echocardiography**

There were no differences in preoperative data among the 4 groups (Fig. 3). No rats presented mitral regurgitation as detected by two-dimensional color Doppler imaging. Two-way repeated measures ANOVA for EDD, ESD, FAC, and AL showed high group and time effects and a strong group-by-time interaction, respectively (Table 1).

EDD in groups III and IV showed the smallest levels at the first week and rising trends since then. Whereas EDD in group III showed no differences from that in group I at the third and fourth week ($P=.39$, .31, respectively), EDD in group IV was still smaller than that in group I even in the late period (0.89±0.02 versus 1.02±0.02 cm, $P<0.0010$ at the third week; 0.92±0.02 versus 1.05±0.03 cm, $P<0.0010$ at the fourth week). EDD in group IV was even smaller than that in group III in that period (versus 0.99±0.03 cm, $P=0.0055$ at the third week; versus 1.01±0.03 cm, $P=0.0090$ at the fourth week). The similar results were recognized in ESD (0.62±0.02 versus 0.74±0.04 cm, $P=0.0093$ at the fourth week).

The graphs of FAC and those of EDD or ESD showed roughly symmetrical trends about a horizontal line between them. FAC in groups III and IV showed peak levels at the first week and declining trends thereafter. At the fourth week, FAC in groups III and IV were higher than that in group I (42.0±3.5, 49.7±3.4 versus 31.8±2.0%, $P=0.023$, .0002, respectively), but showed no differences from that in group II ($P=.45$, .32, respectively) FAC at the fourth week in group IV seemed higher than that in group III but did not reach statistical significance ($P=0.061$).

The graphs of AL revealed different patterns from those of the variables mentioned above. In groups I and II, AL demonstrated almost the same values constantly. Focusing on the late period (at the second week and later), 2-way repeated measures ANOVA for AL showed a high group effect but no time effect or group-by-time interaction (Table 2). AL in groups III and IV was decreased compared with that in groups I and II. AL during the late period remained even smaller in group IV than in group III (14.3±1.2 versus 19.1±1.3%, $P<0.036$).

**Cardiac Catheterization**

One-way factorial ANOVA showed high group effects in $E_s$ and $\tau$ Table 3. $E_s$ in group IV was higher than that in groups I, II, and III (0.61±0.10 versus 0.19±0.03, 0.30±0.09, 0.33±0.07 mm Hg/µL, $P=.0002$, .0037, and .0042, respectively). $\tau$ in groups II and IV were lower than that in control group (18.3±0.8, 18.5±0.4 versus 21.0±0.7 ms, $P=0.017$ in each). LVEDP showed no significant differences among the groups (Fig. 4).

**Histologic Study**

Four weeks after the second surgery, in groups II and IV, transplanted cardiomyocytes were detected through microscopic examination in the scar and peri-infarct area, with

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<th>TABLE 1. Two-Way Repeated Measures ANOVA for EDD, ESD, FAC, and AL</th>
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$AL$ = the percentage of akinetic endocardial length to the whole left ventricular endocardial circumference; $EDD$ = end-diastolic dimension; $ESD$ = end-systolic dimension; $FAC$ = fractional area change.
those cells labeled by PKH26 red fluorescent dye. In group IV, infarct or peri-infarct regions were newly developed around the suture line, where transplanted cells were clearly identified (Fig. 5).

Discussion

The results demonstrated in the present study are summarized as follows. Cell transplantation showed a marginal benefit in the nonsurgical animals.18 The beneficial effects on LV dimension and function initially seen after repair surgery were attenuated as time passed. In contrast, repair surgery combined with CM-TX brought longer-lasting improvement in systolic and diastolic LV function. By adding CM-TX, the initial benefits of LV repair seemed to be maintained even in the late period.

We speculate the reasons for the LV dilation and dysfunction after surgical repair in this model as follows 3,5: 1) The infarct and/or marginal area left unrepaired or newly developed is supposed to have caused LV remodeling in the postoperative course. In addition, they may have been influenced by LV repair surgery itself; and 2) Because of the considerable loss of normal LV mass or architecture resulting from infarction, perfect repair might not maintain the improved LV function in the long term.22 We consider that 1 of the inherent problems of LV repair surgery may be that it cannot recover the lost myocardium.

Although the precise mechanisms by which transplanted cells improve cardiac function have not been elucidated yet, it is likely that their elastic properties against mechanical stretching prevent LV dilation and remodeling.9,11 In addition, angiogenesis or revascularization induced in ischemic regions by the transplanted cells may also ameliorate cardiac function in the ischemic heart.9–12 Because both decrease in wall stress and induction of angiogenesis are very important factors in inhibiting the evolution of myocardial infarction, cell transplantation may be a promising treatment modality for preventing LV remodeling. Therefore, one may reason-ably expect that cell transplantation should provide beneficial effects on the outcome after repair surgery for LV aneurysm.

In the present study, we injected fetal cardiomyocytes just outside the purse-string suture. The region was thought to be relatively weak and vulnerable, with mechanical stress resulting from surgical procedures applied on it because it was just on the marginal zone of myocardial infarction. Therefore, the following speculation may arise regarding the roles of CM-TX performed concomitantly with LV repair. At least part of transplanted cardiomyocytes probably infiltrates or migrates into such area. They prevent, possibly with their elastic and angiogenetic properties, the ischemic lesion around the suture line from falling into infarction early after the operation and limit the size of infarction that develops in the subsequent remodeling process.23 Then, the infarct size developed in the early phase regulates the evolution of late LV remodeling.24 Consequently, dimensional and functional benefits from CM-TX can last for a long period of time.

TABLE 2. Two-way Repeated Measures ANOVA for AL in the Late Period*

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<td>Group</td>
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<td>Group by time</td>
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*At the 2nd week and later.

AL indicates the percentage of akinetic endocardial length to the whole left ventricular endocardial circumference.

TABLE 3. One-Way Factorial ANOVA for Ees, τ, and LVEDP

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<tr>
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<th>Ees</th>
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Ees=end-systolic elastance; LVEDP=left ventricular end-diastolic pressure; τ=time constant of isovolumic relaxation.

Figure 4. Hemodynamic data in cardiac catheterization. Error bars show SEM. Ees indicates end-systolic elastance; τ, the time constant of isovolumic relaxation; LVEDP, left ventricular end-diastolic pressure; I, group I; II, group II; III, group III; and IV, group IV. *P<0.01 compared with group IV, †P<0.05 compared with group I. The results of other statistical tests are shown in the text.

Figure 5. A, Cryopreserved specimen in group IV 4 weeks after the second operation. B, Fluorescent image of the same specimen. Transplanted cardiomyocytes labeled with PKH26 (red) were detected near the excluded portion (A, Original magnification ×1; B, Original magnification ×100). Scale bars=5 mm and 1 mm for A and B, respectively.
Not to mention the difference in species, there are some limitations to the present study as a clinical simulation. First, we used cardiomyocytes as donor cells for transplantation because our previous studies were based on fetal CM-TX in a rat model, which already an established method in our laboratory. In addition, although we realize that autotransplantation of skeletal myoblasts or bone marrow cells is supposed to be a more practical method in human patients at this stage, it is still unknown whether transplanted skeletal myoblasts connect to host myocardium and contribute to cardiac contraction or whether autologous bone marrow cells can be harvested in enough numbers. Recent progress in regenerative medicine might provide us, in the near future, a new technology in which autologous or allogeneic cardiomyocytes are supplied safely and sufficiently (eg, cardiomyocytes derived from bone marrow cells or embryonic stem cells). Second, we performed a direct purse-string repair rather than a patch plasty, which is generally performed in patients who need repair surgery. Because EVCPP or such type of geometric plasty has been proven to provide better outcome than linear repair does, we chose the purse-string method to mimic the repair in a circulatory fashion. However, the geometric and functional change after repair may be different between our model and clinical settings. Despite these limitations, we believe that the presented model and technique would be sufficient for the purpose of this study to demonstrate the efficacy of cell transplantation performed concomitantly with LV repair surgery.

Conclusions
Additional fetal CM-TX showed preventive effects against late LV dilation and dysfunction after LV repair in the rat myocardial infarction model. The results of this study suggest that repair surgery combined with fetal CM-TX may enhance the surgical benefits for patients with LV aneurysm in the long term.

Acknowledgments
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