Longer preservation of cardiac performance by sheet-shaped myoblast implantation in dilated cardiomyopathic hamsters

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Received 15 March 2005; received in revised form 30 October 2005; accepted 1 November 2005

Time for primary review 27 days

Abstract

Objectives: Cell therapy is a promising strategy for ischemic cardiomyopathy. However, the direct injection method has limitations for generalized cell delivery, especially in dilated cardiomyopathy (DCM). We hypothesized that a sheet-shaped myoblast graft would be superior to direct injection for improving cardiac performance in DCM.

Methods: Male 27-week-old BIO TO-2 (DCM) hamsters that showed moderate cardiac remodeling were used as recipients. Myoblasts isolated from BIO F1B hamsters were cultured on dishes coated with poly(N-isopropylacrylamide), a temperature-responsive polymer, and spontaneously detached as a sheet-shaped graft at 20 °C without enzymatic treatment. Three different therapies were conducted: (1) sheet-shaped myoblast graft implantation (S group, n = 29); (2) myoblast injection (M group, n = 28); and (3) sham operation (C group, n = 28). In the S group, two sheet-shaped myoblast grafts were implanted on the left ventricle (LV) wall, and in the M group, myoblasts were injected into the right ventricle (RV) and LV walls.

Results: After the sheet-shaped myoblast grafts were implanted, echocardiography demonstrated that the dilated LV dimension was significantly reduced, whereas the hearts in other groups showed a progression of LV dilation. The fractional shortening in the M and C groups decreased significantly while that in the S group was maintained at the preoperative level for 3 months after the operation. Histological examination demonstrated that in the S group, the LV wall thickness was increased, with viable myoblasts, and myocardial fibrosis was decreased compared with the other groups. Immunohistochemical staining demonstrated alpha-sarcoglycan and beta-sarcoglycan expression on the basement membrane of the cardiomyocytes in the S group but not in the other groups. Life expectancy was significantly prolonged in the S group.

Conclusion: Sheet-shaped myoblast graft implantation improved cardiac performance and prolonged life expectancy, associated with a reduction in myocardial fibrosis and re-organization of the cytoskeletal proteins in DCM hamsters. Thus, sheet-shaped autologous myoblast graft implantation may induce restoration of the heart in DCM.

Keywords: Cardiomyopathy; Myoblast; Sheet

1. Introduction

Dilated cardiomyopathy (DCM) is one of the most common causes of chronic heart failure [1]. Contemporary medical therapy has dramatically improved the prognosis of
heart failure, and a substantial number of patients remain candidates for aggressive approaches, such as left ventricular remodeling operations, implantable defibrillators, and cardiac resynchronization [2]. However, the mortality remains high, and cardiac transplantation and mechanical support using a left ventricular assist device (LVAD) are the accepted ultimate lifesaving means of supporting these patients [3–5]. Unfortunately, cardiac transplantation has limitations, such as donor shortages, rejection, and infection, and the usefulness of LVAD is limited by its durability [5]. Therefore, novel strategies are still desired.

The DCM pathology is characterized by global myocardial remodeling, which mainly consists of myocardial fibrosis associated with changes in the cytoskeletal and sarcolemmal proteins in cardiomyocytes, leading to a reduction in the number and function of these cells [6]. Consequently, cardiac remodeling chronically progresses with ventricular dilation and thinning, leading to progressive congestive heart failure.

Recently, myoblast transplantation has been investigated as a treatment for ischemic cardiomyopathy, and a functional benefit of this procedure has been demonstrated in animal models [7,8]. A clinical trial of autologous myoblast transplantation has begun [9]. For DCM, however, there have been only a few preclinical reports concerning the effects of myoblast transplantation, and they indicated a low functional benefit for the left ventricle [10–12].

There are two reported methods for delivering myoblasts to treat non-ischemic cardiomyopathy: intra-coronary injection and direct myocardial injection [10,11]. However, both methods have limitations for clinical application. In the intra-coronary injection method, the number of myoblasts for grafting is limited because of the risk of coronary embolism [10]. With the direct myocardial injection method, it is necessary to make injections at many sites in the ventricle to supply myoblasts evenly into a globally dilated myocardium. Indeed, it was reported that myoblast transplantation by needle injection into six ventricular sites did not reduce the myocardial fibrosis in a BIO 14.7 DCM hamster model [12]. Moreover, neither method avoids disrupting the micro-intercellular communication and extracellular matrix of the transplanted cells, which play a key role in cell adhesion, migration, proliferation, differentiation, and death [13].

The use of tissue engineering to provide the cell supply might overcome these disadvantages. Although the scaffolds used for tissue engineering might be supportive for myocardial regeneration, problems with biocompatibility, biodegradability, and cytotoxicity, including the inflammatory response and loss of surface adhesive molecules, can limit their efficacy [14,15]. By eliminating such hindrances, we have established a cell sheet engineering technology that does not use scaffolds [16,17]. These cell sheets show preserved cellular communication junctions, endogenous extracellular matrix (ECM), and integrative adhesive agents. The non-ligature implantation of these sheet-shaped neonatal cardiomyocyte grafts into infarcted myocardium resulted in their integration with the impaired myocardium and improved cardiac performance [18].

Given this body of evidence, we hypothesized that the implantation of sheet-shaped myoblast grafts would preserve cardiac performance better than the transplantation of myoblasts by direct injection in DCM. In this study, we used a hamster model of DCM to ask specifically whether the implantation of sheet-shaped myoblast grafts could 1) preserve cardiac performance, 2) decrease the extent of myocardial remodeling, and 3) prolong life expectancy.

2. Methods

2.1. Experimental animals

Twenty-seven-week-old male BIO TO-2 hamsters (Bio Breeders, Fitchburg, MA, USA) were used for this study. Humane animal care was used, in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). The animals were randomly divided into three treatment groups: the first was treated with implantation of sheet-shaped myoblast grafts (S group, $n=29$), the second received injections of myoblasts (M group, $n=28$), and the third received only thoracotomy (C group, $n=28$). A total of 13 hamsters in each group were used for histological analysis at 1, 2, and 8 weeks after the operation. The number of animals sacrificed at each time point is given in the figure legends. The remaining hamsters ($n=16$ for the S group, $n=15$ for the M group, and $n=15$ for the C group) were used for the evaluation of cardiac function or mortality.

2.2. Isolation of skeletal myoblasts

Myoblasts were isolated from the skeletal muscles of both sides of the inferior limbs from BIO F1B hamsters. The hamsters were euthanized, then their skeletal muscles were excised and washed with PBS (NaCl, 136.9 mmol/L; KCl, 2.7 mmol/L; Na2HPO4, 8.1 mmol/L; KH2PO4, 1.5 mmol/L; pH 7.3). After the removal of as much fibrous tissue, tendons, and fat tissue as possible, the muscles were minced, weighed, and enzymatically dissociated with collagenase (Gibco BRL, Rockville, MD, USA) (5 mg/mL) for 40 min. The cells were collected by centrifugation (10 min at 1200 × g), then the enzyme reaction was arrested by adding 20% FBS (ICN Biomedicals Aurora, OH, USA), and the cells were spun again. The supernatant was discarded and the cells were resuspended in culture medium composed of DMEM (Gibco BRL) with 20% FBS and 1% penicillin–streptomycin (Gibco BRL). The initial plating was performed in 100-mm collagen-coated culture dishes (IWAKI, Japan), and the cells were grown in humid air
supplemented with 5% CO2. The next day, the cells were washed and the culture medium was changed. Five days after the initial plating, and before confluence, the cells were harvested by trypsination and washed in DMEM. The cultures contained around 50% desmin-positive cells (Fig. 1A). The recovered cells were counted and delivered to DCM hearts either by direct injection or by constructed sheet-shaped myoblast grafts.

To construct the sheet-shaped myoblast grafts, temperature-responsive culture dishes (provided by CellSeed, Tokyo, Japan) were used. Specific procedures for the preparation of these “intelligent” culture dishes have been previously described [17]. Briefly, N-isopropylacrylamide (IPAAm) monomer solution was spread onto commercial tissue culture polystyrene dishes. These dishes were then subjected to an electron beam, resulting in the polymerization and covalent bonding of IPAAm to the dish surface. The surfaces of the dishes are hydrophobic and cell-adhesive at 37 ºC, but they become hydrophilic and non-cell-adhesive at temperatures below 32 ºC. Cell suspensions at a density of 5 x 10⁶ were plated onto the temperature-responsive cell culture dish and kept in a 37 ºC incubator for 1 day. After 1 day, the cells were induced to spontaneously detach by placing the plates at 20 ºC for 1 h, yielding a scaffold-free sheet-shaped monolayer of myoblasts that could be used as a graft. These myoblast grafts were 1.00 ± 0.05 cm² in area and 60 ± 10 μm in thickness (Fig. 1B, C). Finally, two myoblast grafts were layered to make a thicker sheet for grafting.

2.3. Operative methods

Hamsters were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg body weight). The anesthetized hamsters were intubated, and positive-pressure ventilation was maintained with a ventilator (model SN-480-7 Shinano, Tokyo). The respiratory rate was set at 50 cycles/min, with a tidal volume of 2.0 mL of room air. The heart was exposed through a 2.5-cm left lateral thoracotomy. In the M group, approximately 0.2 mL of cell suspension (containing 10 x 10⁶ of the cultured cells) was injected with a 30 G tuberculin syringe into one site in the RV and 3 sites in the LV wall (anterior free wall, apex, and posterior wall). In the S group, the same number of cells was delivered by implanting the sheet-shaped myoblast graft directly over the anterior free wall to the posterior free wall without sutures. In the C group, only the left thoracotomy was performed.

2.4. Measurement of hamster cardiac function

Isoflurane was used for anesthesia during the echocardiographic measurements. The concentration of the anesthetic was reduced to 1% and maintained for 20 min to stabilize the hemodynamics. Echocardiography was performed with a commercially available echocardiograph, SONOS 5500 (Agilent Technologies, USA). A 12-MHz annular array transducer was used. M-mode echocardiograms were recorded and the left ventricular end-systolic dimension (Ds), left ventricular end-diastolic dimension (Dd), and fractional shortening (Fs) were determined. The reader was blinded to the study group.

2.5. Histological analyses

LV myocardium specimens were obtained 1, 2, and 8 weeks after the operation. Transverse sections of the hearts (2 μm thick) were fixed with 10% buffered formalin, embedded in paraffin, and subjected to hematoxylin and eosin (HE) and Masson’s trichrome staining. To label vascular endothelial cells, we used immunohistochemical staining with anti-factor VIII-related antigen coupled with horseradish peroxidase (EPOS Anti-Human von Willebrand factor/HRP, DAKO, Carpinteria, CA, USA) following the manufacturer’s protocol. The signals were visualized with diaminobenzidine/hydrogen peroxide. Frozen sections (4....
Three-step staining was performed for the following proteins [15]. The primary antibodies used were anti-skeletal myosin (fast isoform), anti-alpha-sarcoglycan, and anti-beta-sarcoglycan (clone: MY-32 [Sigma, Saint Louis, MO, USA], Ad1/20A6 and bSarc/5B1 [Novo Castra, UK], respectively). The second antibody was biotinylated rabbit anti-mouse antibody (K0675, DAKO), followed by staining with fluorescein isothiocyanate (FITC)-conjugated streptavidin (F0422, DAKO). Each protein was viewed using an ECLIPSE TE 2000-U (Nikon, Japan) confocal microscope.

More than five sections were prepared for each specimen, and every stained sample was evaluated by two independent pathologists in a blinded manner. The percentage of the total area that was fibrotic, as determined by Masson’s trichrome staining, was calculated by image analysis of the sections using a planimetric method with Windows MetaMorph software (Universal Imaging Corporation, Downingtown, PA, USA). The immunohistochemical pictures shown in Fig. 4 were taken under the same conditions, and the percentage of the total area for which the green intensity was above half of its maximum intensity, was calculated by the same planimetric method. The obtained values were expressed as a percentage of the value in F1B controls. At least 10 low-power fields per section were analyzed by the software.

2.6. Evaluation of prognosis after the operation

We evaluated the life-saving effect of the implantation of the sheet-shaped myoblast grafts on the TO-2 hamsters compared with cell injection and the sham operation. The animals were randomly allocated to each treatment group and housed for 60 weeks after the operation. The survival rates of the animals in the M, C, and S groups were calculated by the Kaplan–Meier method using SPSS Ver. 11.0 (SPSS Inc, Chicago, IL, USA), and the significance of the difference among the groups at 60 weeks was tested by log-rank analyses.

2.7. Statistical analysis

All values were expressed as the mean±S.E.M. and subjected to multiple analysis of variance (ANOVA) using the StatView 5.0 program (Abacus Concepts, Berkeley, CA, USA). Echocardiographic data were first analyzed by two-way repeated measure ANOVA for differences across the whole time course, and one-way ANOVA with the Tukey–Kramer post-hoc test was used to verify the significance for the specific comparison at each time point. Other numerical data except for survival were analyzed by one-way ANOVA with the Tukey–Kramer post-hoc test. A p value of less than 0.05 was considered significant.

3. Results

3.1. Effect of myoblast transplantation on cardiac performance

Fig. 2 shows the cardiac function evaluation by echocardiography. The Dd, Ds, and Fs at 27 weeks of age were not significantly different among the three groups. After the operation, both the Dd and Ds were gradually enlarged and the Fs was decreased in hamsters of the C group. Although the Dd and Ds of hamsters in the M group appeared slightly smaller than in the C group, they were also gradually enlarged. Hamsters in the M group showed a significantly higher Fs during the first 4 weeks after the operation compared with the C group. On the other hand, the progressive enlargement of the Dd and Ds of hamsters in the S group was markedly reduced and the Fs was maintained at the preoperative level for at least 12 weeks after the operation. (Solid line=S group; dotted line=M group; dashed line=C group; *P<0.05 vs. before the operation [27 weeks old]; super ★P<0.05 vs. the M and C groups; super ♦P<0.05 vs. the C group.)
appeared slightly smaller than in the C group, they were also gradually enlarged. The progressive enlargement of the Ds observed in the C group, however, was delayed in the M group, and hamsters in the M group showed significantly higher Fs during the first 4 weeks after the operation compared with the C group. The effect of the myoblast injection was transient, and the Fs began to decrease by 8 weeks after the myoblast injection. On the other hand, the progressive enlargement of the Dd and Ds of hamsters in the S group was markedly reduced and the Fs was maintained at the preoperative level for at least 12 weeks after the operation.

3.2. Morphological improvement of cardiomyocytes after myoblast sheet implantation

Fig. 3 illustrates typical examples of HE staining and staining for the fast isoform of skeletal myosin in the heart.

![Fig. 3](image_url)

**Fig. 3.** Myocardial changes in TO-2 hamsters 1 week after the operation. Tissue sections were stained with HE or for the fast isoform of skeletal myosin. (A) HE staining; (B) fast isoform of skeletal myosin; (C) factor VIII staining. Enlargement of the left ventricular wall was observed in the hearts of the S group as compared with the M and C groups. This enlargement was widespread (arrows) and contained many skeletal myosin-positive cells in the hearts of the S group. In this enlarged area, an increased number of new blood vessels was observed (n=3 for each group).

![Fig. 4](image_url)

**Fig. 4.** Suppression of fibrotic change in the LV of TO-2 hamsters by implantation of the sheet-shaped myoblast graft. The fibrous area was quantified by image analysis of tissue sections stained with Masson-trichrome. The percent fibrosis in the hearts of DCM hamsters was significantly suppressed by the implantation of the sheet-shaped myoblast graft but was not affected by the needle injection of myoblasts (n=5 for each group 8 days after the operation, *P<0.05).
from hamsters in the S, M, and C groups 1 week after the operation. Although the HE staining did not show obvious differences between the M and C groups, it showed enlargement of the left ventricular wall in the hearts of the S group compared with the M and C groups. This enlargement was widely observed in the hearts of the S group, and staining for the fast isoform of skeletal myosin indicated that the cells observed within this enlargement area were muscle-like. Fig. 3C shows a marked increase in

the number of factor VIII-positive arterioles and capillaries in this enlarged area of the S group.

Fig. 4A shows representative Masson’s trichrome staining results for each group 8 weeks after the operation. Quantification of the fibrotic area showed that although there was no significant difference between the C and M groups, the myocardial fibrosis of hamsters in the S group was significantly suppressed compared with that in the C and M groups (Fig. 4B).

![Images of immunostaining for alpha- and beta-sarcoglycans in cardiomyocytes.](image)

Fig. 5. Immunostaining for alpha- and beta-sarcoglycans in cardiomyocytes. (A) Alpha-sarcoglycan; (B) beta-sarcoglycan. F1B; 27-week-old F1B hamster. Alpha- and beta-sarcoglycans were clearly detected in the sarcolemma of the F1B strain hamsters. In the C and M groups, neither type of sarcoglycan was detected. In contrast, in the S group, cardiomyocytes expressed alpha- and beta-sarcoglycans 2 weeks after the operation; moreover, this expression was still evident 8 weeks after the operation.

![Images of quantitative results.](image)

Fig. 6. Quantitative results of the immunohistological signals shown in Fig. 5. (A) Alpha-sarcoglycan; (B) beta-sarcoglycan. Although the signals for the two proteins had not reached the normal levels observed in F1B hamsters 2 weeks after the operation, they were significantly higher in the S group than in the M and C groups (n = 5 for each group, *P<0.05).
Fig. 5 shows representative immunohistochemical staining results for alpha- and beta-sarcoglycans in the cardiomyocytes from F1B-strain hamsters. Alpha- and beta-sarcoglycans were clearly detected in the sarcolemma of the F1B hamsters. In contrast, the TO-2 strain, in which the promoter region of the delta-sarcoglycan gene is deleted, failed to express not only delta-sarcoglycan but also alpha-, beta-, and gamma-sarcoglycans [19]. We confirmed these results in the TO-2 hamsters of the C and M groups (Fig. 5). Interestingly, in the S-group of TO-2 hamsters, alpha- and beta-sarcoglycans were detected 2 weeks after the operation. Moreover, these proteins were still detectable in the S group even 8 weeks after the operation.

Fig. 6 shows the quantitative analysis of the immunohistological signals shown in Fig. 5. The area in which there were above-background green signals in the indicated group was compared with that of F1B hamsters, and the relative level of expression for each protein is shown. Although none of the signals for any of the three proteins reached the normal levels observed in F1B hamsters 2 weeks after the operation, the signals were significantly higher in the S group than in the C and M groups. Thus, weak but significant expression of all three proteins was observed 2 weeks after the myoblast sheet implantation, and that expression continued for at least 8 weeks after the implantation of sheet-shaped myoblast grafts.

3.3. Prolongation of life expectancy after myoblast sheet transplantation

Hamsters treated with myoblast injection or a sham operation (M or C group) started to die 1 week after the operation, and the number of surviving animals decreased gradually over the 4- to 10-week period after the operation (Fig. 7). There was no significant difference in survival rate between the C and M groups ($p=0.5835$). In contrast, although the hamsters treated with implantation of sheet-shaped myoblast grafts (S group) also started to die 1 week after the operation, and the number of surviving animals decreased gradually over the 4- to 8-week period after the operation, longer survival was observed and the S group showed significantly better survival. The survival percentage for the S group was significantly higher than that of the C and M groups ($p=0.0025$ for S vs. C, $p=0.0055$ for S vs. M). Therefore, we conclude that the life expectancy of the TO-2 hamsters was markedly prolonged by the implantation of sheet-shaped myoblast grafts.

4. Discussion

The present study demonstrated that progressive dilation of the left ventricle was significantly decreased and cardiac performance was preserved in adult cardiomyopathic hamsters by the implantation of sheet-shaped myoblast grafts. This favorable effect on cardiac performance was associated with a re-expression of cytoskeletal proteins and a reduction in myocardial fibrosis, and it led to a prolonged life expectancy in these adult cardiomyopathic hamsters. The favorable effect on cardiac performance and the histological improvements suggest that the implantation of sheet-shaped myoblast grafts is a potential therapeutic strategy for restoring the impaired heart in human DCM.
The TO-2 hamster strain is a representative model of human hereditary DCM in which the promoter region of the delta-sarcoglycan gene is deleted; this hamster fails to express alpha-, beta-, gamma-, or delta-sarcoglycan [20–22]. In this hamster strain, the number of cardiomyocytes decreases progressively because of apoptosis after birth, and cardiac remodeling, which mainly consists of myocardial fibrosis, occurs with ventricular dilation and thinning, leading to progressive congestive heart failure; these animals start to die when they are around 30 weeks old [23]. Treatment by genetic intervention has severe limitations, including the ethical issues associated with gene delivery. Therefore, we speculated that an ideal therapeutic strategy for this disease would be to reduce the myocardial remodeling and prevent the ventricular dilation caused by the numerical and functional cell loss.

Recently, myoblast transplantation has been investigated as a treatment for ischemic cardiomyopathy, and its functional benefit has been established in animal models [7,8]. In contrast, for DCM there have only been a few preclinical reports on the effects of myoblast transplantation, and those reports presented a low functional benefit for the left ventricle [10–12]. Although the precise mechanism has not been elucidated, when skeletal myoblasts are used for cellular cardiomyoplasty, the sequence of events appears to be the following: cells transplanted into the myocardium first impact the diastolic dysfunction. Subsequently, when the transplanted cells are sufficiently organized into myotubes and myofibers, the systolic performance improves. Implanted cells orient themselves against cardiac stress, preventing thinning and dilation of the injured region [9]. Thus, myoblasts might have the beneficial effect of providing mechanical support against cardiac stress. Therefore, if myoblasts could be delivered to the generally impaired heart of DCM more evenly and effectively, an even greater beneficial effect of such mechanical support might be expected.

In this study, although hamsters receiving the direct injection of myoblasts at four ventricular sites showed a significant improvement in systolic function, they did not show prevention of ventricular dilation. Also, in a recent report, myoblast transplantation by direct injection at six ventricular sites ventricle did not decrease the ventricular dilation in a BIO 14.7 DCM hamster model [12]. These results indicate that the method of direct injection may be inadequate to provide generalized cell delivery, especially in DCM. On the other hand, in hamsters receiving the implantation of sheet-shaped myoblast grafts, the progression of the ventricular dilation was prevented for 3 months after the operation. Two advantages associated with the sheet-shaped myoblast grafts might play an important role in this long-term prevention of ventricular dilation progression. One is the rapid adhesion of the sheet-shaped myoblast graft to the host tissue, because this graft has ECM on the basal surface, which can act as an adhesive agent for its attachment to other cell sheets or even to host tissues [24]. This rapid adhesion to host tissues may avoid the cell dislocation typically seen with the needle injection of cells [16]. The second is the efficient transplantation of myoblasts. Shimizu et al. previously reported that cell sheets allow for more effective transplantation because the large-area constructs are not associated with cell loss, in comparison to isolated cell injections, which often result in central tissue necrosis [16]. Therefore, the implantation of sheet-shaped myoblast grafts might be a superior method for delivering myoblasts effectively and widely to the generally impaired heart, leading to the prevention of ventricular dilation in DCM.

Recently, many reports have shown that myoblasts have the ability to secrete growth factors, such as hepatocyte growth factor (HGF), insulin-like growth factor (IGF), fibroblast growth factor (FGF), and others [25]. Recent reports have revealed that angiogenesis caused by HGF or VEGF, or an anti-fibrotic effect caused by HGF would be beneficial to the impaired DCM hearts [21,26]. Moreover, we reported that HGF causes the re-organization of cytoskeletal proteins, such as alpha-dystroglycan, and alpha- and beta-sarcoglycan, which have a mechanical function to strengthen the plasma membrane during heart muscle contraction and an important role in signal transduction [27]. In this study, a decrease in myocardial fibrosis and the re-expression of alpha- and beta-sarcoglycan were observed only in hamsters receiving the transplantation of sheet-shaped myoblasts. The decrease in myocardial fibrosis and the re-expression of cytoskeletal proteins associated with the improvement in cardiac performance of the impaired DCM heart were probably induced by some cytokines including HGF secreted from effectively transplanted myoblasts in the S group.

Several reports have shown that the life expectancy of TO-2 hamsters is significantly improved by delta-sarcoglycan gene transfection and oral therapy with an angiotensin-converting enzyme inhibitor, enalapril [12,21]. In these reports, the treatments were started at 5 or 6 weeks of age, before the number of cardiomyocytes decreased and cardiac function was impaired. Another treatment, cellular cardiomyoplasty using myoblasts, smooth muscle cells, or cardiomyocytes, was started at around 15 weeks of age, at which time the cardiac remodeling, such as the loss of cardiomyocytes or fibrosis, had begun to progress and cardiac function was mildly impaired [28–30]. However, those reports on cellular cardiomyoplasty did not show morphological improvement or prolonged life expectancy. Here, the implantation of sheet-shaped myoblast grafts or direct injection of myoblasts was performed when the hamsters were 27 weeks old. It is very interesting that, while hamsters treated with myoblast injection did not show prolonged life expectancy, those treated with sheet-shaped myoblast grafts showed re-organization of the cytoskeletal proteins, reduction of myocardial fibrosis, and prevention of dilation of the left ventricle, leading to prolonged life expectancy and a longer preservation of cardiac perfor-
mance in the impaired heart, even at such a late phase of DCM.

The results of the present study need to be regarded in the light of some limitations. One is the origin of myoblasts. In this study, we used myoblasts from F1B hamsters because the skeletal myoblasts are phenotypically normal in this strain, although in the TO-2 strain hamsters, the skeletal myoblasts are genetically affected but phenotypically spared by a large deletion of the delta-sarcoglycan gene [31]. However, in the clinical situation, autologous myoblasts can be mainly used when the DCM is caused by a genetic muscle disease. The second is the potential arrhythmic risk after myoblast transplantation [32]. This risk has been associated with ischemic settings and is related to the possible setup of reentry circuits resulting from the differences in electrical membrane properties between skeletal muscle and cardiac muscle cells [33,34]. This arrhythmic risk might be one of the reasons for the early death of hamsters in the S and M groups, although the cardiac performance was preserved in these groups for the first 4 weeks after the operation. Although this risk is equal in ischemic versus non-ischemic myocardial disease, further investigation is needed to determine the best approach for clinical application. The last limitation is that it is still unclear precisely how the myoblast sheets improve the host cardiac function, which leads to the histological improvement of the host myocardium.

In summary, transplantation with sheet-shaped myoblast grafts induced a re-organization of cytoskeletal proteins and anti-fibrotic effect and preservation of cardiac performance, which increased the life expectancy in a hamster model of DCM.

Acknowledgments

We wish to thank Akiko Nishimura, Masako Yokoyama, and Aiko Miki for their excellent technical assistance.

References


[34] Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. Proc Natl Acad Sci U S A 2003;100:7808–11.