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Original Article

Smooth Muscle Cells Transplantation is better than Heart Cells Transplantation for Improvement of Heart Function in Dilated Cardiomyopathy

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Muscle cell transplantation may delay or prevent cardiac dilation in dilated cardiomyopathy. The present study was designed to compare the effects of the heart function of smooth muscle cell (SMCs) auto-transplantation and heart cell (CMs) allo-transplantation in dilated cardiomyopathic hamsters, and to determine which cells are better for cell transplantation. CMs and SMCs were isolated from BIO 53.58 hamsters, and cultured for transplantation. CMs, SMCs (4×10^6 cells each) or culture medium were transplanted into 17 weeks old BIO 53.58 hamsters to achieve CM transplantation (CMTx), SMC transplantation (SMCTx), and controls (Con) (N=10 each). Cyclosporine (5 mg/Kg) was administered subcutaneously to CMTx. Healthy hamsters (sham, N=6) were used to compare heart functions. Four weeks after transplantation, heart function was evaluated in all groups using a Langendorff perfusion apparatus. Histology demonstrated severe focal myocardial necrosis in the dilated cardiomyopathic hearts. CMTx and SMCTx formed huge muscle tissue in the dilated myocardium. Sham, SMCTx, and CMTx had a better heart function than Con ($p<0.01$), and SMCTx had a better peak systolic pressure ($p<0.05$) and developed pressure ($p<0.05$) than CMTx at any balloon volume. However, sham and SMCTx were not statistically different. SMCTx and CMTx formed muscle tissue and produced better heart function in the cardiomyopathic hearts, and SMCTx showed better systolic and developed pressures than CMTx, even though they were similar in other functions. Significantly, SMCTx had heart functions, which were similar to those of healthy hamster's hearts.

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INTRODUCTION

Heart failure is becoming an increasing medical challenge as life expectancy continues to increase. Currently it affects 0.4 - 2% overall and 10% of elderly subjects,[1] and heart muscle disease (cardiomyopathy) is one of the major causes of heart failure. Taking the WHO categorization of cardiomyopathy, the most common cause of the clinical syndrome of heart failure is secondary or primary dilated cardiomyopathy, which is characterized by dilatation and impaired contraction of the left ventricle or both ventricles.[2]

During the past 7 years, there has been a relatively equal representation from both cardiomyopathy and coronary patients for adult heart transplantation.[3] Although cardiac transplantation provides excellent therapy for some patients with terminal heart failure, results are limited by the scarcity of donor organs, reduced long-term survival, and co-morbid conditions.[4,5]

Recently cell transplantation in the failing heart has been extensively investigated to improve heart function in the infarcted myocardium. However, most of these investigations have focused on improving the function of hearts in which myocardial infarction or cryoinjury has resulted in regional ventricular dysfunction.[6-9] Yoo[10,11] and Scorsin[12] studied cell transplantation in dilated cardiomyopathy proved that trans-

planting several types of cells into infarcted myocardium or dilated cardiomyopathy improves heart function. However, much less is known about the effect of cell transplantation on dilated cardiomyopathy.

In our present study, we compared the effect of transplanting heart cells and smooth muscle cells on the histology and function of the hearts of adult hamsters with dilated cardiomyopathy, to determine which cell type is better suited for cell transplantation.

MATERIALS AND METHODS

Experimental animals

The experimental animals used were male BIO 53.58 hamsters (BIO Breeders, Fitchburg, Mass., USA) and healthy Syrian hamsters (Charles River Canada Inc, Quebec, Quebec, Canada). All procedures involving animals were approved by the Animal Care Committee of the Toronto General Hospital, and carried out in compliance with the "Guide to the Care and Use of Experimental Animals" issued by the Canadian Council on Animal Care and the "Guide for the Care and Use of Laboratory Animals" published by the National Institute of Health (NIH publication 85-23, revised 1985).

Isolation and culture of cells

At 4 weeks of age, young BIO 53.58 hamsters underwent sternotomy to remove the heart, and at 13 weeks of age BIO 53.58 hamsters underwent laparotomy to remove the ductus deferens, from which the cultured heart and smooth muscle cells were obtained. The hamsters were anesthetized with ketamine (20 mg/kg, intramuscular) and pentobarbital (30 mg/kg, intraperitoneally). The anesthetized hamsters were ventilated by mask with oxygen-supplemented room air. The heart and ductus deferens were exposed and removed by sternotomy and a transverse lower abdominal incision. After abdominal closure, the hamsters were recovered from surgery, electrocardiographically monitored for 4 hours, and given Penlong XL (benzathine penicillin G 150,000U/mL and procaine penicillin G 150,000 U/mL, 0.2 mL intramuscularly) every 3 days and buprenorphine (0.01 to 0.05 mg/kg subcutaneously) every 12 hours for the first 48 hours after surgery.

The excised heart and ductus deferens were washed with phosphate-buffered saline (PBS) (NaCl, 136.9 mmol/L; KCl, 2.7 mmol/L; Na_2HPO_4, 8.1 mmol/L; KH_2PO_4, 1.5 mmol/L; pH 7.3), then minced and incubated in 10 mL of PBS containing 0.2% trypsin, 0.1% collagenase, and 0.02% glucose for 30 minutes at 37℃,[13] and the digested tissue repetitively pipetted in enzyme solution to disperse the tissue clumps. The supernatant, containing suspended cells, was transferred into a 20 mL of Iscove's modified Dulbecco's medium (Gibco Laboratory, Life Technologies, Grand Island, NY, USA) containing 10% fetal bovine serum, 0.1 mmol/L β-mercaptoethanol, 100 U/mL penicillin, and 100μg/mL streptomycin. This suspension was then centrifuged at 600 × g for 5 minutes at room temperature, and the cell pellet resuspended in cell culture medium and plated on a culture dish. The cells were cultured and passaged once, over a 2-week period for heart cells and a 4 week period for smooth muscle cells.

Identification of transplanted cells

To facilitate the identification of the transplanted cells in the recipient myocardium, cultured cells at 50% confluence were labeled with 25μL of a 0.4% solution of the thymidine analog 5-bromo-2'-deoxyuridine (BrdU) (Zymed Lab. Inc., South San Francisco, CA, USA) for 3 days prior to transplantation (N=2). BrdU labeling efficiency was confirmed by staining two randomly selected dishes. The BrdU-labeled cells were transplanted and identified as described below. Four weeks after transplantation, the hamsters were sacrificed and the hearts were examined. Immunohistochemical staining identified the BrdU-labeled cells in the region of transplantation as described previously.[9] Cyclosporine, at a dose of 5 mg/kg of body weight, was subcutaneously administered daily to the heart cells transplanted group after surgery.

Preparation and transplantation of cultured cells

Dishes of cultured cells were washed three

times with PBS to remove dead cells. The adherent cells were then detached by adding 0.05% trypsin in PBS to culture dishes for 2 minutes. After adding 10 mL of the culture medium, the cell suspension was centrifuged at 580 × g for 3 minutes. The cell pellet so obtained was resuspended in the culture medium at a concentration of 100×10^6 cells/mL, and 4×10^6 cells or culture medium was used for the subsequent transplantation into cell transplanted or control hearts, respectively.

At 17 weeks of age, the BIO 53.58 hamsters were again anesthetized as previously described. The anesthetized animals were intubated and ventilated with oxygen-supplemented room air with a Harvard ventilator (Model 683, Harvard Instruments, South Natick, Mass., USA) at a rate of 60 breaths per minute and a tidal volume of 1.5 mL. The heart was exposed through a 3 cm left lateral thoracotomy. The cell suspension (heart cells transplantation and smooth muscle cells transplantation, N=10 respectively) or culture medium (control, N=10) was injected using a tuberculin syringe into a single site on the anterior aspect of the left ventricular free wall. Sham operated animals (N=6) underwent thoracotomy without injection. After chest closure, the animals were allowed to recover from surgery and treated with antibiotics and analgesics as previously described.

Evaluation of left ventricular function

Four weeks after cell transplantation, the hamsters were anesthetized and heparin sodium (100 units) was administered intravenously. Hearts were quickly excised and perfused in a Langendorff apparatus in filtered Krebs-Henseleit buffer (NaCl, 118 mmol/L; KCl, 4.7 mmol/L; KH_2PO_4, 1.2 mmol/L; $CaCl_2$, 2.5 mmol/L; $MgSO_4$, 1.2 mmol/L; $NaHCO_3$, 25 mmol/L; glucose, 11 mmol/L; pH 7.4), which was equilibrated with 5% carbon dioxide and 95% oxygen. A latex balloon was passed into the left ventricle across the mitral valve and connected to a pressure transducer (model p10EZ; Viggo-Spectramed, Oxnard, CA, USA) and a differentiator amplifier (model 11-G4113-01; Gould Instrument System Inc, Valley View, OH, USA). Coronary flow was measured in triplicate by timed collection in the empty beating heart. After 30 minutes of stabilization, balloon volume was increased in 0.005 mL increments from 0.005 to 0.025 mL by the stepwise addition of saline. Heart rate, systolic and diastolic left ventricular pressures, and maximum +dp/dt and -dp/dt were recorded at each balloon volume. Developed pressure was calculated as the difference between the systolic and diastolic pressures. After completing all measurements, the hearts were arrested in diastole by perfusing with 5 mL of a 20% KCl solution. Passive left ventricular diastolic pressures were recorded over a range of balloon volumes from 0.005 to 0.035 mL, in 0.005 mL increments, and the hearts subsequently weighed.

Histology

Heart sections were fixed in 5% glacial acetic acid in methanol, embedded in paraffin, and cut into 10 μm thick sections. The sections were stained with haematoxylin and eosin as described by the manufacturer's specifications (Sigma Diagnostics, St. Louis, MO, USA). Sections were also immunohistochemically stained[9] for BrdU, troponin I and alpha smooth muscle actin.

Statistical analysis

Data are presented as the means ± standard deviation unless otherwise indicated. Statistical analysis was carried out using the SAS software package (SAS Institute, Cary, NC, USA). Continuous variables of more than two groups were compared by one-way analysis of variance. If the F ratio was significant, differences were specified by Duncan's multiple-range t test. A p value of less than 0.05 was considered statistically significant.

Left ventricular function was evaluated by covariance analysis, with intraventricular volume as the covariate and systolic, diastolic, and developed pressures, and maximum and minimum dp/dt as dependent variables. The main effects were group, heart volume, and the interaction between group and heart volume. When analysis of covariance indicated a significant effect group or interactive effect, differences were specified by multiple pair-wise comparisons.

RESULTS

Labeling of cultured cells

The efficiencies of labeling cultured heart cells (Fig. 1A) and smooth muscle cells (Fig. 1B) with BrdU, defined as positive BrdU staining, were 56.8 ± 4.7% (N=3) for heart cells and 49.2 ± 5.4% (N= 3) for smooth muscle cells.

Histology

Sham animals showed normal histologic myocardium (Fig. 2A), but severe focal myocardial necrosis and fibrosis were noted throughout the myocardium of the 21-week-old hamsters in the other groups (Fig. 2B). Four weeks after cell transplantation, a block of muscle-like tissue was noted at the site of transplantation (Fig. 2C and 2D). This tissue stained positively for BrdU (Fig. 3A and 3B), troponin I and alpha-smooth muscle actin (Fig. 4A and 4B), but the surrounding native myocardium did not. Despite being injected at only a single site, the volume of transplanted cells was sufficient to occupy approximately one third of the area of the left ventricular free wall. Blood vessels were apparent in the cell-transplanted region (Fig. 2C and 2D). No similar muscle-like tissue or capillary ingrowth was noted in control hearts injected with culture medium alone. Sham animals showed normal hamster myocardium.

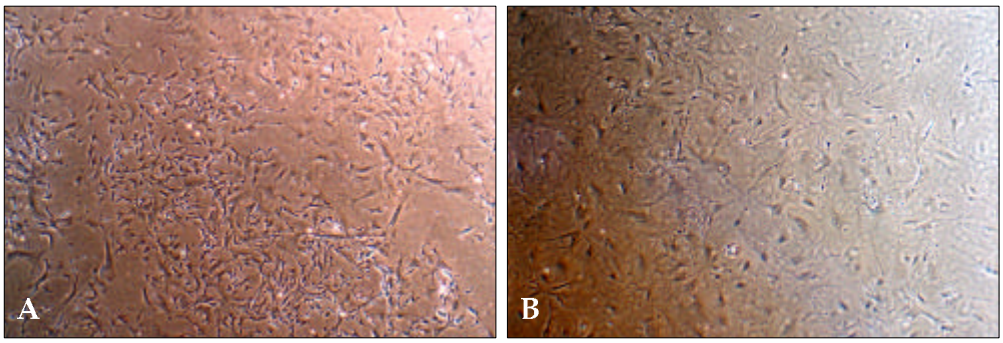

Fig. 1. Photomicrographs of cultured heart cells (A) (40 ×) and smooth muscle cells (B) (40

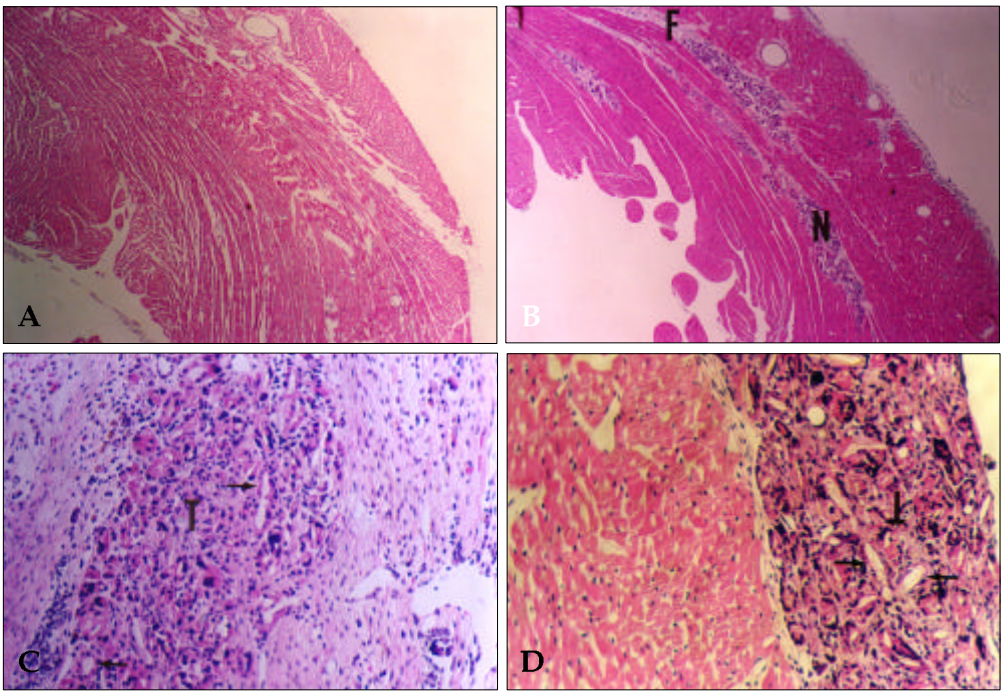

Fig. 2. Photomicrographs of sham operated animal (2A, 40 ×), control (2B, 40 ×), heart cells transplanted heart (2C, 200 ×), and smooth muscle cells transplanted heart (2D, 200 ×) of 21-week-old cardiomyopathic hamsters (haematoxylin and eosin). The cardiomyopathic host myocardium shows severe focal necrosis (N) and fibrosis (F). Transplanted heart cells and smooth muscle cells formed muscle-like tissue (T), in which multiple small- caliber blood vessels were noted (arrows).

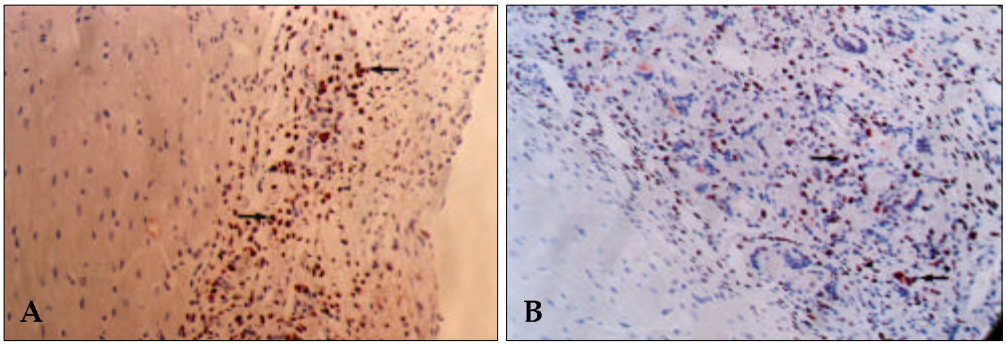

Fig. 3. Photomicrographs of transplanted heart cells (3A, 200 ×) and smooth muscle cells (3B, 200 ×) in which the BrdU-prelabeled cells were stained with haematoxylin and immunohistochemically for BrdU. Muscle-like tissue, which stained positively for BrdU (arrows), was found in both cells transplanted hearts.

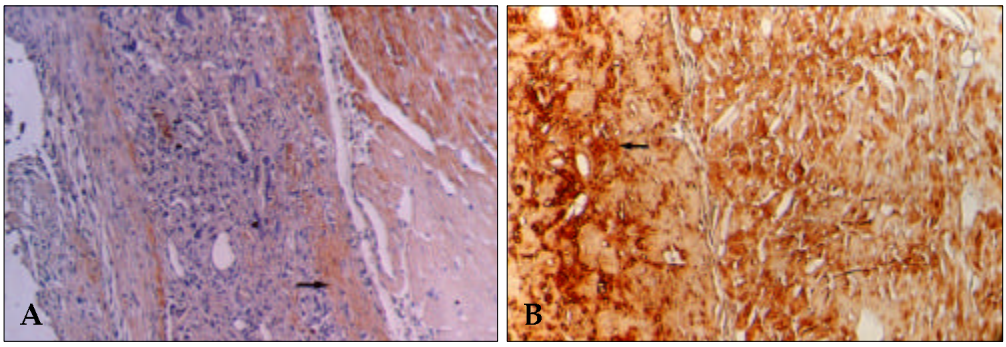

Fig. 4. Photomicrographs of the muscle-like tissue formed by the transplanted heart cells (4A, 200 ×) and smooth muscle cells (4B, 200 ×), stained immunohistochemically for Troponin I and alpha-smooth muscle actin (arrow).

Left ventricular function

Heart rate, coronary flow, and the ratio of heart weight to body weight were similar for the sham operated, control, heart cell and smooth muscle cell transplantation groups.

The evaluation of left ventricular function in our Langendorff apparatus demonstrated significantly greater systolic ($p<0.01$) and developed pressures ($p<0.01$) in both cell transplanted hearts and sham-operated animals, compared to control hearts. In addition, smooth muscle cell transplantation hearts had better peak systolic pressure ($p<0.05$) and developed pressure ($p<0.05$) than heart cell transplantation hearts at any balloon volume (Fig. 5A and 5B). Maximum +dp/dt was also significantly greater in both cell-transplanted animals and in sham-operated animals than in control animals ($p<0.01$) (Fig. 6A). No difference between smooth muscle cell transplantation hearts and sham-operated animals was noted in terms of these parameters of systolic function.

Maximum -dp/dt was significantly lower in both cell-transplanted animals and in sham-operated animals than in control animals ($p<0.01$) (Fig. 6B). Both the active ($p<0.01$) and passive

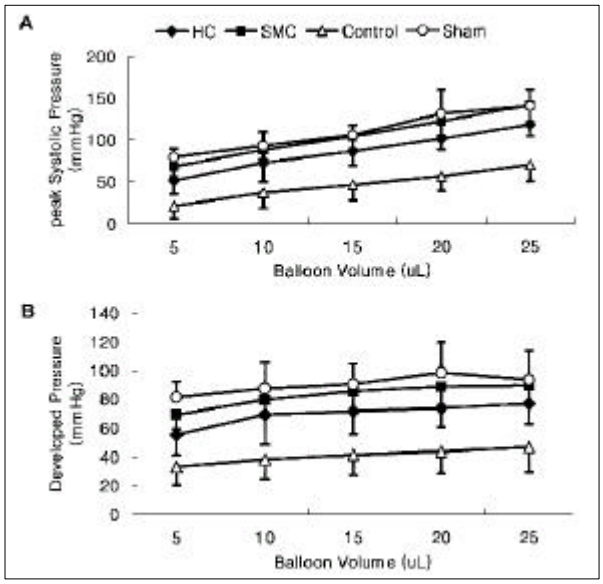

Fig. 5. Peak systolic (A) and developed left ventricular pressures (B) in heart cell and smooth muscle cell transplanted, control, and sham operated heart over a range of intraventricular balloon volumes (means ± SD). Peak systolic and developed pressures in the cell transplantations and in the sham-operated heart were significantly higher than in the control ($p<0.01$). Smooth muscle cells transplantation and sham animals had higher systolic pressure and developed pressure than heart cell transplantation animals ($p<0.05$). No significant differences were found between smooth muscle cell transplantation and sham-operated hearts.

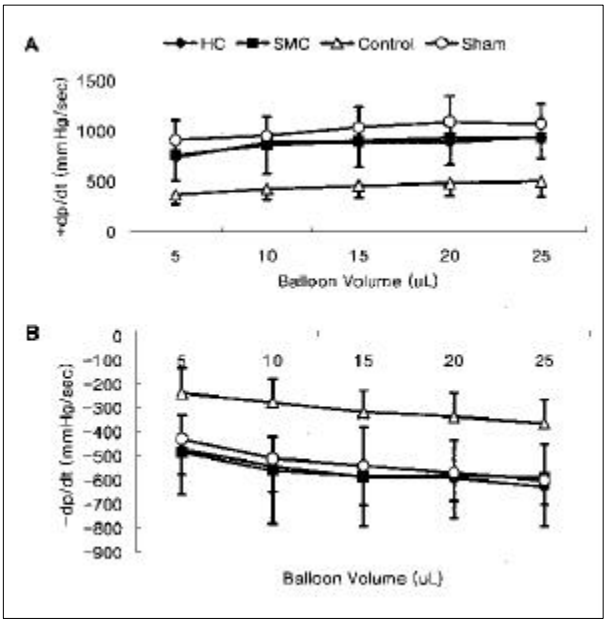

Fig. 6. Maximum left ventricular +dp/dt (A) and -dp/dt (B) in the heart and smooth muscle cell transplanted, control, and sham operated hearts over a range of intraventricular balloon volumes (means ± SD). Maximum +dp/dt was significantly higher, and maximum -dp/dt was significantly lower, in the cell transplantations and the sham-operated hearts than in the controls ($p<0.01$). There were no significant differences between the cell transplantations and the sham-operated hearts.

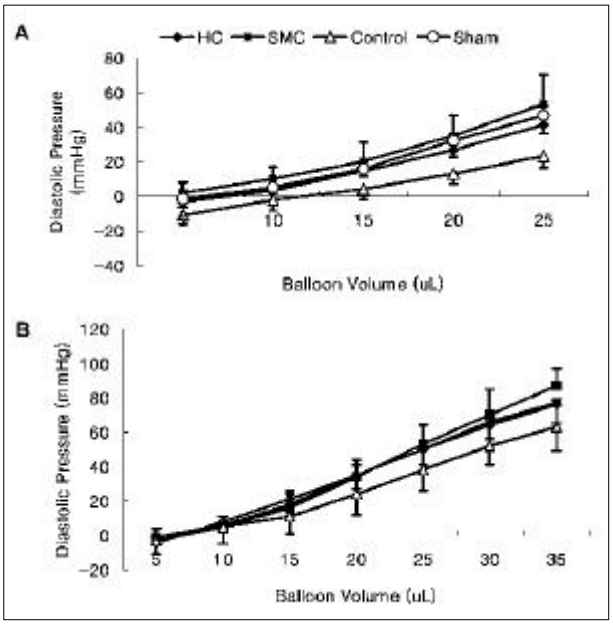

Fig. 7. Active (A) and passive (B) left ventricular diastolic pressures in heart and smooth muscle cell transplantations, and control, and sham-operated hearts over a range of intraventricular balloon volumes (means ± SD). Diastolic pressures in the cell transplantations and sham operated hearts were significantly higher, at any intraventricular balloon volume, than in the controls ($p<0.01$). No significant differences were found between cell transplantations and sham-operated hearts.

diastolic left ventricular pressures ($p<0.01$) were significantly greater, at any intraventricular balloon volume, in cell-transplanted animals and sham-operated animals than in control animals ($p<0.01$) (Fig. 7A and 7B). Cell-transplanted animals and sham-operated animals did not differ significantly in terms of diastolic function.

Comments

Since the pioneering studies by Soonpaa et al in which cultured AT-1 and fetal cardiomyocytes were transplanted into syngeneic mouse hearts,[14,15] significant progress has been achieved during the past decade in the field of cell transplantation. We have previously evaluated the effects of the allo-transplantation of heart cells and auto-transplantation of smooth muscle cells in BIO 53.58 cardiomyopathic hamsters. Both transplanted cell types formed muscle-like tissue and improved heart function.[10,11]

In this study, we used BIO 53.58 Syrian hamsters,[16-19] which display hereditary abnormalities of the cardiac and skeletal muscles, which are inherited as an autosomal recessive trait. They develop a dilated cardiomyopathy with ventricular dilatation and focal myocytolysis, leading to progressive congestive heart failure. Histologic changes begin at approximately 10 weeks of age, and the disease becomes clinically apparent at about 17 weeks. Theoretically, heart cells are the most ideal candidates for cell transplantation into diseased heart but even the auto-transplantation of already cardiomyopathic heart may be unfavorable in this animal model like a skeletal muscle. However, smooth muscle has several characteristics, including the ability to maintain prolonged tonic contraction with very low energy requirement, and often with higher maximum contraction force than that of skeletal muscle.[20] Another important characteristic is the ability of smooth muscle to return nearly to its original contraction force seconds or minutes after it has been elongated or shortened beyond a normal

range. We showed improved heart function after heart cell allo-transplantation in this strain even though we used cyclosporine. We also showed that smooth muscle cell auto-transplantation improved heart function in this strain. However, it is still unclear which cells are better than others, and the degree of heart function versus the normal hamster heart. We therefore employed this strain to compare the effects of different cell transplantations with each other and with normal hamster heart. We selected 4.0×10^6 cultured cells, which represent a maximal number for transplantation in the small hamster's heart without causing deterioration of left ventricular function, and transplanted them into a site on the left ventricular free wall.

Both transplanted BrdU prelabeled cells survived in the host myocardium, which was confirmed by BrdU positive staining, and they also formed muscle-like tissue which retained its own characteristics since the muscle-like tissue stained positively for troponin I and alpha smooth muscle actin. The engrafted tissue also contained numerous capillaries, which were noted in the region of cell implantation. Angiogenesis may have improved regional perfusion, and thereby, augmented native cardiomyocyte contractility, and also helped the survival of transplanted cells. Since smooth muscle cells can proliferate *in vivo*,[9] some of the muscle-like tissue observed may have been formed by post-implantation hyperplasia of the transplanted cells. The arrangement of the transplanted cells, however, was clearly disorganized by light microscopy, and thus the effective contractility of the transplanted cells seems unlikely. Both cell-transplanted animals showed improved systolic and diastolic heart function compared to control animals with cardiomyopathic hearts. The reason for the improved heart function was not determined in this study. We did not observe the contraction of hamster heart cells and smooth muscle cells *in vitro* during culture or *in vivo* after transplantation. The diastolic pressures of transplanted hearts rose and reached rapidly physiologic left ventricular volume versus control hearts. We attributed changes noted in the diastolic function of the cell-transplanted hearts to increased wall thickness, which limits ventricular dilatation. Improved diastolic function is reflected in improved systolic performance of the heart. In particular, the significantly greater maximum +dp/dt and -dp/dt in the transplanted hearts indicated an improved force-velocity relationship suggesting that cell transplantation favorably affected systolic shortening and active relaxation.

Smooth muscle cell transplantation and sham animals had higher systolic pressures and developed pressure than heart cell transplantation animals. The muscle-like tissue formed by heart cells implantation gradually decreased in size with time, which we attribute to chronic rejection despite immunosuppression. That therefore might potentially have made differences. Sham animals showed slightly better heart function than smooth muscle cell transplantation but this was not statistically significant. Cell auto-transplantation therefore appeared to be an ideal way to achieve the beneficial effects of cell transplantation without stimulating a chronic immune response.

Although both cell transplantations improved heart function irrespective of use of cyclosporine in a hamster model of dilated cardiomyopathy, we did not evaluate the long-term survival of these animals or large animal models. In addition, it is still unclear as to which characteristics of smooth muscle cells are responsible for the improved ventricular function versus heart cell allo-transplantation. Further studies upon muscle cell transplantation, in a large animal model of dilated cardiomyopathy are needed in order to further define its effect.

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