Cell transplantation with a catheter-based approach: an efficient method for the treatment of heart failure with multiple lesions

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Abstract. Cell transplantation is emerging as a potential therapeutic approach for the treatment of heart failure. At present, popular methods of cell delivery may not be efficient in perfusing cells through the whole myocardium. We have developed a novel catheter-based method for global transplantation of cells. Heart failure was induced in rabbit by intravenous administration of doxorubicin. Autologous bone marrow mononuclear cells were transplanted into failing hearts via the root of the aorta. Bilateral sinus aortae and coronary arteries were visualized by angiography during the cell transplantation procedure; there was no intraprocedural death. Four weeks after cell transplantation, there was an improvement in the mean left ventricular ejection fraction from baseline 72.13% to 81.54% ($P = 0.034$). Transplanted cells were observed throughout the cardiac layers of left and right ventricles. In conclusion, cell transplantation through the root of the aorta is a useful approach to globally supply cells into the heart.

INTRODUCTION

Cell transplantation is emerging as a potential novel therapeutic approach for the treatment of heart failure. Popular methods for cell delivery include direct intramuscular injection (Menasché *et al.* 2003; Smits *et al.* 2003) and selective intracoronary transplantation (Assmus *et al.* 2002; Strauer *et al.* 2002). However, cells infused by direct intramuscular injection usually produce localized islet-like formations, resulting in limitation of cell-to-cell interaction between grafted cells and native cardiomyocytes. This may affect the integration of grafted cells into the native myocardium, restricting efficacy of cell transplantation and bearing the potential risk of pro-arrhythmia (Makkar *et al.* 2003; Menasché *et al.* 2003; Smits *et al.* 2003). Cells delivered by selective intracoronary transplantation are confined in localized regions supplied by any selected coronary artery (Taylor *et al.* 1997). This regional confinement of distribution of infused cells may limit the usefulness of cell transplantation for severe heart failure when it is dominated by multiple scattered lesions. In the present study, we have developed a novel catheter-based method for global dissemination of cells into the myocardium.

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MATERIALS AND METHODS

Animals

The investigation was performed within the directives of the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (1985) and within institutional guidelines.

Male Japanese white rabbits (14–16 weeks old) weighing 2.5–3.0 kg were used in the experiment. Twenty-four rabbits were assigned to a healthy control group, a sham operation group and an autologous bone-marrow mononuclear cell (MNC) transplantation group.

Generation of doxorubicin-induced cardiomyopathy

Doxorubicin-induced heart failure was generated as previously described (Arnolda *et al.* 1985). Doxorubicin (Haizhen Pharma Co, China) was administered intravenously (2 mg/kg in saline) to rabbits once a week over a period of 8 weeks. The rabbits in the healthy control group received injections of equal volumes of saline. The animals were observed for 4 weeks after the final injection. At the end of the 4-week post-treatment period, MNCs were isolated.

Isolation and labelling of MNCs

MNCs were isolated according to the modified method of Papadaki *et al.* (2002). In brief, each rabbit was anaesthetized with ketamine (20 mg/kg). A bone marrow aspirate was collected from the femoral bone into a syringe containing 3000 U heparin. The marrow sample was immediately diluted 1 : 1 in D-Hanks' solution supplemented with 100 IU/ml penicillin and 100 IU/ml streptomycin. The diluted samples were centrifuged in Lymphoprep solution (density 1.077 g/ml; TBD, Tianjing, China) at 460 *g* for 30 min at room temperature. MNCs were collected from the interface. They were then diluted with two volumes of D-Hanks' solution and re-collected by centrifugation at 362 *g* for 5 min. The cells were labelled with sterile bromodeoxyuridine (BrdU, 10 mg/l) (Barbash *et al.* 2003) in growth medium composed of Dulbecco's modified eagle's medium (DMEM), 5% foetal bovine serum, 10% bovine serum, 100 IU/ml penicillin and 100 IU/ml streptomycin. Cells were cultured overnight at 37 °C in a humidified atmosphere with 5% carbon dioxide and were then rinsed three times in D-Hanks' solution to remove all excess, unbound BrdU. MNCs were collected (approximately 1×10^7 cells for each transplantation) and were re-suspended in 10 ml serum-free DMEM.

Catheterization procedure for transplantation

Autologous MNC transplantation was performed as described in succeeding discussions. Each rabbit was anaesthetized with ketamine (20 mg/kg). The left common carotid artery was exposed. It was then punctured and cannulated with a 21-gauge sheathed needle. Two guide wires (100-cm, 0.014-inch J-tipped wire) were passed into the sheath, and then two percutaneous transluminal coronary angioplasty (PTCA) balloons (3.5-mm diameter, 8-mm-long dilating balloon) were advanced across the guide wires, respectively. With the aid of fluoroscopic guidance, the tip of each balloon was directed to the ascending aorta and was adjusted just above the sinus of Valsalva, visualized by angiography. To allow for infusion of cells into the coronary artery while both balloons were simultaneously inflated at 8–10 atmospheres and to block downstream blood flow in aorta for 1.5 min, 3.3 ml of MNC suspension was infused distally into the aortic root through the side port of one occluding balloon catheter. This manoeuvre was repeated three times to accommodate infusion of the total 10 ml MNC suspension, interrupted by 3 min of reflow by deflating the balloons. After completion of MNC suspension infusion, the left common carotid artery was ligated, and the animal was returned to the animal care facility. All

surgical procedures were performed aseptically. In the sham operation group, 10 ml of serum-free DMEM was infused as described earlier.

Functional assessment of the heart

Before MNC primary isolation and 4 weeks after cell transplantation, the animals' left ventricular ejection fraction (LVEF) was studied by echocardiography. Parasternal long axis two-dimensional echocardiography was performed with an 8.5 MHz transducer, and the two-dimensionally guided M-mode echo was recorded. When a correct image was obtained with well-defined continuous interfaces for the septum and posterior wall at the higher frame rate, a numeric acquisition was performed on the hard disk of the echocardiograph. Left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV) were determined by using the single-plane area-length method at end-diastole (at the time of apparent maximal cavity dimension) and at end-systole (at the time of maximum anterior motion of the posterior wall). LVEF was calculated as $LVEF (\%) = (LVEDV - LVESV) / LVEDV \times 100$.

Immunofluorescence study

Four weeks after cell transplantation, each rabbit was killed by administering an overdose of ketamine. The heart was removed and rapidly rinsed with phosphate buffer saline solution. Each ventricle was cut into seven segments including right ventricular outflow tract, right ventricular free wall, right ventricular apex, left ventricular outflow tract, left ventricular free wall, left ventricular apex and anterior ventricular septum. Paraffin wax-embedded sections, 5 μ m in thickness, were prepared from each segment to determine the number of cells labelled with BrdU. The sections were also used for haematoxylin eosin staining.

To detect BrdU, the sections were incubated with a 1 : 100 dilution of anti-BrdU polyclonal antibody (Abcam, Cambridge, UK) for 2 h at 37 °C. This was followed by 1 h of incubation with a 1 : 300 dilution of fluorescein isothiocyanate-conjugated secondary antibody. All tissue sections were counterstained with 0.02% 4',6-diamidino-2-phenylindole (DAPI) for 5 min at room temperature. Each section was examined by confocal microscopy at three randomly chosen optical fields. Cells stained with DAPI were regarded as cell nuclei and cells costained with BrdU and DAPI were regarded as MNC-derived cells. The mean percentage of MNC-derived cells was calculated as the total number of MNC-derived cell nuclei of all cells in the three optical fields ($\times 100$ magnification). In the heart function assessment and immunofluorescence study, observations were carried out blind to experimental conditions.

Statistical analysis

All values are presented as mean $\pm$ SEM. Heart function for each group and the percentages of MNC-derived cells at different ventricular sites were compared by ANOVA/Tukey test. The change of LVEF after cell transplantation was analysed using pair *t*-testing. Statistical analysis was performed with SPSS (version 10.0, SPSS Inc). $P < 0.05$ was considered as statistically significant.

RESULTS

Heart failure model of doxorubicin-induced cardiomyopathy

Compared to the healthy control group, LVEF was significantly reduced after administration of doxorubicin in either the MNC transplantation group or the sham operation group (Table 1). In doxorubicin-treated hearts, myocardial fibre disarray and interstitial fibrosis were widely

Table 1. Comparison of heart function

Group	LVEF (%)		LVEDD (mm)	
	Pre-	Post-	Pre-	Post-
Healthy (<i>n</i> = 8)	81.50 ± 1.52		1.15 ± 0.03	
Sham (<i>n</i> = 8)	72.38 ± 2.10*	70.46 ± 4.05	1.31 ± 0.08	1.51 ± 0.08†
MNCs (<i>n</i> = 8)	72.13 ± 2.50*	81.54 ± 1.95†‡	1.22 ± 0.07	1.25 ± 0.06‡

Data are mean ± SEM. **P* < 0.05 versus healthy control group (healthy), ‡*P* < 0.05 versus sham operation group (sham) and †*P* < 0.05 post-transplantation (post-) versus pre-transplantation (pre-). MNCs represent MNC-transplantation group, LVEF represents left ventricular ejection fraction, LVEDD represents left ventricular end-diastolic diameter.

demonstrated in haematoxylin- and eosin- stained sections. These data indicate doxorubicin-induced cardiomyopathy and heart failure.

Catheter-based transplantation

No arrhythmic events, significant haemodynamic deterioration or death of the animals were observed during catheter-based transplantation. Curable infection or minor haematomas around the surgical incision of several rabbits were discovered 2–3 days after catheterization. Within the 4-week post-catheterization period, no neurological events (such as paralysis) were observed.

In the balloon inflation procedure, bilateral sinus aortae and coronary arteries were visualized during angiography, suggesting that blood at the root of the aorta was forced into the bilateral coronary arteries (Fig. 1).

Heart function assessment after cell transplantation

Mean LVEF showed a significant increase of 9% over the 4-week follow-up period in the MNC transplantation group. In contrast, LVEF deteriorated further in the sham operation group. Meanwhile, dilation of the left ventricular end-diastolic diameter was retarded after MNC transplantation (Table 1).

Figure 1. Static angiogram of sinus aortae and coronary arteries. (a) Angiogram of left sinus aortae and left coronary artery (left sinus aortae, left main coronary artery, proximal left anterior descending artery and proximal left circumflex coronary artery were observed); (b) Angiogram of right sinus aortae and right coronary artery (right sinus aortae and the ostium of right coronary artery were observed).

Figure 2. Immunofluorescent findings of BrdU-labelled cells in transplanted heart. Cells (a, magnification $\times 400$, arrow) costained BrdU (green) and DAPI (blue) were found in MNC transplantation heart but not in sham operation heart (b, magnification $\times 400$).

Figure 3. Comparison of percentage of MNC-derived cells between different ventricular sites. * $P < 0.05$ versus left ventricular apex (LVA), # $P < 0.05$ versus left ventricular outflow tract (LVOT), \$ $P < 0.05$ versus left ventricular free-wall (LVF). RVA-right ventricular apex, RVF-right ventricular free-wall, RVOT-right ventricular outflow tract and septum-ventricular anterior septum.

Immunofluorescence findings

Four weeks after transplantation, MNC-derived cells, positively costained with BrdU and DAPI were observed in all cell-transplanted hearts (Fig. 2a), whereas no positive cells were found in sham operation hearts (Fig. 2b).

In MNC transplantation hearts, BrdU-labelled cells were widely dispersed throughout the cardiac layers of left and right ventricles, although more cells were found in the left ventricle (Fig. 3).

DISCUSSION

Direct intramuscular injection and selective intracoronary transplantation have been popularly adopted for cell transplantation to treat heart failure. After these procedures, however, the cells are mostly confined to a restricted anatomical region. In heart failure dominated by multiple scattered lesions, the efficacy of cell transplantation may be reduced owing to restricted distribution of infused cells. In the present study, we have first developed a method for cell transplantation through the root of the aorta. As demonstrated in this trial, infused cells were widely disseminated into the heart through bilateral coronary arteries, and global heart function was dramatically increased. Meanwhile, there was no intraprocedural rabbit death. Accordingly, cell transplantation through the root of the aorta has proved to be a safe and useful procedure for the treatment of heart failure, especially when there are multiple scattered lesions. In this study, more grafted cells were observed in the left ventricle than in the right. We hypothesize that this can most likely be attributed to different mechanisms of blood supply and level of damage induced by doxorubicin between left and right ventricles (Sacco *et al.* 2003).

In this study, two common PTCA balloons were employed to obstruct downstream blood flow in the aorta, yet incomplete occlusion of blood was found. Although distribution characteristics of infused cells could not be influenced, the treatment capability of cell transplantation would be partly reduced by escaping cells. Thus, we think that further improvement of heart function will be achieved if a custom balloon is used.

In conclusion, we have developed a novel method for efficient cell transplantation into the heart by infusion of cells through the root of the aorta. This method will be useful in disseminating cells globally into the heart, particularly when there are extensive lesions.

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