Therapeutic approach for global myocardial injury using bone marrow-derived mesenchymal stem cells by cardiac support device in rats

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Abstract

Bone marrow-derived mesenchymal stem cells (BMSCs) have been considered a promising therapeutic approach to cardiovascular disease. This study intends to compare the effect of BMSCs through a standard active cardiac support device (ASD) and intravenous injection on global myocardial injury induced by isoproterenol. BMSCs were cultured in vitro, and the transplanted cells were labeled with a fluorescent dye CM-Dil. Isoproterenol (ISO) was injected into the rats; 2 weeks later, the labeled cells were transplanted into ISO-induced heart-jury rats through the tail vein or ASD device for 5 days. The rats were sacrificed on the first day, the third day, and the fifth day after transplantation to observe the distribution of cells in the myocardium by fluorescence microscopy. The hemodynamic indexes of the left ventricle were measured before sacrificing. H&E staining and Masson’s trichrome staining were used to evaluate the cardiac histopathology. In the ASD groups, after 3 days of transplantation, there were a large number of BMSCs on the epicardial surface, and after 5 days of transplantation, BMSCs were widely distributed in the ventricular muscle. But in the intravenous injection group, there were no labeled-BMSCs distributed. In the ASD + BMSCs-three days treated group and ASD + BMSCs -five days-treated group, left ventricular systolic pressure (LVSP), the maximum rate of left ventricular pressure rise (+dP/dt), the maximum rate of left ventricular pressure decline (-dP/dt) increased compared with model group and intravenous injection group (P < 0.05). By giving BMSCs through ASD device, cells can rapidly and widely distribute in the myocardium and significantly improve heart function.

Keywords Global myocardial injury · BMSCs · Active cardiac support device (ASD) · Stem cell treatment · Epicardial delivery

1 Introduction

Cardiovascular diseases (CVD) have the highest mortality rate throughout the world (Naveed et al. 2020). Myocardial hypertrophy and fibrosis can cause the formation of cicatricial tissue in the heart, leading to the death of myocardium, which can affect heart function and finally result in heart failure (Naveed et al. 2018a; Opie et al. 2006). Therefore, the
prevention of myocardial fibrosis may slow the progression of heart failure. Recent studies have shown that mesenchymal stem cells (MSCs) can promote angiogenesis, inhibit cell apoptosis, and promote tissue regeneration (Bernstein and Srivastava 2012; Cai et al. 2015). Thus, MSC transplantation has become a promising alternative treatment to heart transplantation (Doppler et al. 2013; Forte et al. 2011). There are many kinds of cells used in transplanting, among these are bone marrow stem cells (BMSCs) which show superiority to other cells for they can differentiate into multiple cells and released the growth factors into the damaged myocardium which will improve the symptoms of heart failure and increase the blood flow of the infarcted area, thereby promoting myocardial regeneration (He et al. 2018; Heldman et al. 2014; Orlic et al. 2003; Sawa 2013). The safety and feasibility of BMSCs transplantation has been studied and documented by some clinical trials (Seth et al. 2010; Strauer 2010).

As a right donor cell for myocardial transplantation, MSCs are mostly injected through epicardium, endocardium with a catheter, intracoronary or intravenous, but they have some shortcoming that can’t be avoided (Campbell and Suzuki 2012; Fukushima et al. 2008). This study aims to investigate whether an innovative epicardial delivery method (Li et al. 2020a; Li et al. 2020b; Liu et al. 2020), active hydraulic ventricular attaching support system (ASD), can increase the storage and engraftment of stem cells and repair global myocardial injury.

The primary techniques for BMSC administration include 1. Intravenous infusion: This is the simplest way to transplant cells with the ability of multiple infusions. 2. Epicardial injection: BMSCs are injected into the myocardial infarction area when the thoracic surgery is performed. 3. Endocardial injection: Cells are inserted into the endocardium through a catheter. 4. Intracoronary injection: This method needs the application of the OTW balloon catheter to the infarcted coronary artery, then cells are sent through it to the ischemic myocardium (Dib et al. 2011; Hristov 2006; Strauer and Steinhoff 2011). However, current studies focus more on local delivery of stem cells in myocardial infarcted areas, but these local delivery methods will not be effective in global myocardium injury. Our research provided a novel approach to deliver BMSCs into the epicardial using ASD, which is a net cover made of silicone covering both ventricles of the heart. ASD can provide sufficient contact time between BMSCs and epicardium and can widely spread the cells.

In this research, we investigate whether the delivery of BMSCs by ASD can improve cardiac function after ISO-induced global myocardial injury. Compared with intravenous injection, the systemic delivery method can also deliver cells multiple times.

2 Materials and methods

2.1 Experimental animals

Zhejiang Laboratory Animal Center provided a total of 40 male Sprague-Dawley (SD) rats weighing from 220 to 250 g. During the experiment, animal handling and disposal conform to the criteria of the China Pharmaceutical University Institutional Animal Care and Use Committee. All animals were placed in the environment of free food and water for 1 week before the experiment. Isoproterenol was used to induce global myocardial injury, as described before, with little modification (Teerlink 1994). Briefly, isoproterenol was administered with saline to a solution of 85 mg/ml and injected into the rats at 170 mg/kg for two consecutive days. The control group was administered subcutaneously with 0.5 ml of saline. Forty rats were randomly divided into two groups, 8 rats were selected randomly as the (i) Control group, and the other rats were modeled with isoproterenol according to the method mentioned above. Two weeks later, the survived 30 rats were divided into 5 groups randomly, (ii) ISO group (model group), (iii) BMSC+ASD-one day treated group (iv) BMSC+ASD-three days treated group (v) BMSC+ASD-five days treated group, (vi) BMSC+IV treated group. BMSCs were given through the tail vein in IV-treated group and were delivered through the outside tube of ASD in the ASD-treated groups, once a day for 5 days.

2.2 Isolation of BMSCs

Three to four weeks old rats with the weight of 60-80 g were sacrificed. BMSCs were isolated from tibia and femur under sterile conditions, and cultured in low glucose DMEM (Glenview, USA) containing 10% FBS (Hyclone, USA) and 1% penicillin-streptomycin (Gibico, USA) in plastic culture flasks with the concentration of 1 × 10^7 at 37 °C in 5% CO_2 saturated wet incubator. Four days later, the whole culture solution was changed, the unattached cells were washed off, and the adherent cells propagated for 3 to 4 passages before transplantation. Then the culture solution was changed every 2 days. The cells grow to 80%–90% confluence, trypsinized (0.25% trypsin/1 Mm EDTA, Solarbio, China), and expanded in a ratio of 1:2.

2.3 Identification of BMSCs

Harvesting the third generation BMSCs, and analyzing them by flow cytometry. Trypsinize and prepare the single-cell suspension in phosphate-buffered saline (PBS) per 1 × 10^6 cells, 2.5 μg/ml, 2 antibodies, including FITC anti-rat CD45 antibody, APC anti-mouse/rat CD29 antibody (Biolegend, USA) were added and incubated on ice for 30 min in the dark. Cells were washed with 2 ml PBS twice and centrifugated at
350 rpms for 5 min. Flow cytometric analysis was performed, and the quantitative analysis results were obtained.

2.4 Labeling of BMSCs

To track the BMSCs after transplantation, cells were labeled with cell tracker CM-Dil (Invitrogen, USA). Cells were suspended at a density of $1 \times 10^6$/ml in the serum-free culture medium, then 5 μl cell labeling solution was added into the suspension with very gentle mixing. The labeled cells were incubated at 37 °C for 20 min, then washed twice with 2 ml PBS at 1000 rpms for 5 min. The labeled cells were observed under a fluorescence microscope and analyzed by flow cytometry, then cultured in expansion medium for transplantation.

2.5 Designing and manufacturing of ASD

Active hydraulic ventricular attaching support system (ASD) is a net cover made of silicone, a biocompatible material with a heart-shaped configuration consisting of flexible interconnected hollow tubules; it is surgically fixed to the outer wall of the heart and covers both ventricles of the heart providing physical support (Naveed et al. 2018c). ASD is manufactured as a previously described method (Naveed et al. 2018b). Briefly, the 3D printed wax model of ASD was made at first, and covered it with silicone evenly, then heated it at 100 °C for 1 h to melt the wax and obtained the pure silicone ASD (Fig. 1a, b, c).

2.6 Implantation of ASD

Two weeks after the last ISO injection, rats were sedated with 10% chloral hydrate by intraperitoneal injection (0.03 ml/kg). The small animal ventilator was used to keep breathing. After confirming the ventricular position, the pericardium was separated, and the heart was exposed. ASD was fixed on the surface of the heart with suture and covered both ventricles, and with a connection of silicone tube entered the chest wall from the second intercostal space, it pierced the skin from the back of the ear (Fig. 1d, e).

2.7 Implantation of BMSCs

The BMSCs labeled by CM-Dil were harvested and suspended at a density of $2 \times 10^5$/ml with PBS. Rats in intravenous injection group and ASD groups received $2 \times 10^5$ cells once a day for 5 days. In this study, the rats in the ASD group were sacrificed on 1, 3, and 5 days and the IV injection group was sacrificed on 5 days.

2.8 Cardiac performance measurement

Before sacrificing, the rats were anesthetized with 10% chloral hydrate (0.3 g/kg) by intraperitoneal injection. II electrocardiograph was used to monitor ECG continuously. In order to evaluate the left ventricular function, the right carotid cannula method was used, generally speaking, one end of the heparin saline (500 U/ml)-filled polyethylene tube was inserted into the left ventricle and the other end connected to the pressure...
sensor and then connected to the BL-420S biologic function experiment system, and the hemodynamic indexes of the left ventricle were measured including heart rate, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), the maximum rate of LV pressure rise (+dP/dtmax) and a maximum speed of LV pressure decline (-dP/dtmax).

2.9 Cardiac histopathology

After hemodynamic evaluation, the animals were sacrificed, and the heart was quickly removed. Washed in saline and cut into two transverse segments paralleling to the atrioventricular groove, then one was placed in 10% formalin for 24 h, and the other was stored at −80 °C in OCT. For Masson’s trichrome and hematoxylin and eosin (H&E) staining, the formalin segment was made into a wax block and cut into slices with a thickness of 5 μm. Digital pathology was used, and the extent of fibrosis was quantified by ImageJ software. The other part was made into a frozen section with a thickness of 5 μm, observing labeled cells by fluorescence microscope.

2.10 Statistical analysis

All experimental data were presented as mean ± SD and analyzed by statistical software for the social sciences (SPSS21.0). The LSD-q test to compare the values between the groups and the one-way analysis of variance (ANOVA) test was used between groups. P values <0.05 were considered statistically significant.

3 Results

In this experiment, three rats died after the injection of ISO in 24 h; one died after the ASD implant surgery. Therefore there were six rats left in each group.

3.1 Morphology and characterization of MSCs

After the cells were inoculated into the culture flask, the cells were round and of different sizes, suspended them in culture medium (Fig. 2a). The number of cells was increased at 72 h after the extraction, with the shape of round or spindle (Fig. 2b). After 14 days of culture, the cells increased in a large number, which was uniform spindle-shaped (Fig. 2c).

BMSCs were labeled with CM-Dil at 3 passages and detected by a fluorescence microscope before delivery (Fig. 2d). After the first passage of the labeled cells, there was no change in the cell morphology and the fluorescence intensity (Fig. 2e). The flow cytometry results showed CM-Dil labeling rate was >90% (Fig. 2f), and the isolated cells expressed the MSC marker CD29 but didn’t have the pan leukocyte marker CD45 (Fig. 2g).

3.2 Effects of BMSCs transplantation on heart function

In this experiment, there was no significant difference in heart rate. Two weeks after the last ISO injection. Compared with the ISO group, LVSP, LV + dP/dtmax, and LV-dP/dtmax (P < 0.05) were significantly increased in ASD-three days treated group and ASD-five days treated group. As compared to the BMSCs+iv group, the ASD-five days treated group also showed significantly increased LVSP and LV + dP/dtmax (P < 0.05) (Table 1, Fig. 3).

3.3 Distribution of CM-Dil labeled cells

After transplantation, labeled cells were observed in ASD + BMSCs -three days treated and ASD + BMSCs -five days treated groups, but not in other groups (Fig. 4).

The labeled cells in BMSCs+ASD-3 day’s group were mainly distributed on the epicardium, BMSCs were observed near the middle layer of the myocardium in BMSCs+ASD-5 days group. There were no BMSCs that could be found in the BMSCs+IV group.

3.4 Histopathological examination of heart tissues

We investigated the cardiac histopathology by H&E staining and Masson’s trichrome staining. In the group of rats undergoing myocardial injury, H&E staining showed a significant loss of myocardial structure and the infiltration of inflammatory cells (Fig. 5). Cardiac muscle fibers of the model group degenerated in BMSCs+ASD-1 day group, BMSCs+IV group (Fig. 5c, f). There were a large number of inflammatory cells infiltrated and distributed diffusely. Myofibrils were arranged neatly near endocardium on BMSCs+ASD-3 days group (Fig. 5d). In BMSCs+ASD-5 days group, myocardium was arranged tightly, and less inflammatory cells dispersed in the endocardium (Fig. 5e).

Masson’s trichrome staining showed a massive increase in the collagen fibers among the degenerating muscle fibers in the model group, BMSCs+ASD-1 day group, BMSCs+IV group (Fig. 6). After 5 days’ treatment, the collagen fibers decreased (P < 0.05), especially in the medium and endocardium of the myocardium.

4 Discussion

John et al. (1994) have confirmed that subcutaneous injection of ISO (> 85 mg/kg) can cause diffuse myocardial fibrosis after 2 weeks, and gradually lead to heart failure. Compared with ischemic myocardial infarction, this method can reflect the natural process of HF, which is similar to that in patients (Li et al. 2002).
In this study, we established the rat model of extensive myocardial injury by subcutaneous injection of ISO at a dose of 170 mg/kg for 2 consecutive days. The changes in hemodynamic parameters often used to evaluate heart function (Lee et al. 2009; Ren et al. 2018). Two weeks later, our results showed that in comparison with the control group, the left ventricular function reduced in the groups received ISO injection ($P < 0.05$).

| Groups            | HR       | LVSP mmHg | LVEDP mmHg | +dP/dt_{max} (mmHg/s) | -dP/dt_{max} (mmHg/s) |
|-------------------|----------|-----------|------------|-----------------------|-----------------------|
| control           | 380.83 ± 30.82 | 105.34 ± 4.06 | 17.18 ± 7.16 | 2566.94 ± 279.73 | -2528.8 ± 495.23 |
| model             | 423.67 ± 11.41 | 52.12 ± 9.56  | 11.45 ± 8.04 | 1378.91 ± 162.38 | -1317.87 ± 181.75 |
| BMSCs+ASD-1 day   | 421.17 ± 21.65 | 29.58 ± 4.96  | 13.84 ± 7.59 | 1062.22 ± 52.23 | -1019.61 ± 109.9 |
| BMSCs+ASD-3 days  | 415.16 ± 20.67 | 57.64 ± 11.47 | 13.13 ± 7.21 | 1794.03 ± 261.66 | -1741.13 ± 360.46 |
| BMSCs+ASD-5 days  | 425.5 ± 34.56 | 77.34 ± 15.03 | 10.44 ± 6.32 | 2082.03 ± 283.48 | 2093.75 ± 413.74 |
| BMSCs+iv          | 411.33 ± 51.89 | 57.16 ± 9.18  | 8.6 ± 5.70   | 1566.77 ± 287.43 | 1634.32 ± 500.77 |

Data are presented as mean ± SD. #significant difference vs. model group, ###P value <0.01, ##P value <0.05, *significant difference vs. BMSCs+iv group, **P value <0.01, *P value <0.05 (Each group n = 6). HR, Heart rate; LVSP, Left ventricular systolic pressure; LVEDP, Left ventricular end-diastolic pressure; +dP/dt_{max}, Maximum rate of left ventricular pressure rise; -dP/dt_{max}, Maximum rate of left ventricular pressure decline.

Table 1  The effects of BMSCs transplantation on HR, LVSP, LVEDP, and +dP/dt_{max}.
Bone marrow-derived mesenchymal stem cells (BMSCs) are multipotent stem cells that exist in the bone marrow and can differentiate into various mesodermal cells, such as bone cells, fat, and matrix tissue cells. BMSCs were cells that can secrete a variety of hematopoietic factors to support hematopoiesis and immune regulation ability (Tropel et al. 2004).

Fig. 3 Effects of BMSCs transplantation on left ventricular (LV) hemodynamic parameters in the experiments. #significant difference vs. model group, ###P value <0.01, ##P value <0.05, *significant difference vs. BMSCs+IV group, ***P value <0.01, *P value <0.05 (Each group n = 6). HR, Heart rate; LVSP, Left ventricular systolic pressure; LVEDP, Left ventricular end-diastolic pressure; +dP/ dt max, Maximum rate of left ventricular pressure rise; -dP/dt max, Maximum rate of left ventricular pressure decline

Fig. 4 BMSCs engraftment in myocardium. The CM-Dil labeled BMSCs were detected under fluorescence microscopy. a BMSCs+ASD-1 day group, b BMSCs+ASD-3 days group, c BMSCs+ASD-5 days group, d BMSCs+IV group, 40× magnifications, scale bar 100 μm
BMSCs can express a variety of surface antigens, but no specific surface antigens. According to reports, CD29, CD44, CD59 are positive for surface antigen expression, while hematopoietic stem cell markers CD45, CD14, and CD117 are negative. After 24 h of culture, only a few cells grew adherently, the spindle cells were scattered growth. 72 h later, the number of adhered cells increased, and grew into small pieces. In this experiment, CD29 and CD45 were selected, and it was found that adherent cells express CD29^+CD45^−, combining with the results of morphology, we confirmed that the isolated and cultured cells were BMSCs. BMSC therapy is a viable option for heart renovation. There are many ways to deliver BMSCs, but traditional methods of transportation always have some problems such as low storage rate, low cell survival rate, and inability to be administered multiple times (Hastings et al. 2015). Intravenous injection of BMSCs has been thought to be useful for diffuse myocardial disease, which is also the most common method in clinic, while other local delivery methods are mainly for myocardial infarction, and IV injection can achieve the purpose of multiple administrations which is similar to ASD delivery. Before engrafting in the infarcted myocardium, cells will go through the systemic circulation and receive the local signal of injured tissue (Barbash et al. 2003). In recent studies, epicardium delivery of BMSCs has been considered a promising method (Smart et al. 2012; Wei et al. 2015), which can not only avoid myocardial injury and inflammation caused by intravenous injection. ASD is a kind of device that can deliver the drug or cells into the epicardium improving delivery efficiency (Naveed et al. 2017; Yue 2018). Besides, it can provide physical support to prevent ventricular enlargement. Our objective is to compare the effectiveness of multiple BMSC deliveries through veins and ASD. In this experiment, the increase of LVSP, and ±dP/dtmax in BMSCs+ASD-3 days group and BMSCs+ASD-5 days group indicated that the cardiac contraction improved. In our previous research, ASD can not only deliver drugs but also provide pressure. For the BMSCs+ASD-1 day group, the decrease of these indicators may be caused by the thoracotomy. There was no difference in these indicators in the BMSCs+IV group compared with the model group. The results indicated that although IV injection was a more convenient delivery method, the treatment effect was not evident. According to another study, the cells would be caught by other organs such as the liver, lung, or spleen (Carr 2008). CM-Dil is a kind of lipophilic non-toxic live-cell dye that can track cells in vivo or vitro steadily (Staffend and Meisel 2011). Moreover, it can’t transfer to other unlabeled cells. The labeled cells could be injected into vivo to show the migration and differentiation of transplanted cells effectively. In this
 experiment, CM-Dil marked BMSCs were observed under a fluorescence microscope in the BMSCs+ASD group. In ASD + BMSCs groups, as the time and the frequency of transplantation increased, the cells were more widely distributed and gradually migrated to the endocardial as the transplants increased, which will be beneficial for extensive myocardial damage. Our previous study has confirmed that the delivery of fat-soluble fluorescent dyes through ASD was mainly distributed in the ventricles (Mikrani et al. 2020), while there was almost no distribution in lung, liver, and spleen.

In many cases of heart failure, increased content, and cardiac fibrosis can be detected and trigger a series of changes in ventricular function (Li et al. 2008; Whelan et al. 2010). The New York heart association (NYHA) reported that myocardial apoptosis in patients with heart failure of cardiac service III and IV accounted for 0.12%–0.70% of myocardial tissue, while that in normal myocardium accounted for only 0.001%–0.01% (Orogo and Gustafsson 2013). Masson’s trichrome is one of the primary methods for displaying fiber staining in tissues. It is mainly used for the differential analysis of collagen and smooth muscle and one of the classic methods for collagen fiber staining, dyed blue. In our experiment, the collagen deposition area increased significantly after two weeks of ISO injection. We also observed that compared with the model group and IV injection group, the myocardial collagen area and the perivascular collagen area of the left ventricular endocardium in the BMSCs+ASD-treated groups were significantly decreased (P < 0.01), indicating that the BMSCs grafted by the ASD can repair myocardial fibrosis effectively.

This study focused more on how long can the improvement of heart function occur and proved that the cells can implanted in the myocardial rapidly by ASD for it can extend administration time and expand contact area. From the distribution of labeled cells, we can find that after 5 days delivery of BMSCs by ASD, the cells survived more. BMSCs can survive in an ischemic and hypoxic environment, and the expression of some factors related to angiogenesis is also up-regulated. This also proves that cells can survive after entering the surface of the heart, and multiple delivery of cells is also to increase cell survive. The actual mechanism of BMSCs will be further studied.

5 Conclusions

In this experiment, we firstly provide a kind of cell delivery method for global myocardial injury. This study demonstrated ASD increased BMSCs distribution area and significant improvement in cardiac function compared with the IV injection
group. The results of this research indicate that multiple times the delivery of BMSCs by ASD is more effective for global injured myocardium. Further study should focus on integrating engrafted cells with the myocardium and enhancing cell survival by delivering with other substances.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

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