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Direct implantation versus platelet-rich fibrin-embedded adipose-derived mesenchymal stem cells in treating rat acute myocardial infarction

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ABSTRACT

Background: This study tested whether adipose-derived mesenchymal stem cells (ADMSC) embedded in platelet-rich fibrin (PRF) scaffold is superior to direct ADMSC implantation in improving left ventricular (LV) performance and reducing LV remodeling in a rat acute myocardial infarction (AMI) model of left anterior descending coronary artery (LAD) ligation.

Methods: Twenty-eight male adult Sprague Dawley rats equally divided into group 1 [sham control], group 2 (AMI only), group 3 (AMI + direct ADMSC implantation), and group 4 (AMI + PRF-embedded autologous ADMSC) were sacrificed on day 42 after AMI.

Results: LV systolic and diastolic dimensions and volumes, and infarct/fibrotic areas were highest in group 2, lowest in group 1 and significantly higher in group 3 than in group 4, whereas LV performance and LV fractional shortening exhibited a reversed pattern ($p < 0.005$). Protein expressions of inflammation (oxidative stress, IL-1β, MMP-9), apoptosis (mitochondrial Bax, cleaved PARP), fibrosis (Smad3, TGF-β), and pressure-overload biomarkers (BNP, MHC-β) displayed a pattern similar to that of LV dimensions, whereas anti-inflammatory (IL-10), anti-apoptotic (Bcl-2), and anti-fibrotic (Smad1/5, BMP-2) indices showed a pattern similar to that of LV performance among the four groups (all $p < 0.05$). Angiogenesis biomarkers at protein (CXCR4, SDF-1α, VEGF), cellular (CD31+, CXCR4+, SDF-1α+), and immunohistochemical (small vessels) levels, and cardiac stem cell markers (C-kit+, Sca-1+) in infarct myocardium were highest in group 4, lowest in group 1, and significantly higher in group 3 than in group 2 (all $p < 0.005$).

Conclusion: PRF-embedded ADMSC is superior to direct ADMSC implantation in preserving LV function and attenuating LV remodeling.

1. Introduction

Although the incidence of acute myocardial infarction (AMI) has recently been reported to be steady worldwide with an improved in-hospital mortality [1,2], the short-term and long-term mortality rates remain notably high [3,4], especially in the aged patients [4]. AMI-induced myocardial necrosis and the subsequent progressive left ventricular (LV) remodeling eventually lead to LV chamber dilatation and pump failure that accounts for the poor clinical outcomes [5–7].

Despite the wide acceptance of primary percutaneous coronary intervention and thrombolytic therapy as the gold standards in rescuing the ischemic/jeopardized myocardium [8–10], the loss of myocardium after AMI is always irreversible. Of paramount importance is that the severity of pump failure depends on the extent of myocardial loss and the degree of myocardial ischemia [5–7,11,12]. Maximizing the mass of viable myocardium after infarction through myocardial repair/regeneration of functional myocardium, therefore, is the ultimate therapeutic goal.

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Abundant studies from both experimental models and clinical trials have established the safety and effectiveness of stem cell therapy in improving heart function in the settings of ischemia-related LV dysfunction and myocardial infarction [13–18]. On the other hand, therapeutic success of stem cell treatment against AMI depends on several essential factors, including the type and number of stem cells, time point of stem cell treatment, autologous versus allogenic stem cell utilization, and the route of the stem cell transplantation. Interestingly, the most common method for stem-cell implantation in experimental studies is direct myocardial needle injection of cell suspensions [15,16,19,20]. Albeit simple, this procedure is associated with rapid cell loss from leakage through the injection site, needle-mediated direct tissue damage, or even heart perforation with uncontrollable hemorrhage due to infarct-related thinning and fibrosis of LV wall. For these reasons, the clinical applicability of direct myocardial injection of cell suspensions has its limitations. Accordingly, the development of a more suitable method for cell administration not only can avoid needle-related complications, but can also ensure more secure contact between the ischemic myocardium and the introduced cells to augment therapeutic outcome.

Growing evidence has suggested biological scaffolding with tissue engineering as a potentially applicable strategy for effective delivery of mesenchymal stem cells (MSC) for myocardial repair after myocardial infarction [21,22]. Application of this "patching" technique using autologous grafts embedded with autologous MSCs/endothelial progenitor cells (EPCs) may, therefore, improve LV function and suppress LV remodeling by at least two mechanisms: 1) myocardial regeneration from enhanced angiogenesis, paracrine effects, and possible myocardial differentiation from MSCs/EPCs, and 2) mechanical support from ventriculoplasty to reduce chamber size and, therefore, the wall stress according to the Laplace's law. Moreover, studies have recently shown that adipose tissue is a promising source of autologous stem cells [e.g., adipose-derived mesenchymal stem cell (ADMSC)] in the treatment of ischemia-related organ dysfunction [23,24]. Therefore, using a rodent AMI model, this study attempted to test the following hypotheses: 1) The scaffold made from platelet-rich fibrin (PRF), when patched on the infarct area, might preserve LV function and suppress LV remodeling; 2) Patching of infarcted myocardium with ADMSCs embedded in PRF (i.e., autologous ADMSC graft) may be superior to direct myocardial implantation of ADMSCs in improving LV function and ameliorating LV remodeling.

2. Materials and methods

2.1. Ethics

All animal experimental procedures were approved by the Institute of Animal Care and Use Committee at Chang Gung Memorial Hospital-Kaohsiung Medical Center (Affidavit of Approval of Animal Use Protocol No. 2010122405) and performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85–23, National Academy Press, Washington, DC, USA, revised 1996).

2.2. Animal grouping and isolation of adipose tissue for culturing adipose-derived mesenchymal stem cells

Pathogen-free, adult male Sprague–Dawley (SD) rats (n = 28) weighing 325–350 g (Charles River Technology, BioLASCO Taiwan Co. Ltd., Taiwan) were randomized and equally categorized into group 1 (sham controls receiving thoracotomy only, n = 7), group 2 (AMI induction only, through left coronary artery ligation, n = 7), group 3 (AMI + autologous ADMSC, n = 7), and group 4 (AMI + autologous ADMSC-embedded PRF scaffold, n = 7). The dosage of ADMSC (2.0×10^6) was chosen according to our previous studies with minor modifications [15,20,24].

Rats in groups 3 and 4 were anesthetized with inhalational 2% isoflurane 14 days before AMI induction for harvesting peri-epididymal adipose tissue. The procedure and protocol for the culture and identification of ADMSCs were described in our recent reports [24,25]. Briefly, the adipose tissue surrounding the epididymis was carefully dissected and excised. Then 200–300 μL of sterile saline was added to every 0.5 g of tissue to prevent dehydration. The tissue was cut into $<1\ mm^3$ pieces using a pair of sharp, sterile surgical scissors. Sterile saline (37 °C) was added to the homogenized adipose tissue in a ratio of 3:1 (saline: adipose tissue), followed by the addition of stock collagenase solution to a final concentration of 0.5 units/mL. The tubes with the contents were placed and secured on an orbital shaker and incubated with constant agitation for 60 ± 15 min at 37 °C. After 40 min of incubation, the content was triturated with a 25 mL pipette for 2–3 min. The cells obtained were placed back to the rocker for incubation. The contents of the flask were transferred to 50 mL tubes after digestion, followed by centrifugation at 600 ×*g*, for 5 min at room temperature. The fat layer and saline supernatant from the tube were poured out gently in one smooth motion or removed using vacuum suction. The cell pellet thus obtained was resuspended in 40 mL saline and then centrifuged again at 600 ×*g* for 5 min at room temperature. After being resuspended again in 5 mL saline, the cell suspension was filtered through a 100 μm filter into a 50 mL conical tube to which 2 mL of saline was added to rinse the remaining cells through the filter. The flow-through was pipetted to a 40 μm filter into a new 50 mL conical tube. The tubes were centrifuged for a third time at 600 ×*g* for 5 min at room temperature. The cells were resuspended in saline. Isolated ADMSCs were cultured in a 100 mm diameter dish with 10 mL DMEM culture medium containing 10% FBS for 14 days.

2.3. Preparation of PRF, ADMSC labeling, AMI induction, and patching

By day 14 after ADMSC isolation and culture, CM-Dil (Vybrant™ Dil cell-labeling solution, Molecular Probes, Inc.) (50 μg/mL) was added to the culture medium 30 min before AMI induction for ADMSC (1.0×10^6 cells per batch) labeling as previously reported [24,25]. At the same time, 3.0 mL of blood was drawn through cardiac puncture from rat and placed in an Eppendorff tube, followed by centrifugation (model 5415D; Eppendorf) at 400 g for 10 min at room temperature. The suspension of white jelly-like component, so-called "PRF scaffold", was collected after centrifugation (Fig. 1A, B, & C). The scaffold was then cut into two pieces each of which was placed at the bottom of an Eppendorff tube. Two batches of ADMSCs (0.5×10^6 each) previously labeled with CM-Dil were pipetted onto the two pieces of PRF in the Eppendorff tubes, respectively, before being centrifuged at 800 g for 20 min. This procedure helped in the embedment of ADMSCs into the PRF scaffold.

During the preparation of PRF scaffold and ADMSC grafts, all animals were anesthetized by inhalational 2.0% isoflurane and placed in a supine position on a warming pad at 37 °C. Under sterile conditions, the heart was exposed via a left thoracotomy. AMI was induced in group 2 to 4 animals by left coronary artery ligation 3 mm distal to the margin of left atrium with a 7–0 prolene suture. Regional myocardial ischemia was verified by observing a rapid color change from pink to dull red over the anterior surface of the LV and rapid development of akinesia and dilatation over the affected region. Rats receiving thoracotomy only without AMI induction served as sham controls (group 1).

For group 4 animals, ADMSCs were embedded into the PRF scaffolds through centrifugation of the scaffolds in an Eppendorf tube with ADMSCs placed on top of the graft so that the cells sank into the graft through the gravitational force of centrifugation. Care was then taken to place the upper side of the graft (i.e. the side with direct contact with ADMSCs during centrifugation) on the heart before securing the graft in place with interrupted 7/0 prolene sutures over the edge of the graft so that the embedded cells were in direct contact with the heart. Two pieces of ADMSC-embedded grafts were used to ensure maximum cell embedding during centrifugation and also to completely cover the infarct surface of LV myocardium to avoid undue tension of using a single graft that may restrict cardiac dilatation (Fig. 1D, E, & F). For group 3 animals, ADMSCs were directly implanted into the infarct region at four different points with two over the upper and two over the lower zones. After the procedure, the thoracotomy wound was closed and the animals were allowed to recover from anesthesia in a portable animal intensive care unit (ThermoCare®) for 24 h.

2.4. Functional assessment by echocardiography

All animals underwent transthoracic echocardiography under general anesthesia in supine position at the beginning and end of the study. The procedure was performed by an animal cardiologist blind to the experimental design using an ultrasound machine (Vevo 2100, Visualsonics). M-mode standard two-dimensional (2D) left parasternal-long axis echocardiographic examination was conducted. Left ventricular internal dimensions [end-systolic diameter (ESD) and end-diastolic diameter (EDD)] were measured at mitral valve and papillary levels of left ventricle, according to the American Society of Echocardiography leading-edge method using at least three consecutive cardiac cycles. LVEF was calculated as follows: $\text{LVEF}\ (\%) = [(\text{LVEDD}^3 - \text{LVEDS}^3) / \text{LVEDD}^3] \times 100\%$.

2.5. Western blot analysis of heart tissue

The procedure and protocol for Western blot analysis were based on our recent reports [20,23,24]. Briefly, equal amounts (50 μg) of protein extracts were loaded and separated by SDS-PAGE using acrylamide gradients. After electrophoresis, the separated proteins were transferred electrophoretically to a polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences). Nonspecific sites were blocked by incubation of the membrane in blocking buffer [5% nonfat dry milk in T-TBS (TBS containing 0.05% Tween 20)] overnight. The membranes were incubated with the indicated primary antibodies [Bax (1:1000, Abcam), cleaved poly (ADP-ribose) polymerase (PARP) (1:1000, Cell Signaling), Bcl-2 (1:200, Abcam), phosphorylated (p)-Smad3 (1:1000, Cell Signaling), p-Smad1/5 (1:1000, Cell Signaling), bone morphogenetic protein (BMP) 2 (1:5000, Abcam), transforming growth factor (TGF)-β (1:500, Abcam), interleukin (IL)-1β (1:1000, Cell Signaling), matrix metalloproteinase (MMP)-9 (1:3000, Abcam), IL-10 (1:5000, Abcam), CXCR4 (1:1000, Abcam), stromal cell-derived factor (SDF)-1α (1:1000, Cell Signaling), endothelial nitric oxide synthase (eNOS) (1:1000, Abcam), vascular endothelial cell growth factor (VEGF) (1:1000, Abcam), brain natriuretic peptide (BNP) (1:600, Abcam), myosin