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Original article

Implantation of cardiac progenitor cells using self-assembling peptide improves cardiac function after myocardial infarction

Masakuni Tokunaga [a,1], Mei-Lan Liu [a,1], Toshio Nagai [a], Koji Iwanaga [a], Katsuhisa Matsuura [b], Toshinao Takahashi [a], Masato Kanda [a], Naomichi Kondo [a], Pin Wang [a], Atsuhiko T. Naito [a], Issei Komuro [a,c,*]

[a] Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan
[b] Department of Cardiology and Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan
[c] Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

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ABSTRACT

Implantation of various types of cells into the heart has been reported to be effective for heart failure, however, it is unknown what kinds of cells are most suitable for myocardial repair. To examine which types of cells are most effective, we injected cell–Puramatrix™ (PM) complex into the border area and overlaid the cell–PM patch on the myocardial infarction (MI) area. We compared cardiac morphology and function at 2 weeks after transplantation. Among clonal stem cell antigen-1 positive cardiac progenitors with PM (cSca-1/PM), bone marrow mononuclear cells with PM (BM/PM), skeletal myoblasts with PM (SM/PM), adipose tissue-derived mesenchymal cells with PM (AMC/PM), PM alone (PM), and non-treated MI group (MI), the infarct area of cSca-1/PM was smaller than that of BM/PM, SM/PM, PM and MI. cSca-1/PM and AMC/PM attenuated ventricular enlargement and restored cardiac function in comparison with MI. Capillary density in the infarct area of cSca-1/PM was higher than that of other five groups. The percentage of TUNEL positive cardiomyocytes in the infarct area of cSca-1/PM was lower than that of MI and PM. cSca-1 secreted VEGF and some of them differentiated into cardiomyocytes and vascular smooth muscle cells. These results suggest that transplantation of cSca-1/PM most effectively prevents cardiac remodeling and dysfunction through angiogenesis, inhibition of apoptosis and myocardial regeneration.

1. Introduction

Cell therapy has been expected to be a new therapy for severe heart failure [1]. Various types of cell sources such as skeletal myoblasts, bone marrow mononuclear cells and mesenchymal stem cells have been examined in both basic and clinical studies [2]. Although there have been reports showing that transplanted cells improve the function of ischemic heart, the effects of cell therapy are variable among the clinical trials and it is still unknown how they show beneficial effects and what kinds of cells are suitable for myocardial repair [3,4]. A low rate of engraftment of transplanted cells, which stem from leakage and wash out during injections and massive death of cells, hampers the efficiency of cell transplantation within the host tissue [5,6]. Biological scaffolds are expected to circumvent the loss of grafted cells as they confer the three-dimensional microenvironment for the cells and support their survival, proliferation and function [7]. Many kinds of scaffolds have been designed at present; however, there are many unsolved issues in terms of their matching to engrafted cells, compatibility with host tissue, and clinical safety.

Self-assembling nanopeptides consist of alternating hydrophilic and hydrophobic amino acid residues that can adopt ß-sheet structures and forms a stable three-dimensional hydrogel consisting of >99.5% water depending upon pH, salt, and time [8]. The hydrogel has been shown to promote cell survival, proliferation and differentiation of many different cell types in culture, including neural stem cells [9], osteocytes [10], and endothelial cells [11]. When self-assembling peptides are injected into the tissue, they form a nanofiber network, which confers three-dimensional microenvironments for the endogenous cells, leading to angiogenesis and neuronal axon regeneration [12,13]. Self-assembling nanopeptides have been reported to be useful for protein delivery system. Charged assembled peptides can directly bind to platelet-derived growth factor (PDGF) or can be designed to conjugate with insulin-like growth factor-1 (IGF-1). Injection of PDGF or IGF-1 with self-assembling peptides after myocardial infarction has been reported to decrease infarct size, improve cardiac function and increase survival of co-transplanted neonatal rat cardiomyocytes [14,15]. These findings suggest that self-assembling nanopeptides are effective as a biological scaffold for various types of cells, which are currently investigated as candidates for transplantation to the diseased heart.

* Corresponding author. Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. Tel.: +81 43 226 2097; fax: +81 43 226-2557.
E-mail address: komuro-tky@umin.ac.jp (I. Komuro).
[1] Masakuni Tokunaga and Mei-Lan Liu equally contributed to this work.

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In this manuscript, we transplanted self-assembling nanopeptides with various types of cells such as bone marrow mononuclear cells (BM), skeletal myoblasts (SM), adipose tissue-derived mesenchymal cells (AMC) or clonal stem cell antigen-1 positive cardiac progenitors (cSca-1), into mouse hearts after producing myocardial infarction (MI) and determined the most effective cell sources to reduce infarct size and prevent cardiac dysfunction. In addition, we examined the potential mechanisms of beneficial effects of a complex of cardiac progenitors and self-assembling nanopetides.

2. Material and methods

2.1. Animals

Wild mice (C57Bl/6J, 10–12 weeks) were purchased from Japan SLC (Shizuoka, Japan). Adult GFP transgenic mice [16] were purchased from Japan SLC, Japan. All protocols were approved by the Institutional Animal Care and Use Committee of Chiba University Graduate School of Medicine and the Japanese Government Animal Protection and Management Law (no. 105).

2.2. Preparation of cells

cSca-1 are a cell line of cardiac progenitor cells established from adult mouse heart Sca-1 positive cells as described previously [17,18]. AMC were isolated from adult mice as described previously [19] with a few modifications. Briefly, white adipose tissues were digested at 37 °C in PBS with 2.5 mg/ml of dispase (Invitrogen, Carlsbad) for 45 min. After filtration through 25-μm filters and centrifugation, isolated AMC were suspended in Iscove's modified Dulbecco's medium (IMDM, Invitrogen) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin, and cultured on 1% gelatin-coated dishes. AMC after passages 3 to 5 were used. SM were isolated from the hind limbs of adult mice as described previously [20]. In brief, minced muscle tissues were digested in 0.05% trypsin-EDTA and cultured in F-10 medium (Invitrogen) with 20% horse serum and 2.5 ng/ml basic fibroblast growth factor (bFGF, Promega Madison) for 4 days. SM were expanded in the medium with 20% FBS and used within 2 to 3 passages. BM were harvested from adult mice. Mononuclear cells were subsequently separated using Histopaque 1083 (Sigma-Aldrich Japan, Tokyo, Japan) and suspended in IMDM supplemented with 10% FBS and subsequently used for cell transplantation. To obtain the growth curve of cSca-1, AMC and SM, the number of cultured cells was measured by Countess (Life Technology Corp.) at an appropriate time period. Green fluorescence protein (GFP)- and red fluorescence protein (RFP)-expressing retroviral stocks were generated as described previously [21]. cSca-1 were infected with the GFP- or RFP-expressing retroviral vector. Infected cells were selected for growth in the presence of 500 μg/ml of neomycin (Sigma-Aldrich) for 2 weeks. The efficiency of transfection of GFP and RFP was >95%.

2.3. Animal surgery and cell transplantation

The mice were anesthetized by intra-peritoneal injection of 50 mg/kg of sodium pentobarbital and ventilated with a volume-regulated respirator. MI was produced by ligation of the left anterior descending artery with a 10-0 Prolene suture. Puramatrix™ (PM; peptide sequence AcNRADARADARADARADA-CNH2, RAD16-I) was kindly provided by 3-D Matrix, Ltd. (Tokyo, Japan). PM was dissolved in 295 mM sucrose solution (1% W/V) and sonicated before use [22]. The cells were suspended in 295 mM sucrose solution (1% W/V) and mixed with 1% PM/sucrose solution at the ratio of 1:1 or 1:9. Thus the final concentration of PM in the cell mixture was 0.1% or 0.5%, respectively. Within 5 min after ligation of the left anterior descending artery, 10 μl of 0.1% PM–cell mixture containing 2×10^4 cells was directly injected into the border zone of the myocardium two times (Supplemental Figs. 1A and C). Subsequently, 20 μl of 0.5% PM–cell mixture containing 2×10^4 cells was disseminated onto the surface of the infarct area (Supplemental Figs. 1B and C). PM polymerized within minutes when it encountered neutral body fluid, which contains salt (Supplemental Fig. 1D). When cSca-1 were transplanted without PM, 10 μl of 295 mM sucrose solution containing 2×10^4 of cSca-1 cells was injected directly into the border zone of the myocardium two times. To neutralize the vascular endothelial growth factor (VEGF), cSca-1/PM with 100 ng/ml of goat anti-mouse VEGF antibody (R&D systems) or normal goat IgG (R&D systems) were injected.

2.4. Morphological examination and infarct size measurement

The hearts were fixed in 10% PBS buffered formalin (Wako, Osaka Japan), embedded in paraffin and several transverse sections (4 μm) were collected every 0.4 mm from the apex, mid and base. Fibrous infarct area was detected visually by Masson Trichrome staining. The lengths of scar and of noninfarcted muscle for both the endocardial and epicardial surfaces of each histological section were measured. The final infarct size was expressed in percentage as the average of the endocardial and epicardial lengths of scar of surface circumferences times one hundred [23]. For area at risk (AAR) and infarct size (INF) assessment, mice were anesthetized and the left anterior descending artery was re-occluded at 2 weeks after transplantation. Evan's blue dye (2.5%, Wako Japan) was injected from left ventricular apex to delineate the in vivo AAR. The heart was rapidly excised, frozen and sectioned into 2 mm transverse sections from the apex to base. Following defrosting, the slices were incubated at 37 °C with 2% triphenylte-trazolium chloride in phosphate buffer (pH 7.4) for 30 min, fixed n 10% formaldehyde solution and photographed with a digital camera (Leica Germany). AAR and INF were expressed as percentage of ventricle surface (AAR/LV) and AAR (INF/AAR), respectively.

2.5. Echocardiography

Echocardiography in spontaneous breathing mice was performed using a Vevo770 (Visual Sonics, Toronto, Canada) with a 25-MHz imaging transducer. Two-dimensional images and M-mode tracing were recorded from the parasternal long axis view at midpapillary level to determine the left ventricular internal diastole diameter (LVIDD) and left ventricular internal systolic diameter (LVISD). LV fractional shortening (FS) was calculated as $\%FS = [(LVIDD - LVISD)/LVIDD]\times100$.

2.6. Immunohistochemistry and cytochemistry

Paraffin-embedded heart tissues were sectioned at 4 μm thickness and stained overnight at 4 °C with anti-von-Willebrand factor (anti-vWF) (DAKO, Kyoto, Japan) and anti-alpha-smooth muscle cell actin (anti-SMA, clone 1A4; Sigma-Aldrich). Detection of antibodies was performed by using VECSTATIN ABC kit (Vector Laboratories Inc. CA) according to the manufacturer's instructions. Nuclei were stained with hematoxylin. For the detection of TUNEL positive cardiomyocytes and endothelial cells, fresh frozen sections (4 μm) were fixed with acetone for 5 min at 4 °C and then TUNEL staining was performed by using in situ Apoptosis Detection kit (TAKARA BIO Inc. Sigma, Japan) or TMR red (Roche Diagnostics Suisse) according to the manufacturer's instructions. The sections were subsequently stained with anti-alpha-sarcomeric actinin (anti-SA) (clone EA-53; Sigma-Aldrich) and anti-CD31 (clone MEC 13.3; BD Biosciences CA) primary antibodies and then with FITC or Cy3 conjugated secondary antibodies (Jackson Immuno Research Laboratory, MO). For the detection of transplanted GFP- or RFP-expressing cSca-1, fresh frozen sections were fixed with 4% paraformaldehyde and double-stained with anti-