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Longitudinal monitoring adipose-derived stem cell survival by PET imaging hexadecyl-4-^{124}I-iodobenzoate in rat myocardial infarction model

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ABSTRACT

This study aims to monitor how the change of cell survival of transplanted adipose-derived stem cells (ADSCs) responds to myocardial infarction (MI) via the hexadecyl-4-^{124}I-iodobenzoate (^{124}I-HIB) mediated direct labeling method in vivo. Stem cells have shown the potential to improve cardiac function after MI. However, monitoring of the fate of transplanted stem cells at target sites is still unclear. Rat ADSCs were labeled with ^{124}I-HIB, and radiolabeled ADSCs were transplanted into the myocardium of normal and MI model. In the group of ^{124}I-HIB-labeled ADSC transplantation, in vivo imaging was performed using small-animal positron emission tomography (PET)/computed tomography (CT) for 9 days. Twenty-one days post-transplantation, histopathological analysis and apoptosis assay were performed. ADSC viability and differentiation were not affected by ^{124}I-HIB labeling. In vivo tracking of the ^{124}I-HIB-labeled ADSCs was possible for 9 and 3 days in normal and MI model, respectively. Apoptosis of transplanted cells increased in the MI model compared than that in normal model. We developed a direct labeling agent, ^{124}I-HIB, and first tried to longitudinally monitor transplanted stem cell to MI. This approach may provide new insights on the roles of stem cell monitoring in living bodies for stem cell therapy from pre-clinical studies to clinical trials.

1. Introduction

Myocardial infarction (MI) occurs when blood supply reduced to a part of heart tissue, and is the leading cause of mortality and morbidity in developed country [1–3]. MI induced loss and necrosis of resident cardiomyocytes, and the injured myocardium replaced with scar tissue by fibrosis to maintain structural rigidity. Stem cell therapy shows promise in patients with heart disease, including in those with acute MI and chronic ischemia. Various types of stem cells can be isolated from different tissues such as bone marrow, skin, amniotic fluid, and adipose tissue [4–8]. In particular, adipose-derived stem cells (ADSCs) have been a focus of study due to their general abundance, easy cell isolation, and ability to proliferate and differentiate [9–11]. Injected ADSCs can be engraft and differentiate into cardiomyocytes and endothelial cells in MI model [9], and ADSCs therapy for MI repair has shown improvement on cardiac function through elevation of angiogenesis via paracrine factor [12], and cardiac remodeling attenuation [13]. To date, numerous studies including preclinical study and clinical trials have demonstrated that transplantation of circulating progenitor cells (CPCs) or mesenchymal stem cells can recover

Abbreviations: ADSCs, adipose-derived stem cells; CT, computed tomography; DMSO, dimethyl sulfoxide; ^{18}F-FDG, 2-^{18}F-fluoro-2-deoxy-D-glucose; ^{18}F-HFB, hexadecyl-4-^{18}F-fluorobenzoate; ^{124}I-HIB, hexadecyl-4-^{124}I-iodobenzoate; HPLC, high performance liquid chromatography; MI, myocardial infarction; PET, positron emission tomography; ^{99m}Tc-HMPAO, ^{99m}Tc-hexamethyl propylene amine oxime; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling.

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regional perfusion and improve cardiac function in the myocardial infarction [14–16]. However, the monitoring of the fate of transplanted stem cells at target sites is poorly understood. Although, histological evaluation can provide the information about effect on stem cell therapy, but they cannot monitor longitudinal change for transplanted stem cells. Thus, serial monitoring the fate of transplanted stem cells for cardiac repair is an important part of regenerative medicine.

Positron emission tomography (PET) provides high sensitivity, good spatial resolution, and cell tracking imaging for transplanted stem cells [17]. Therefore, PET can be applied for a noninvasive imaging technique to monitor for longitudinal stem cell tracking.

In this study, we first tried to monitor the fate of the transplanted stem cells with PET using ^{124}I-HIB for 9 days in normal and MI model.

2. Materials and methods

2.1. Cell isolation and characterization

ADSCs were isolated from male Sprague–Dawley (SD) rats (250 ± 10 g, Narabio, Seoul, Korea) euthanized via carbon dioxide (CO_2) inhalation. Visceral fat encasing the stomach and intestine was dissected and minced to 1–3 mm. The isolated tissue was dissociated for 15 min at 37 °C using 0.1% (w/v) collagenase type I (Worthington Biochemical Corp., Lakewood, NJ). The solution was passed through 70 μm nylon mesh, neutralized using Dulbecco's Modified Eagle's Medium (DMEM) (WELGENE Inc., Daegu, Korea) with 10% (v/v) fetal bovine serum (FBS) (JRScientific, Inc., Woodland, CA), and centrifuged at 250×*g* for 5 min. The cell pellet was re-suspended in DMEM (WELGENE Inc.) containing 10% (v/v) FBS (JRScientific, Inc.) and 1% (v/v) penicillin/streptomycin solution. Cultures were maintained in a 37 °C incubator with 5% CO_2, and the medium was changed every 3 days.

Expression of stem cell-specific surface markers using fluorescein isothiocyanate (FITC)-conjugated CD44 (LSBio, Seattle, WA), phycoerythrin (PE)-conjugated CD90, PE-conjugated CD31, and FITC-conjugated CD45 antibodies (eBioscience, Inc., San Diego, CA) was evaluated with a FACSCalibur flow cytometer (BD Bioscience, San Jose, CA). Isotype control antibodies were used as a negative control.

2.2. Radiochemical synthesis and in vitro study

The radioactive cell labeling agent using in this study, hexadecyl-4-tributylstannylbenzoate, is covered by the patent (publication number: WO 2010074532 A2). ^{124}I-NaI was produced at the Korea Institute of Radiological & Medical Sciences (KIRAMS) via 50 MeV cyclotron irradiation. ^{124}I-NaI (20–92 MBq)-added 50 μL 1 N HCl was added to a mixture of hexadecyl-4-tributylstannylbenzoate (50 μg) in 50 μL of ethyl acetate and 50 μL of 3% H_2O_2, respectively. The mixture reacted for 10 min at room temperature (RT), and then the reaction was terminated by adding 100 μL of saturated $NaHSO_3$. The radioactive ^{124}I-HIB was purified through high performance liquid chromatography (HPLC) (Luna C8 column, 5 μm, 4.6 × 50 mm, mobile phase 95% acetonitrile/H_2O, flow rate of 1 mL/min). The collected pure ^{124}I-HIB was completely dried under a vacuum and dissolved in 20% dimethyl sulfoxide (DMSO)/phosphate buffered saline (PBS) solution for subsequent cell labeling studies. The radiolabeling yield and purity were determined with radio-thin layer chromatography (TLC) using a mobile phase of hexane: EtOAC (20:1 v/v) on a silica plate.

A solution of ^{124}I-HIB (6.3–22.2 MBq, 200 μL) in 20% DMSO/PBS or 2-^{18}F-fluoro-2-deoxy-D-glucose (^{18}F-FDG) (37–74 MBq, 200 μL) was added to a suspension of 5 × 10^6 rat ADSCs in 1 mL PBS, and the mixture was incubated at 37 °C for 1 h. After centrifugation (250×*g*, 5 min), the supernatant was removed, and the cells were washed twice with PBS. The radioactive content of the isolated pellet and supernatant was measured to calculate radiolabeling efficiency using a radioisotope calibrator (CRC®-127R; Capintec, Inc., Ramsey, NJ). This procedure was repeated three times to ensure accurate and efficient cell labeling.

Cell viability of ^{124}I-HIB-labeled ADSCs (n = 3) was determined via the trypan blue dye exclusion test. Leakage ratio of ^{124}I-HIB-labeled ADSCs (9.3–18.5 MBq) (n = 3) in culture medium was investigated by checking radioactivity in the supernatant and cell pellets at various times for 24 h in vitro. Also, the release of ^{124}I-HIB (n = 3) from cell death by distilled water (D.W.) treatment was calculated from the radioactivity ratio of the supernatant and cells.

2.3. Differentiation of ^{124}I-HIB-labeled ADSCs

^{124}I-HIB-labeled ADSCs (3.7–4.0 MBq) were differentiated into osteogenic, chondrogenic, and adipogenic lineages using a STEMPRO® Osteogenesis, Chondrogenesis, and Adipogenesis Differentiation Kit (Gibco, Carlsbad, CA). Each differentiation procedure was performed according to the manufacturer's instructions.

After differentiation, the each cell was fixed with 4% paraformaldehyde for 30 min for the staining procedure. Alizarin Red S, Alcian Blue, and Oil Red O staining were used to investigate osteogenic, chondrogenic, and adipogenic differentiation, respectively. Cardiomyogenic differentiation was processed as described by Carvalho et al. [18].

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA from cardiomyogenic induced radiolabeled ADSCs and rat heart were extracted using TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH). Five microgram of total RNA were reverse-transcribed using SuperScript III Reverse Transcriptase (Invitrogen, Grand Island, NY) and random hexamers to generate cDNA. GATA binding protein 4 (GATA-4), NK2 transcription factor related locus 5 (Nkx2-5), ventricular myosin light chain type 2 (MLC-2v), alpha-myosin heavy chain (α-MHC) and β-actin expression were analyzed. Primers for RT-PCR were shown in Supplementary Table 1. After a denaturation phase of 5 min at 94 °C, amplification was performed at an annealing temperature of 56–65 °C for 30 s for 30–35 cycles, then elongation performed at 72 °C for 5 min.

2.5. MI induction and cell transplantation

Female SD rats (250 ± 10 g, Narabio) were used, and the animals were anesthetized with 2% isoflurane (Foran, Choongwae Pharma Co., Seoul, Korea), intubated and maintained on a ventilator. The animals underwent left thoracotomy, and a MI was induced by permanent ligation of the left anterior descending coronary artery. Cell transplantation performed by single intramuscularly injection at the myocardium. ^{124}I-HIB-or ^{18}F-FDG-labeled ADSCs were suspended in PBS (5 × 10^6 cells/50 μL) and kept on ice until transplantation. The ^{124}I-HIB-labeled (1.18–1.48 MBq, n = 3) or ^{18}F-FDG-labeled ADSCs (1.18–1.48 MBq, n = 2) were intramuscularly injected to left myocardium of the normal model. The ^{124}I-HIB-labeled (1.18–1.48 MBq, n = 4) or ^{18}F-FDG-labeled ADSCs (1.18–1.48 MBq, n = 2) were transplanted into the left myocardium, at the infarct site.