In vivo cell tracking imaging of hexadecyl-4-[$^{123, 124}$I]iodobenzoate labeled adipose derived stem cells (ADSCs) in rat heart

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1. Introduction

Monitoring of transplanted stem cells for cardiac repair is important part in regenerative medicine. Direct cell labeling techniques using [^{18}F]FDG, [^{64}Cu]PTSM and [^{99m}Tc]-HMPAO have been developed for *in vivo* imaging [1,2]. Especially, ^{18}F-labeled derivates have been widely used for direct labeling agent [3]. But the ^{18}F has short half life ($T_{1/2}$=~2 h), thus this imaging agent has limitation of *in vivo* imaging. We used ^{123}I or ^{124}I which has relative long half life, to track the transplanted stem cells for a long-term imaging. This study is aimed to track the transplanted adipose derived stem cells (ADSCs) in rat heart using hexadecyl-4-[$^{123, 124}$I]iodobenzoate ([$^{123, 124}$I]HIB) mediated direct labeling method *in vivo*.

2. Methods and Results

2.1 Isolation of adipose derived stem cells (ADSCs)

ADSCs were isolated from adult rat Sprague-Dawley rats euthanized by CO_2 inhalation. Visceral fat encasing stomach and intestine was aseptically dissected and minced by 1-3 mm size with sterilized surgical blade. Isolated tissue was enzymatically dissociated for 15 min at 37℃ using 0.1% (w/v) collagenase type I. The solution was passed through 70 ㎛ nylon mesh to remove undissociated tissues, then neutralized using DMEM including 10% (v/v) fetal bovine serum (FBS) and centrifuged at 250g for 5min. The stromal cells pellet was resuspended in DMEM containing 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin solution. Cultures maintained in a 37℃ incubator with 5% CO_2 and medium was changed every 3 days.

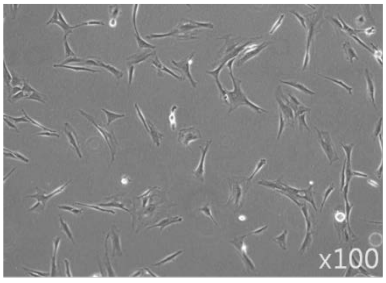

Fig.1. Microscopic image of adipose derived stem cells (Magnification: x100).

2.2 Flow cytometry (FACS) analysis of isolated ADSCs

Expression of stem cell specific surface markers including CD44H and CD90, was investigated by flow cytometry (FACS) analysis. For negative markers of ADSCs, CD31 and CD45, were also investigated.

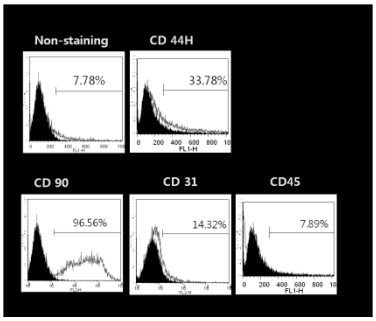

Fig. 2. The cell surface protein profile of ADSCs was analyzed by FACS. CD44H and CD90, positive markers of stem cells were highly expressed in ADSCs, while negative for CD31, CD45 such as endothelial and hematopoietic markers.

2.3 Cell labeling with [$^{123, 124}$I]HIB

A solution of 200 μL of [$^{123, 124}$I]HIB in 20% DMSO/PBS was added to a suspension of 0.5 million rat ADSCs in 1 mL serum-free media and the mixture incubated at 37 ℃ for 1 h. After centrifugation (250 x g, 5 min), the supernatant was removed and the cells were washed with twice with PBS. The radioactivity content of the isolated cell pellet and supernatant was measured using a dose calibrator.

Table I: Radiolabeling & cell labeling efficiency

	[^{123}I]labeled ADSCs	[^{124}I]labeled ADSCs
Radiolabeling yield	99.46%	97.39%
Radiochemical purity	98.07%	98.09%
Cell labeling efficiency	55%	50%

2.4 In vitro stability and cell viability.

Cell viability following radio-labeling was determined by trypan blue exclusion test for 24 h *in vitro*. Leakage ratio of [^{123}I]HIB labeled ADSCs was investigated by activity check in culture medium for 24 h *in vitro*.

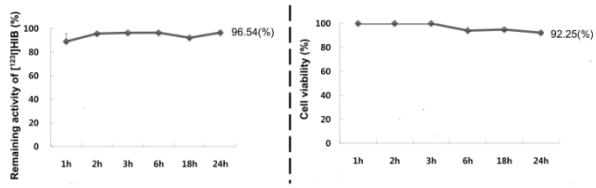

Fig. 3. *In vitro* stability (left) and cell viability (right) of [^{123}I]HIB labeled ADSCs for 24 h.

2.5 Cell transplantation

Sprague-Dawley rats (300-350g) were anesthetized with 2% isoflurane and intubated with a 16-gauge angiocath for ventilation. Unlabeled and [$^{123,\ 124}$I]HIB labeled ADSCs were harvested by trypsin treatment and resuspended in serum-free media at a cell concentration of 5x10^6 cells/100μL. The harvested cells were kept on ice until transplantation. The heart was exposed by thoractomy and the cells were intramuscularly injected at left myocardium. The chest was closed with 4-0 silk suture and the animal was allowed to recover.

2.6 In vivo imaging of [$^{123,\ 124}$I]HIB-labeled cells in rat myocardium.

In vivo monitoring of transplanted cells was followed each for 60 and 30 min by imaging using small animal single photon emission computed tomography (SPECT)/computed tomography (CT) and positron emission tomography (PET)/CT. Subsequently, SPECT/CT imaging was performed for 5 days and PET/CT imaging was performed for 18 days post transplantation, respectively. Through longitudinal quantification of cell survival, on the guess that initial transplanted cells were 100%, 84.1% of transplanted [^{123}I]HIB labeled ADSCs remained in rat myocardium at day 2, and 23.83% of transplanted [^{124}I]HIB labeled ADSCs remained in rat myocardium at day 9.

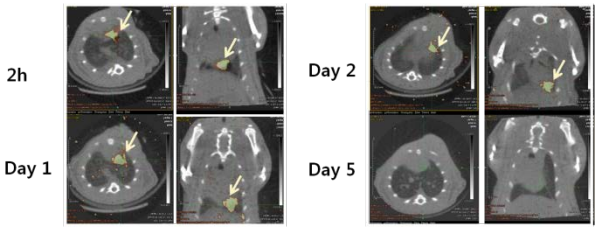

Fig. 4. Animal SPECT/CT image of transplanted [^{123}I]HIB labeled ADSCs in rat myocardium.

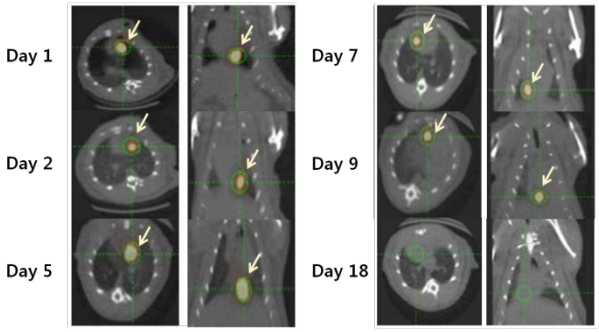

Fig. 5. Animal SPECT/CT image of transplanted [^{124}I]HIB labeled ADSCs in rat myocardium

3. Conclusions

[$^{123,\ 124}$I]HIB was efficiently labeled to ADSCs and radio-labeled ADSCs was successfully engrafted in cardiac muscle and possible monitoring of transplanted cells for 2 days ([^{123}I]HIB) and 9 days ([^{124}I]HIB). These results suggested that [^{123,124}I]HIB can be used as direct labeling agent for early cell tracking of transplanted stem cells

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