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Technical note

MR tracking of transplanted glial cells using poly-L-lysine-CF_3

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Abstract

Magnetic resonance (MR) imaging using super-paramagnetic iron oxides (SPIOs) is a powerful tool to monitor transplanted cells in living animals. Since, however, SPIOs are negative contrast agents, positive agents have been explored. In this study, we examined the feasibility of FITC-labeled poly-L-lysine-CF_3 (PLK-CF_3) using glial cells. FITC-labeled PLK-CF_3 was easily internalized by neuroblastoma cells and glia as adding it into culture medium. No toxicity was seen at the concentration of less than 80 μg/ml. MR images positively detected labeled cells transplanted in the brain of living mouse. The results indicate that FITC-labeled PLK-CF_3 is a useful positive contrast agent for MR tracking.

Keywords: Glia; Cell therapy; Molecular imaging; Magnetic resonance; Transplantation

1. Introduction

Neural cell transplantation and glial cell therapy have received some attention for the treatment of neurological disease, but few non-invasive techniques exist for monitoring the cells after administration. Magnetic resonance (MR) tracking is one of the methods to visualize transplanted cells in living animals (Bulte et al., 2002; Hoehn et al., 2002; Modo et al., 2002; Frank et al., 2002). Since native cells are not detected by MR images, it is necessary to label cells with a contrast agent for MR tracking. The most popular agents for MR tracking are super-paramagnetic iron oxides (SPIOs) (Frank et al., 2002). MR images using SPIOs display a high sensitivity (Toyoda et al., 2004; Song et al., 2005). Since, however, SPIOs are negative contrast agents, they reduce MR signals at their surrounding area and remove MR information obtained from other sources. Therefore, positive contrast agents have been explored.

ncerning positive contrast agents, there are several methods proposed. Gadolinium is often used (Modo et al., 2005; Vuu et al., 2005). However, gadolinium may be toxic and be retained in tissues for a long time after cell death. Cunningham et al. have tried to use SPIOs as a positive contrast agent using a new measuring method (Cunningham et al., 2005). SPIOs combined with fluorescent materials were also used (Hauger et al., 2006). Recently, perfluoropolyether has been reported as a positive contrast agent (Ahrens et al., 2005). However, it is difficult to detect perfluoropolyether by histochemistry. We designated a novel positive contrast agent based on peptide, poly-L-lysine-CF_3 (PLK-CF_3) labeled with FITC for MR microscopy. In this study, we examined the feasibility of FITC-labeled PLK-CF_3 using glial cells.

2. Materials and methods

2.1. Synthesis of PLK-CF_3 and its ^{19}F NMR spectra

For the synthesis of PLK-CF_3, 100 mg of poly-L-lysine hydrobromide (M_W 1000–4000) was dissolved in a mixture of water (0.5 ml) and methanol (0.5 ml). The mixture was added by 0.5 ml of methanol solution containing 72 mg of

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4-(trifluoromethoxy)benzyl bromide and 130 mg of solid potassium carbonate. The mixture stirred for 19 h at room temperature. Fifteen ml of ethanol was added to the mixture and the insoluble material removed by filtration. The mixture was added by 1 ml of 1 mM ethanolic hydrogen chloride was added to the filtrate and the mixture concentrated to about 2 ml. The required PLK-CF_3 was precipitated out by adding 50 ml of diethyl ether to the solution.

For labeling of PLK-CF_3 with fluorescein isothiocyanate (FITC), a mixture of PLK-CF_3 (1 mol), FITC (0.1 mol) and tributylamine (1.2 mol) in methanol was stirred for 5 h at room temperature. Addition of diethyl ether to the mixture gave the PLK-CF_3 combined with the fluorescent group.

2.2. Analysis fluorescent labeling of culture cells

Mouse astrocyte (C8-D1A, astrocyte type I clone), microglia cell lines (EOC 13.31) and PC12 cells were purchased from ATCC (Manassas, VA, USA). PC12 cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, UK) with 4 mM L-glutamine, 1.5 g/l sodium bicarbonate and 4.5 g/l glucose containing 10% fetal bovine serum (FBS, Gibco, UK), 5% horse serum (Gibco, UK), 1% penicillin/streptomycin mixture (Nacalai Inc., Osaka, Japan). The same medium without the horse serum was used for astrocytes. Microglia were prepared in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 20% Ladmac supernatant (ATCC), and 1% penicillin/streptomycin mixture. Glia and PC12 cells were grown with 80% confluency at 37 °C, 5% CO_2.

Immediately before use, we solved FITC-labeled PLK-CF_3 with sterilized distilled water at a concentration of 8 mg/ml. And we added the solution into the culture medium in culture dishes. The final concentration of PLK-CF_3 varied from 5 to 160 μg/ml. The incubation time also varied from 2 to 48 h.

After the incubation with FITC-labeled PLK-CF_3, culture dishes were gently washed three times with 10 mM phosphate-buffered saline (PBS), pH 7.4, and then fixed for 10 min with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, at room temperature. After washing with PBS (−), fluorescent signals were observed by a confocal microscopy (LSM510 META, Germany) or fluorescent microscopy (IX 70, OLYMPUS Co., Tokyo, Japan). The images were analyzed by the Meta Morph imaging system (Universal Imaging Co., PA, USA).

The labeling intensity was calculated using the following formula; labeling intensity = fluorescent intensity × labeling area.

For measuring cell toxicity, we employed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Denizot and Lang, 1986). In brief, cells (10^4 cells per well) were grown in 96 wells. After adding PLK-CF_3, for 24 h in culture medium at various concentrations, cells were washed with PBS (−) and applied to MTT assay.

2.3. MR imaging of PLK-CF_3 in the brain of living mouse

The experimental procedures were approved by the Committee on Animal Care of the Shiga University of Medical Science. Four male ICR mice (40–50 g) were used. Glial cells were labeled for 24 h with 80 μg/ml of FITC-labeled PLK-CF_3. After washing cells with PBS (−), cells were incubated for 10 min with 1.25% trypsin (Nacalai Inc., Osaka, Japan) at 37 °C. Cells were recovered into Falcon tube and added by the same volume of culture medium containing FBS to stop the reaction. Labeled cells were collected by the centrifugation for 2 min at 180 × *g*. Then, we formed 200 μl of Cellmatrix (Nitta-gelatin Co., Osaka, Japan) containing 8×10^6 of labeled cells. Under an anesthesia with sodium pentobarbital (50 mg/ml, i.p.), the left cranial bone of mice was opened. The left parietal cortex was defected with a drill, and the Cellmatrix containing labeled cells covered the lesioned area.

The magnetic resonance (MR) images were acquired with a 7 T Unity Inova MR scanner (Varian, Palo Alto, CA). A surface coil 20 mm in diameter, which can be tuned to both ^1H and ^{19}F frequencies (300 and 282 MHz), was used for the signal acquisition. First, a gradient echo ^1H image of mouse brain was acquired in the axial plane with 100 ms repetition time (T_R), 5 ms echo time (TE), 45° flip angle, 1 mm slice thickness, 35 mm × 35 mm field of view (FOV) and 256 × 256 matrices. Subsequently, ^{19}F chemical shift imaging (CSI) data was acquired in the same 35×35 mm^2 FOV, 50 ms T_R and 2048 acquisitions for 8 × 8 phase encoding steps. The total acquisition time was 110 min. The raw data was processed by 3D-Fourier transformation with zerofilling and converted to 32 × 32 spectral data sets. The FITC-labeled PLK-CF_3 image was constructed by extracting the signal intensities of FITC-labeled PLK-CF_3 in individual pixels. During the *in vivo* NMR measurement, general anesthesia was maintained with intermittent infusion of sodium pentobarbital through a polyethylene tube inserted intraperitoneally.

3. Results

Fig. 1A and B shows the structure of FITC-labeled PLK-CF_3 and its ^{19}F NMR spectrum, respectively. In our FITC-labeled PLK-CF_3, half of the amino groups were 4-(trifluoromethoxy)benzylated and 10% of the amino groups were combined with FITC. The ^{19}F NMR spectrum showed a sharp peak at *δ*-59.3 ppm (Fig. 1B).

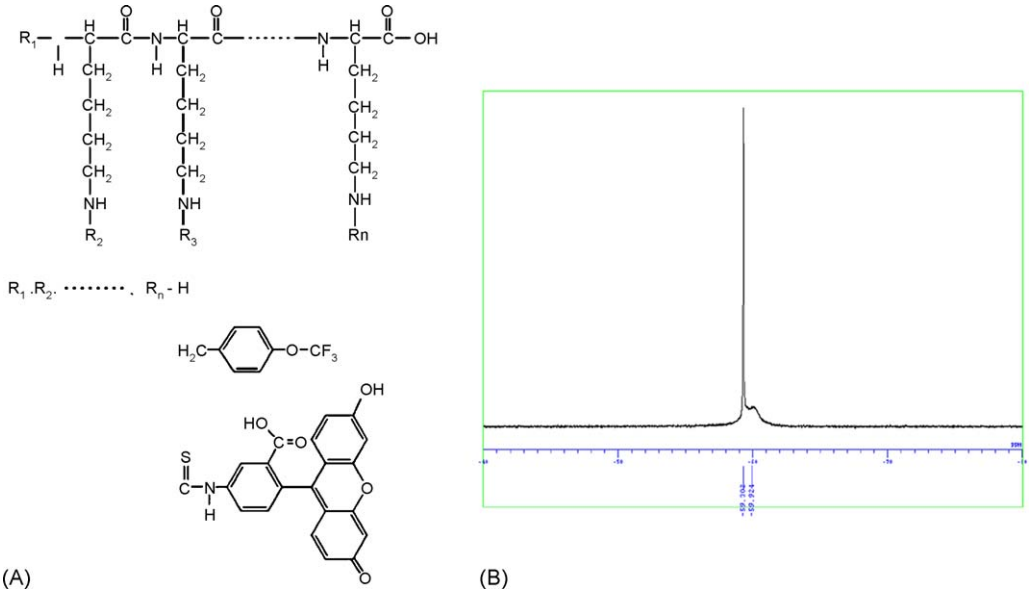

Fig. 1. Structure (A) and ^{19}F NMR spectrum of FITC-labeled PLK-CF_3 (B). (A) Fifty percent of the amino group (R) is 4-(trifluoromethoxy)benzylated. Ten percent of the amino group is replaced by FITC. (B) A sharp peak at *δ*-59.3 ppm and a broad peak at *δ*-59.9 ppm are seen using C_6F_6 as the external standard.