Novel Positive-Charged Nanoparticles for Efficient Magnetic Resonance Imaging of Islet Transplantation

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Significant graft loss immediately after islet transplantation occurs due to immunological and nonimmunological events. Magnetic resonance imaging (MRI) is an attractive potential tool for monitoring islet mass in vivo. Although an efficient uptake of MRI contrast agent is required for islet cell labeling, commercially available magnetic nanoparticles are not efficiently transduced into cells. In this study, we developed six kinds of novel magnetic iron oxide nanoparticles, which are electrically charged by cationic end-group substitution of dextran. Each of the nanoparticles consisted of a small monocrystalline, superparamagnetic iron oxide core that is stabilized by a cross-linked aminated dextran coating to improve stability. We also used three different commercially available nanoparticles for controls. The labeling efficiency of the novel nanoparticles was evaluated, and the feasibility of the imaging by MRI was assessed. The positive-charged nanoparticles were transduced into a β -cell line, MIN6 cells, but not three commercially available nanoparticles. MRI showed a marked decrease in signal intensity on T1- and T2-weighted images at the site of the labeled cells in vitro. These data suggest that novel positive-charged nanoparticles could be useful MRI contrast agents to monitor islet mass after transplantation.

Key words: Cationic nanoparticles; Islet transplantation; Magnetic resonance imaging (MRI); In vivo imaging; Dextran

INTRODUCTION

The Edmonton protocol has markedly improved the outcome for pancreatic islet transplantation as a therapeutic strategy for type 1 diabetes (15,18,28). However, the insulin independence rate after islet transplantation from a single donor remains low. The low frequency of islet grafting results from poor islet recovery from donors (23) and early islet loss during the first hours after grafting (6,30). Potential causes of failure of islet transplants include failure of initial engraftment, instant blood-mediated inflammatory reaction, allo- or auto-immune responses, glucotoxicity, and β -cell toxicity mediated by immunosuppressive agents (3,4,26). At present, the assessment of graft function is dependent on clinical biochemistry, including measurement of C-peptide levels, glucose levels, and oral/intravenous glucose tolerance tests (4).

Therefore, the establishment of a noninvasive technique for quantifying islet graft survival is extremely important for clinical islet transplantation.

Magnetic resonance imaging (MRI) is an attractive potential tool for measuring islet mass in vivo because it is generally noninvasive, it can achieve relatively high spatial resolution, and it can use multiple mechanisms for contrast enhancement (22). It can potentially target extracellular or intracellular enzymes, nuclear transcription factors, cell surface receptors, transporters, or other surface antigens. However, quantification of small amounts of transplanted islets by MRI is currently difficult (24). Although an efficient uptake of MRI contrast agent is required for cell imaging, this process is particularly difficult in nonphagocytic cells (5). Recently, labeling of islet cells has been pursued with magnetic iron oxide particles and has allowed detection of transplanted islets

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(2,8,9,11,16,29). Such a technique could allow real-time, noninvasive imaging of postransplanted viable islet mass and may facilitate the examination of various interventions to promote or sustain islet mass over time. However, commercially available magnetic nanoparticles are not efficiently transduced into cells because of their negative charge, since the cell surface is normally negatively charged. In this study, we developed novel cationic nanoparticles and investigated their detection by MRI.

MATERIALS AND METHODS

Animals

Six-week-old male adult Sprague–Dawley (SD) rats weighing 250–300 g were purchased from SLC Japan. The rats were housed under specific pathogen-free conditions with a 12-h light/dark cycle and had free access to food and water. Rat studies were approved by the review committee of Nagoya University Graduate School of Medicine.

Cell Line

β -cell-derived MIN6 cells, which were kindly provided by Dr. Junichi Miyazaki, were routinely grown in sterile plastic flasks containing Dulbecco's modified Eagle's medium (DMEM) and 25 mM glucose supplemented with 15% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μ g/ml streptomycin, and 5 μ M/L β -mercaptoethanol at 37°C in a humidified atmosphere of 5% CO₂.

Cell Labeling and Estimation of Iron Content in MIN6 Cells

Alkali-treated dextran-coated magnetic iron oxide nanoparticles (ATDM), carboxymethyl dextran-coated magnetic iron oxide nanoparticles (CMDM), carboxymethyl diethylaminomethyl dextran-coated magnetic iron oxide nanoparticles (CMEADM), trimethylamino dextran-coated magnetic iron oxide nanoparticles (TMADM-01 through -05), and diethylaminoethyl dextran-coated magnetic iron oxide nanoparticles (DEAEDM) were kindly provided by MEITO Sangyo Co., Ltd. (Kiyosu, Japan). MIN6 cells were detached from the flasks with trypsin-EDTA and incubated for 1 h at 37°C with each nanoparticle reconstituted in DMEM with 15% FBS. At the end of the uptake experiments, the cells were washed three times in phosphate-buffered saline. Measurement of cellular toxicity was performed by the manual counting method based on the trypan blue exclusion procedure. The iron content of MIN6 cells labeled with each nanoparticle was measured by photon correlation spectroscopy using nuclear magnetic resonance sequence (NMR) [Bruker mq20 Series NMR Analyzer (Bruker, Milton, Ontario, Canada)]. At the end of the uptake experiment, labeled cells were collected in 500 μ l deionized water and homogenized. The volume was

brought up to 1 ml with deionized water and analyzed by pulse NMR.

Islet Isolation and Labeling

Islet isolation was performed as follows: under general anesthesia induced by pentobarbital sodium (50 mg/kg), rats were injected with 10 ml of Hanks' balanced salt solution (Gibco) containing 2 mg/ml collagenase (Sigma; type V) into the common bile duct. The distended pancreas was removed and incubated at 37°C for 16 min. The islets were purified by centrifugation (2,000 rpm for 10 min) with Histopaque 1077-RPMI 1640 medium gradient (Sigma). Individual islets, free of attached acinar, vascular, and ductal tissues, were selected and removed with a Pasteur pipette under a dissecting microscope, yielding highly purified islets. Islets were incubated for 1 h at 37°C with TMADM-03 reconstituted in DMEM with 15% FBS. At the end of the uptake experiments, islets were washed three times in RPMI 1640 medium.

MRI

MIN6 cells labeled with TMADM-03 were collected in 15-ml tubes and set on MRI equipment (MRTechnology, Inc., Tsukuba, Japan), and T1- and T2-weighted images were acquired according to the manufacturer's procedure. All T1- and T2-weighted image data sets were visually evaluated to identify the location of labeled cells.

RESULTS

Novel Magnetic Nanoparticles

We developed six modified dextran-coated magnetic iron oxide nanoparticles (TMADM-01 through -05 and DEAEDM), each of which consists of a small monocrystalline, superparamagnetic iron oxide core that is stabilized by a cross-linked aminated dextran coating to improve stability (Fig. 1). We also used three different commercially available nanoparticles for controls (ATDM, CMDM, CMEADM; Fig. 1). The particle size (Fig. 2A) and end group of the modified dextran (Fig. 2B) influence the zeta potential of the magnetic iron oxide nanoparticles (Fig. 2C).

Cell Labeling With the Novel Magnetic Nanoparticles

In order to determine the labeling efficiency of the novel nanoparticles, 1×10^6 MIN6 cells were incubated for 1 h at 37°C with each nanoparticle (150 μ g Fe/ml) reconstituted in culture medium, and the iron content of MIN6 cells labeled with each nanoparticle was determined using nuclear magnetic resonance (NMR) (Fig. 3A). TMADM-02, -03, -04, and DEAEDM were efficiently transduced into cells because of their positive charge, since the cell surface is normally negatively charged. On the other hand, negative-charged nanoparticles such as ATDM, CMDM, CMEADM, and TMADM-05 were not as efficiently transduced

Figure 1. Schematic diagram of novel magnetic nanoparticles. Alkali-treated dextran-coated magnetic iron oxide nanoparticles (ATDM; top left). Carboxymethyl dextran-coated magnetic iron oxide nanoparticles (CMDM; top right). Carboxymethyl diethylaminomethyl dextran-coated magnetic iron oxide nanoparticles (CMEADM; bottom left). Trimethylamino dextran-coated magnetic iron oxide nanoparticles (TMADM-01-05; bottom right). Diethylaminoethyl dextran-coated magnetic iron oxide nanoparticles (DEAEDM) were coated with dimethylaminoethyl dextran instead of trimethylamino dextran.

Figure 2. Characteristic features of novel magnetic nanoparticles. (A) Diameters of modified dextran-coated magnetic iron oxide nanoparticles. (B) Cationic end-group substitutions of modified dextran-coated magnetic iron oxide nanoparticles. (C) Zeta voltage of modified dextran-coated magnetic iron oxide nanoparticles.

Figure 3. Cell labeling with novel magnetic nanoparticles. (A) Uptake of nine different modified dextran-coated magnetic iron oxide nanoparticles in MIN6 cells. MIN6 cells were incubated for 1 h at 37°C with 150 $\mu\text{g Fe/ml}$ nanoparticles, reconstituted in culture medium. (B) Comparison of the cellular toxicity of MIN6 cells labeled with the nanoparticles. Unlabeled MIN6 cells were used as a control. (C) Relative aggregation rate of the nanoparticles in culture medium.

into cells. One of the positively charged nanoparticles, TMADM-01, did not transduce well. This may be due to its low cationic end-group substitution degree. The total amount of cationic substitution as well as positive charge (positive zeta voltage) may be important to transduce nanoparticles into cells.

We evaluated the cellular toxicity of labeling MIN6 cells with TMADM-02, -03, -04, and DEAEDM (which are efficiently transduced). There was little cellular toxicity from these nanoparticles (Fig. 3B). To test the stability of these four nanoparticles in the culture medium, the relative aggregation rates were measured. TMADM-03 and DEAEDM were stable and did not aggregate, while TMADM-02 and TMADM-04 did aggregate (Fig. 3C). These aggregation rates may show “false positives” when they are transplanted in vivo.

These data suggest that TMADM-03 and DEAEDM could be useful for labeling cells.

Morphology of Labeled MIN6 Cells and Isolated Islets: Uptake of TMADM-03

To investigate the morphology of labeled cells, MIN6 cells and isolated islets from rat pancreata were incubated with 150 $\mu\text{g Fe/ml}$ of TMADM-03 for 1 h. The morphology of both MIN6 cells and isolated islets was similar to that of untransduced cells (Fig. 4A–D). However, the color of both labeled MIN6 cells and islets became darker than that of unlabeled cells (Fig. 4B, D). Since the culture media of Fig. 4B and D contained TMADM-03, the backgrounds were also darker than that of the control. The colors of cell pellets before and after labeling with TMADM-03 are shown in Fig. 4E.

Figure 4. Morphology of labeled MIN6 cells and isolated islets: Uptake of TMADM-03. (A) Morphology of unlabeled MIN6 cells detached from culture flask. (B) Morphology of MIN6 cells labeled with TMADM-03. MIN6 cells were incubated for 1 h at 37°C with 150 μg Fe/ml of TMADM-03 in culture medium. (C) Morphology of unlabeled islets. (D) Morphology of islets labeled with TMADM-03. Islets were incubated for 1 h at 37°C with 150 μg Fe/ml of TMADM-03 in culture medium. (E) Cell pellet of MIN6 cells before and after labeling with TMADM-03.

Ex Vivo MRI of MIN6 Cells Labeled With TMADM-03

To test whether labeled cells were detected by MRI, MIN6 cells were incubated with 150 μg Fe/ml of TMADM-03 for 1 h at 37°C in culture medium containing 15% FBS, and MRI was performed on the cell pellets. The labeled pellet had lower intensity on both T1- and T2-weighted images than the unlabeled pellet (Fig. 5A–D). These data suggest that a 1-h labeling in the medium with 150 μg Fe/ml TMADM-03 could provide sufficient contrast for cell visualization in MR images *ex vivo*.

DISCUSSION

In this study, we showed that the positively charged nanoparticles were efficiently transduced into cells and able to visualize the labeled cells. The potential to achieve near-normal glucose control and possible insulin

independence in islet transplant recipients depends largely on the number of surviving islets that contribute to the total posttransplantation islet mass (5). At present, transplanted or native islets have been visualized mainly indirectly through the detection of magnetically labeled infiltrating T lymphocytes in an autoimmune model (17) or by MR detection of “metabolic footprints,” which are caused by local intrahepatic periportal steatosis as a consequence of insulin production by the transplanted islets (14). For direct visualization, we performed pretransplantation *in vitro* labeling of cells with an appropriate MR contrast agent. Some groups have demonstrated that Feridex labeling of islet grafts is specific, safe, and effective for their localization by MRI (10,12). For islet labeling, we used modified raw materials of the iron nanoparticle contrast agent Resovist® (ATDM), which

Figure 5. Ex vivo MRI of MIN6 cells labeled with TMADM-03. (A) T1- and (B) T2-weighted images of unlabeled MIN6 cell pellet. (C) T1- and (D) T2-weighted images of MIN6 cell pellets labeled with TMADM-03 (arrows, low-intensity area within the dotted lines). High-intensity portions at the upper part of the pellet (arrowheads) were due to residual PBS.

has already been clinically approved for human use. The size of the coated particles seems acceptable (ranging from 45 to 60 nm), and their use requires no further facilitation of iron uptake by the cells. It has been reported that the magnetic iron oxide nanoparticles covered with a modified dextran coating were derivatized with a cell-penetrating peptide (13). Several such peptides have recently been described, including the third helix of the homeodomain of Antennapedia (7,19), a peptide derived from anti-DNA monoclonal antibody (1), VP22 herpes virus protein (25), HIV-1 Tat protein (27), and polyarginine peptide (20,21). Most of these peptides have positively charged amino acids such as arginine and lysine. For cell labeling, we used the iron nanoparticle contrast agent TMADM-03, which is positively charged. The data suggest that cell labeling by the positively charged nanoparticles may depend on macropinocytosis, by which positively charged cell-penetrating peptides are transduced into cells.

TMADM-03, which is a modified form of a commercially available contrast agent of ATDM, could be used as a marker of isolated pancreatic islets for MRI. Labeling with modified dextran-coated magnetic iron nanoparticles enables visualization of their localization and distribution. This approach could potentially be translated into clinical practice for evaluating graft survival and for monitoring therapeutic intervention during graft rejection.

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