Improved islet labeling for longitudinal monitoring by MRI using cationic magnetoliposomes


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Abstract

The transplantation of pancreatic islets (PIs) containing insulin producing beta cells is considered an alternative approach for the treatment of type 1 diabetes mellitus (T1DM) [1]. Currently, different transplantation sites are assessed in clinical trials to improve outcome [2, 3]. To better monitor and validate the success of transplantation, it is desirable to monitor the location of engrafted islets non-invasively [4]. In vivo MR imaging of transplanted islets is one of the most suitable cell tracking methods, however, this requires labeling of the islets with a suitable contrast agent prior to transplantation [5-8]. In this study, we have tested the feasibility of cationic magnetoliposomes (MLs) compared to commercial contrast agents (Endorem and Resovist) by labeling insulinoma cells (INS1E) and freshly isolated rat PIs. It was possible to incorporate MR detectable amounts of MLs in a much shorter time (2 hours) without addition of transfection agents when compared to Endorem and Resovist. MLs did not show negative effects on the PIs viability and functional parameters in vitro. Further, labeled islets were transplanted in the renal sub-capsular region of healthy mice. Hypointense contrast in MR images due to the labeled PIs was detected in vivo upon transplantation. However MRI based detection was not possible when islets were labeled with Endorem and Resovist alone, but after addition of the transfection agent Poly-L-Lysin (PLL). The findings of this study indicate that MLs provides a satisfactory means to image pancreatic islets and have no deleterious effect on their function, which is promising for future pre-clinical and clinical studies involving the assessment of islet transplantation.

Magnetoliposomes (MLs) are built up of nm-sized magnetite (Fe₃O₄) core, in which the original stabilizing lauric acid coat is first replaced by a phospholipid bilayer during incubation and dialysis of the fatty acid coated particle with performed small unilamellar phospholipid vesicles. Mechanistically, it has been proven that the process of ML formation is controlled by spontaneous transfer of phospholipids according to the so-called aqueous transfer model [9,10]. As MLs can be categorized as ‘ultrasmall superparamagnetic iron oxides’ (commonly designated as USPIO’s), a high gradient magnetic field (HGM) is needed to withdraw the MLs from the incubation mixture. For a 14 nm-diameter iron oxide core covered with an intact phospholipid bilayer, typically, a phospholipid/Fe₃O₄ (mmol/g) ratio between 0.7 and 0.8 is calculated. The ligand exchange reaction can also be used to incorporate fluorophores (fluorescent labels for microscopic validation) or targeting molecules (small molecules targeting the GLUT2, monoclonal antibodies and potentially nanobodies) to the MLs double-layer.
Insulinoma (ISN-1E) cells and pancreatic islets (PIs) were then be labeled with different types of MLs (non-functionalized and functionalized) by co-incubation at different concentrations and for different time intervals. Insulinoma (ISN-1E) cells and pancreatic islets (PIs) were freshly isolated from Sprague Dawley rats (female, 200-250 g) with collagenase digestion. They were then labeled with cationic MLs (3,33% DSTAP), and also for comparison with the FDA approved Resovist (Schering AG) or Endorem (Guebert, France). Incubation occurred at a concentration of 50 µg Fe/mL. After incubation for different time intervals (from 4 to 24 hours) the labeled samples were collected for viability tests (PI/FDA staining and LDH test), functional assays (insulin storage and secretion), iron uptake (TEM and Prussian blue staining), iron quantification (ICP-OES) and high resolution MRI scanning.

INS-1E labeled with cationic MLs at 50µgFe/ml for 4 hours indicated MLs adhered on the cell membrane (red arrows) whereas longer incubation (24 hours) resulted in particle uptake and prominent blue staining in the cell cytoplasm (black arrows) (Figure 1A). Also Prussian blue staining images of INS-1E labeled with Endorem and Resovist (at 50µgFe/ml) alone indicated no uptake whereas 100% uptake was observed when conjugated with PLL for 24 hours at 50µgFe/ml of incubation (Figure 1C). ICP-OES measurements of INS-1E labeled with cationic MLs showed marked increase in the iron uptake with incubation time (1.64 ± 0.70 pg/cell at 4 hours and 5.33 ± 1.9 pg/cell at 24 hours) (Figure 1B). Total cell count post labeling was comparable to non-labeled samples (Figure 1D). Viability with FDA/Propidium Iodide staining indicated no negative effect on the cells (Figure 1E). D-glucose stimulated insulin secretion did not alter when labeled with Endorem. But Resovist labeled cells at 1.1mM and MLs labeled cells at 11.1mM glucose concentration showed significant increase in the insulin secretion (Figure 1F).

Iron uptake by PIs was confirmed by Prussian blue staining and TEM when labeled with Endorem + PLL (Figure 2A), Resovist + PLL (Figure 2B), which showed heterogeneous uptake when different islets of the same batch were compared. With Prussian blue staining, one islet indicated almost no uptake (Figure 2A) and other showed blue staining distributed around complete islet (Figure 2B). Presence of particles adhered to the cell membrane is shown by red arrows in Prussian blue and TEM images. Fluorescent MLs showed uptake already after 2 hours and elevated uptake was observed after 18 hours of co-incubation with MLs. (Figure 2E).
Figure 2 Iron uptake by rat islets: (A, B, C) Iron uptake by PIs was confirmed by Prussian blue staining and TEM (D) Iron content in the labeled PIs (n=2) was measured with ICP-OES (E) Fluorescent uptake of Rhodamine-MLs after 2h and 18h hours of labelling. Scale bar: 2µm for TEM; data expressed as Mean ±S.D. (F) Viability of islet cells (FDA/propidium iodide staining) after labeling. Scale bar: 100µm.

To assess the viability of islets labeled with NPs, an FDA/Propidium iodide staining was performed. (Figure 2F) shows fluorescent images acquired to determine the viability of PIs. None of the SPIOs showed significant negative effects on the viability of islet cells after labeling. As viability assays of FDA/Propidium iodide is not a quantitative measure to determine viability, it was necessary to evaluate the effect of the labeling procedure on the functional capacity to secrete insulin. Labeled PIs were subjected to hypo-, normo- and hyperglycemic concentrations using D-glucose. (Figure 3) indicates that there was no effect observed in the insulin secretion of labeled PIs post D-glucose stimulation.

Figure 3 Effect on insulin secretion of labeling PIs: Insulin release from PIs in presence of glucose (1.1, 8.3 and 16.7 mM with/without Theophylline 1.4mM) when labeled with MLs (left panel), Endorem+PLL (middle panel) and Resovist (right panel) at 50µg/ml for 24 hours.

We next investigated the feasibility to monitor transplanted islets in healthy mice by using in vivo MR imaging. We first implanted 200 PIs labeled with Endorem (+PLL), Resovist (+PLL) or cationic MLs under the left kidney capsule of B6 mice. (Figure 4A) indicates shows a marked decrease in the signal intensity (white square) on T2-weighted MR images of PIs labeled with MLs, whereas PIs labeled with Endorem/Resovist (+PLL) showed any or little hypointense signal. In order to confirm the signal intensity reduction was indeed due to the presence of labeled PIs, Prussian blue staining of the kidney sections was performed.
Figure (4B) indicates blue stains in the sub-capsular zone indicating the presence of labeled islets. With histological staining it was possible to detect labeled islets for five days post transplantation for all examined particles. As seen from higher magnifications, staining pattern from islets labeled with MLs was prominently seen compared to Endorem and Resovist (+PLL) labeled PIs.

Figure 4 MR image of labeled PIs in vivo: (A) Hypointense sub-capsular region (highlighted by White Square) indicates PIs labeled with Endorem+PLL, Resovist+PLL and MLs. (B) Kidney tissue subjected to Prussian blue staining clearly indicated the presence of prominent staining was observed due to ML labeling.

The use of lipid bi-layers as coating for iron oxide cores has shown to generate excellent and biocompatible MLs (hydrodynamic size = 40nm) for cell labeling, which can be applied as a versatile MR contrast agent [11, 12]. In contrast, FDA-approved Endorem and Resovist NPs are composed of dextran or carboxy-dextran coated iron cores, comprising multiple crystals with an overall hydrodynamic diameter of 80-120nm and 62nm, respectively. So far, many pre-clinical and also clinical studies have indicated the feasibility of Endorem /Resovist (±PLL) to label and determine the fate of islet graft non-invasively [11-17]. But, prolonged incubation times for islet labeling and the fact that Endorem and Resovist have been withdrawn from the market compelled researchers to investigate alternatives for these contrast agents [11, 12, 14-16]. The flexibility offered by lipid coating allows an efficient and flexible functionalization like the ability for bimodal imaging (addition of fluorescent dye to the outer lipid layer of MLs) or the use different surface charges (anionic, neural or cationic MLs) [17]. MLs have been found to be non-toxic and allow high intracellular iron concentrations in a wide variety of cell types [17, 18]. Thus, studying the feasibility of MLs for PIs labeling and in vivo tracking is a promising alternative to Endorem/Resovist. In conclusion, we have demonstrated that PIs can efficiently be labeled by using cationic MLs with a labeling efficiency higher than that of Endorem and Resovist. The superior characteristics of MLs highlight their potential application for islet (or other types of cells) labeling and monitoring. The results of this preliminary study are encouraging in terms shorter incubation time (thus less stress on the islets) and no negative effect on the viability and functional properties of labeled islets. In future, MLs can be functionalized as a specific contrast agent by attaching a functional group specific for receptor present on the β-cell to determine beta cell mass (BCM) in order to determine the early onset of diabetes with non-invasive imaging.

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References (inclusion of full list of all authors is recommended; if more than 5 authors, abbreviation with “et al” will be tolerated)


