Imaging of islet transplantation in a pre-clinical animal model using an FDA-approved contrast agent: in vitro studies.

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Introduction. Pancreatic islet transplantation has recently emerged as a potential cure for patients with Type I diabetes milletus. In order to monitor transplantation efficiency and graft survival, reliable non-invasive imaging methods are critically needed. We have recently reported on the labeling and in vivo imaging of human pancreatic islets using "in-house"-made superparamagnetic iron oxide nanoparticles (1). This study showed our ability to label human pancreatic islets without causing detrimental toxicity or influencing insulin secretion. In addition, we performed longitudinal in vivo MR imaging of labeled pancreatic islets transplanted under the kidney capsule of diabetic NOD.scid mice. Labeled islets restored normoglycemia as fast as unlabeled islets and allowed for imaging of islet grafts up to 188 days after transplantation (1). However, for translation of this study into a clinical application we need to utilize already existing FDA-approved commercially available iron oxide-based contrast agents for islet labeling and clinically relevant models of islet transplantation, such as intraportal transplantation. Here we report on the use of FERIDEX®, for labeling human pancreatic islets and testing its safety in vitro. An accompanying poster from our laboratory reports on the in vivo MR imaging of FERIDEX®-labeled human islets after transplantation into the liver through the portal vein, which is the most common route of clinical islet transplantation.

Materials and Methods. To test whether human pancreatic islets can be labeled with FERIDEX®, one thousand human islets were incubated with FERIDEX® overnight (200 µg Fe/ml) and subjected to MR imaging. Acquisition of T2-weighted spin echo pulse sequences was based on the following protocol: TR/TE = 2000/15, 30, 45, 60, 75, 90, 105, 120 ms; FoV = 4x4cm^2; matrix size 256x256; resolution 156 x 156 µm; slice thickness = 1 mm, and imaging time of 8 min 32 sec. In order to quantitate the amount of iron uptake in human islets, we utilized an iron-binding assay. One hundred pancreatic islets were incubated overnight with increasing concentrations of the MN-NIRF probe (10-300 mg iron/ml). After incubation, islets were washed and resuspended in 6N HCl. The iron content of labeled islets was determined using a Total Iron Reagent Set (Pointe Scientific, Canton, MI). Incubation with each iron concentration was repeated five times. A similar experimental setup was utilized for the time course studies where islets were incubated with FERIDEX® (200 µg Fe/ml) for 1, 2, 4, 6 and 24 hours. Labeled islets were stained for iron using DAB-enhanced Prussian Blue stain and for insulin using anti-insulin antibodies followed by FITC-labeled secondary antibodies. Islet viability was evaluated using standard a MTT cytotoxicity assay. To ensure that labeling with FERIDEX® does not affect islet function, we performed insulin secretion studies of labeled and non-labeled islets in static incubations in low glucose (1.7 mM) vs. high glucose (16.7 mM). Insulin concentration was measured using a human insulin ELISA kit (Mercodia, Uppsala, Sweden). Non-labeled islets served as controls where applicable.

Results. MR imaging of the phantom containing labeled and unlabeled islets showed a significant decrease in signal intensity on T2-weighted images in labeled islets (Fig. 1A). The layer of medium above the islets did not produce any difference in signal intensity on these images. Considering that an average pancreatic islet consists of 2000 islet cells, iron uptake tested by an iron binding assay resulted in 1.21±0.36 to 12.01±3.3 pg iron/islet cell after incubation with FERIDEX® (Fig. 1B). Time course studies showed the increase in islet iron content with increasing incubation time. Islet viability after overnight exposure to the probe (300 mg iron/ml) remained unchanged compared to non-labeled cultured islets ($p > 0.05$). Labeled islets (Fig. 2, left) showed normal granulation and insulin production with no detectable morphological changes (Fig. 2, right). Glucose-stimulated insulin secretion, as seen by a stimulation index, was unchanged for labeled vs. non-labeled islets (5.89 ± 2.02 vs. 6.184 ± 2.06 respectively; $p > 0.05$).

Conclusion. Our in vitro studies indicated that FERIDEX® could be used for labeling human pancreatic islets and that it produced a change in signal intensity on T2-weighted images. We found that FERIDEX® was not cytotoxic to human islets and their viability was preserved after exposure to the probe. Labeling with the iron oxide contrast agent did not affect insulin secretion in a glucose-stimulated assay. We concluded that this FDA-approved commercially available contrast agent could be safely used for labeling human pancreatic islets. In vivo MR imaging of FERIDEX®-labeled islets transplanted in the liver is presented in the accompanying poster from our laboratory.

(1). Evgenov N, Medarova Z, Dai G, Bonner-Weir S, Moore A. "In vivo imaging of islet transplantation". *Nat Medicine*, 2006: in press.

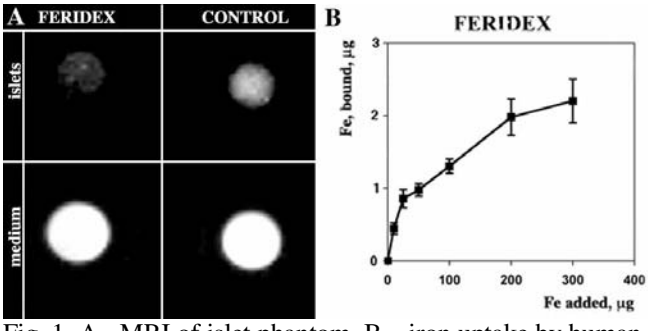

Fig. 1. A - MRI of islet phantom. B – iron uptake by human islets.

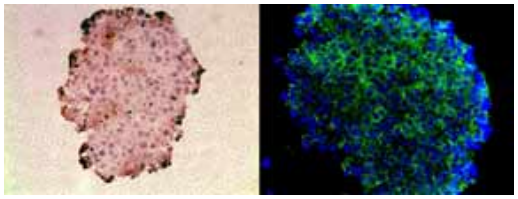

Fig. 2. DAB-enhanced Prussian Blue stain (left) and staining for insulin (right).