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

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Introduction

Islet transplantation has been introduced as an effective therapy for patients with Type 1 diabetes mellitus. In spite of encouraging results, graft fate after transplantation is mostly unknown. Non-invasive magnetic resonance imaging (MRI) of pancreatic islets after transplantation can provide important information regarding graft location and survival. Previously, we have shown that human pancreatic islets could be labeled with "in-house"-made superparamagnetic iron oxide nanoparticles (MN) and detected by in vivo MRI after transplantation under the kidney capsule. Furthermore, we demonstrated that MN resided in endosomes of islet cells without altering their insulin-producing function and produced a sufficient signal intensity change on MR images up to 188 days after transplantation (1). However, for translation of this study into clinical practice 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 in vivo MR imaging of human pancreatic islets labeled with FERIDEX® and transplanted into the liver through the portal vein, which is the most common route of clinical islet transplantation. We used MR imaging to investigate islet rejection in immunocompromised (NOD.scid) and immunocompetent (Balb/c) models and evaluate the input from autoimmunity on islet death after transplantation. The MR imaging data were further corroborated by histological studies.

Materials and Methods

Human donor pancreatic islets were labeled with FERIDEX® overnight (200 μg Fe/ml) and used for transplantation. Balb/c mice (n=8) and NOD.scid mice (n=12) were transplanted with 1000 labeled islets into the liver through the portal vein. In vivo MR imaging of islet graft-bearing livers was performed on days 1, 2, 3, 4, 5, 6, 10, and 14 after transplantation using a 4.7T Bruker horizontal bore scanner (Billerica, MA) equipped with ParaVision 3.0 software. The imaging protocol consisted of T2*-weighted gradient echo (GE) pulse sequences with the following parameters: TR/TE = 200.000/ 8.000 ms, number of averages 32, FOV = 3.2x3.2 cm^2, matrix size 256 x 256, resolution 0.125 x 0.125 mm^2, slice thickness = 0.5 mm, and a total scan time of 27 m 18 s. Quantitative islet scoring was performed by three independent blinded investigators. Animals from both groups were sacrificed on days 1, 2, 3, 4-5, 6-7, 10-11, and 14–15 after transplantation. Islet-bearing livers were excised and stained for apoptosis using deoxynucleotidyl–mediated dUTP nick end-labeling (TUNEL) assay (Apoptag apoptosis detection kit, Chemicon International, Temecula, CA).

Results

On in vivo MR images labeled pancreatic islets transplanted into the liver appeared as dark voids and were detectable in both models for the duration of the study. The number of islets gradually decreased during the course of the study and plateaued between days 10 and 14 in both cases (Fig. 1). This decrease was consistent with islet death after transplantation due to mechanical injury, ischemia and nonantigen-specific inflammatory events (2). However, immunocompetent mice showed a 20% higher rate of islet disappearance on MR images, compared to immunocompromised animals, presumably due to the input from autoimmunity. To correlate our MR imaging data of islet death with apoptotic rates, we performed TUNEL assay on excised livers. In NOD.scid and Balb/c mice apoptosis was most pronounced on day 1 (12.1% and 18.6% respectively). The amount of apoptotic cells in immunodeficient mice decreased to 1.8% by day 4 and stayed at this level for the duration of the study. In immunocompetent mice the number of apoptotic cells gradually decreased to 3.3% on day 4-5 followed by severe antigen-specific rejection, which destroyed up to 13.2% by day 10-11 (Fig. 2). Overall, the results from this study suggested that the disappearance of pancreatic islets on MR images was due to islet death, which was confirmed by TUNEL assay.

Conclusion

The goal of this study was to utilize the FDA-approved commercially available contrast agent FERIDEX® for labeling of human pancreatic islets and subsequent in vivo MR imaging of labeled transplanted islets in a clinically relevant model of islet transplantation. The safety of this agent for islet function and viability is described in the accompanying poster. The present studies showed that it is possible to image islet fate after transplantation in immunocompetent and immunocompromized animal models. Furthermore, we were able to detect non-invasively a greater rate of islet death in the immunocompetent model due to autoimmune rejection. Currently, studies are underway to investigate the fate of iron released after islet death.

Fig. 1

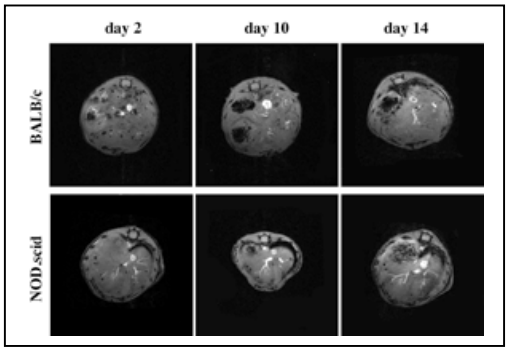
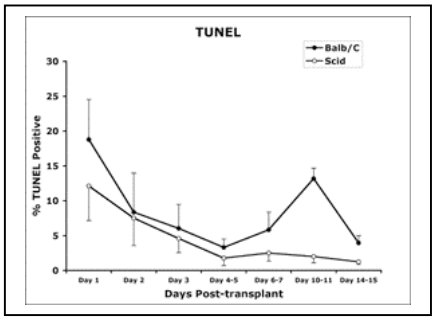


Fig. 2



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