SUPPLEMENTAL DIGITAL CONTENT (SDC)

Longitudinal MR imaging for monitoring allogeneic islet grafts in nonhuman primates

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SDC, Materials and Methods

Islet harvest and mixed lymphocyte reaction

For autologous transplantation under the kidney capsule B255 and B267 underwent partial (70%) pancreatectomy (PPtx). The excised organs were immediately perfused with and immersed in cold University of Wisconsin (UW) solution. For islet isolation, pancreata were perfused with Liberase HI (Roche Biochemicals, Indianapolis, IN) and incubated at 37°C in a static digestion chamber for 45 min. The digested tissue was purified on a continuous Ficoll Gradient using a COBE 2991 cell processor. The total number of islets was calculated as islet equivalents (IEQ) with an average diameter of 150 μm per islet. Islets were cultured overnight in CMRL 1066 supplemented culture media (Cellgro; Mediatech, Herndon, VA) with added 10% FBS and 1% Penicillin/Streptomycin and HEPES buffer (Cellgro; Mediatech, Herndon, VA). Islet count, islet purity, and viability were assessed using standard procedures and described in our previous study (1).

For allogeneic transplantation donor islets were isolated from healthy donor baboons (n = 4) after partial (70–80%) PPtx as described above.

The pre-transplant mixed lymphocyte reaction (MLR) assay was carried out as previously described (2).

Longitudinal magnetic resonance imaging

An initial localizer scan was performed for optimal slice positioning. Slices were positioned so that the middle slice was located 2 to 3 cm above the branching of the portal vein (liver) or the center of the graft covering the entire graft volume (kidney). Imaging sequences include T1 weighted image and T2* weighted map. Imaging parameters were as follows (1):

Kidney:

a) (with respiratory gating) - TR/TE=200/2.1-29.1 ms, slice thickness 3 mm, FoV = 180 × 180 mm^2, matrix size 192 × 192, flip angle = 25°, number of slices 12, number of averages = 4, and in plane resolution 0.9 × 0.9 mm^2.
b) (with navigator) - TR/TE = 2000/4.76-61.3 ms, slice thickness 3 mm, FoV = 180 × 180 mm^2, matrix size 256 × 256, flip angle = 70°, number of slices = 12, number of averages = 1, and in plane resolution 0.7 × 0.7 mm^2.

Liver:

a) (with respiratory gating) - TR/TE = 100/2.3-29.3 ms, slice thickness 3 mm, FoV = 200 × 200 mm^2, matrix size 192 × 192, flip angle = 25°, number of slices = 10, number of averages = 1, and in plane resolution 1 × 1 mm^2.
b) (with navigator) - TR/TE = 2000/4.76-61.3 ms, slice thickness 3 mm, FoV = 200 × 200 mm^2, matrix size 256 × 256, flip angle = 70°, number of slices = 12, number of averages = 1, and in plane resolution 0.78 × 0.78 mm^2.

All images were processed using ImageJ software (1.48g, National Institutes of Health, USA). Two independent investigators performed image analysis blindly. Results were

expressed as an average of the two counts. For the islet grafts in the liver, all sections acquired over the liver were studied. Signal voids representing islet/islet clusters were counted manually. Islet/islets grafts were defined as those having a round or elliptical shape, and appearing in up to three adjacent MRI slices (to exclude the small spots and also avoid transversal projections of the vascular bed) (3). The first number determined 1 day post transplantation was defined as 100%. Results of subsequent scans were presented as percentages of the initial counts. For the islet grafts under the kidney capsule, graft volumes were calculated by counting the area in each slice of the region of interest (ROI) outlining the graft and multiplying by the number of slices. The total number of signal void regions related to transplanted islets was manually calculated in all sections covering the sub-kidney capsule areas of two recipients.

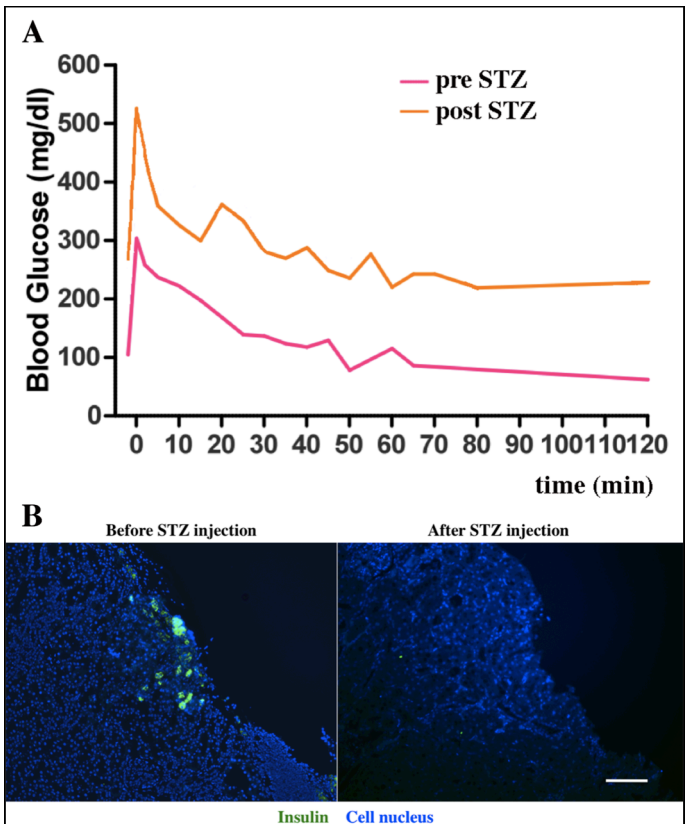
References

1. Medarova Z, Vallabhajosyula P, Tena A et al. In vivo imaging of autologous islet grafts in the liver and under the kidney capsule in non-human primates. *Transplantation* 2009; 87:1659.

2. Griesemer A, Liang F, Hirakata A et al. Occurrence of specific humoral non-responsiveness to swine antigens following administration of GalT-KO bone marrow to baboons. *Xenotransplantation* 2010; 17:300.

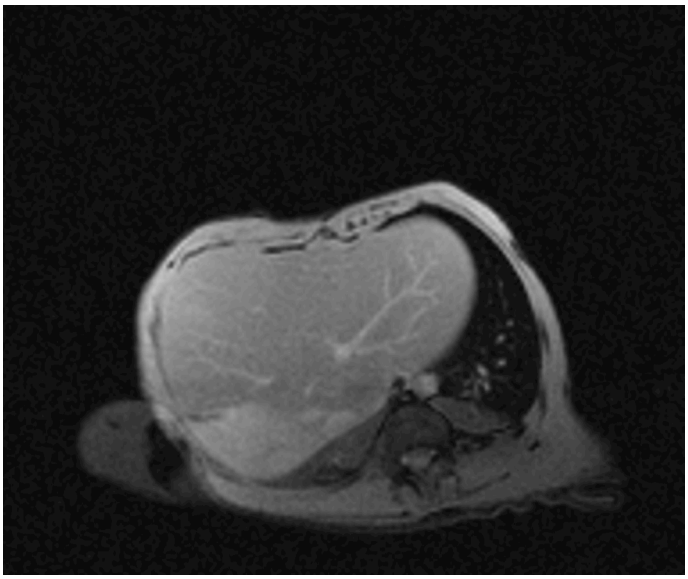
3. Kim JH, Jin SM, Oh SH et al. Counting small hypointense spots confounds the quantification of functional islet mass based on islet MRI. *Am J Transplant* 2012; 12:1303.

SDC, Supplemental Figure 1



A: Intravenous glucose tolerance test (IVGTT) demonstrates abnormal results after STZ injection in animals with autologous transplants. B: Biopsy of the grafts before STZ administration show insulin positive cells under the kidney capsule, which were destroyed after SZT injection as indicated by the staining of the necropsy tissue sections (green – insulin; blue – cell nucleus; magnification bar = 50 µm).

SDC, Supplemental Figure 2



Supplemental Figure 2: T2* weighted MR image of the pre-transplant liver parenchyma demonstrates considerable homogeneity.