

MR Characterization of Isolated Human Pancreatic Islets

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Introduction:

Clinically, transplantation of isolated pancreatic islets from a donor to recipient is one of the therapeutic technologies under development for treatment of diabetes, especially Type I. Relatively new, there are many questions still unresolved which impact the efficacy of this approach. These include isolation regimes, isolation media, number of transplanted islets, functional capacity of transplanted islets and ultimately their site of engraftment. To investigate the potential role of activation dependent Manganese (Mn) enhanced MRI (MEMRI) in answering these questions, we examined isolated human pancreatic islets. If the function of human islets can be visualized in vivo, it may be possible to evaluate different transplantation regimes for effectiveness of engraftment as well as for specifying the site of engraftment. Glucose stimulated influx of calcium into β -cells is necessary for insulin release. When present during glucose stimulation, extra-cellular Mn can enter β -cells through voltage-gated calcium channels and its accumulation alters T1 and T2 relaxation times. As with rodent islets, high-resolution MR micro imaging of glucose activated isolated human islets showed a significant increase in MR contrast. However, while maximum contrast in rat islets was achieved at 2.5 μ M and 25 μ M Mn (1), it could be seen in human islets at 35 μ M Mn and higher (*data not shown*). Contrast MRI provides information on sample properties through the following parameters - spin density, susceptibility, molecular motions resulting from diffusion and perfusion, and T1 and T2 relaxation times between tissues. Preferential enhancement of signal intensity can be obtained by selectively altering these relaxation parameters. Therefore before the study of any contrast agent effects, T1 and T2 of control islets were measured and maps created to establish baseline values. These data were then used to establish parameters for MR image acquisition of activated versus non-activated islets. Analysis of control and activated islets demonstrates the presence of both intra and inter-islet characteristics which will play a key role in the interpretation of functional data in vivo.

Materials and Methods:

Non-stimulated Islets: Isolated human pancreatic islets were first incubated in Krebs Ringer Buffer (KRB) solution at 1.67 mM glucose for 30 minutes and then rinsed three times with KRB. These control islets were then loaded into 2 cm long micro capillary tube of 570 μ m ID. **Stimulated Islets:** Islets were switched to KRB with 35 μ M Mn and 16.7 mM glucose for 30 minutes followed by rinsing. The sample tubes were mounted on a home built double loop Archimedes spiral coil with OD of 750 μ m and inserted into the Bruker Micro 5 Imaging Probe (triple axes gradients of maximum strength 2000 gauss/cm) (2). Spin echo (SE) sequences were used to obtain spin density, T1 and T2 weighted contrast images. All experiments were conducted in a 56-mm vertical bore 11.7 T magnet using a Bruker DRX Avance Spectrometer (Bruker, Billerica, MA). The typical imaging parameters were: TE = 8 ms, TR = 500 ms, Mx = 256, NEX = 15, Slice thickness = 0.3 mm, FOV = 0.3 mm, In-plane resolution = 10 μ m. The MR images acquired were viewed and processed using ImageJ (National Institutes of Health, USA).

Results and Discussion:

Figure 1 illustrates a 2D high resolution spin density MR image of human islets (neither glucose stimulated nor Mn treated). T1 & T2 measurements were performed, exponential equations were used to fit the curve between the recovery time and intensity, and T1 and T2 relaxation maps produced using Matlab (The Mathworks Inc, Natick, MA). From the data shown in figure 2, the T1 values of islets are in the range of ~500-700 msec compared to the surrounding media T1 of ~1150-2000 msec. From the data shown in figure 3 the T2 values from the islets are in the range of ~19-39 msec compared to the surrounding media T2 < 14 msec. To determine if the glucose stimulated uptake of Mn could be seen in human islets, a similar protocol developed by our group for use with rodent islets (1) was implemented. The viability of islets was confirmed using trypan blue before and after imaging. Figure 4A is a high resolution 2D MR image of control islets (left) and stimulated islets (right) that have been glucose activated in the presence of Mn. Contrast was achieved for Mn concentrations ranging between 35 μ M and 100 μ M. As opposed to our previous work with rodent islets for which highest contrast was achieved between 2.5 μ M to 25 μ M, no contrast was seen at lower Mn concentrations. However, despite the higher Mn concentrations employed, in this case as well no toxic effects on insulin secretion were observed (*data not shown*). The activated islets show a pronounced increase in contrast due to the influx of Mn and a resulting change in T1 relaxation. A line profile across the two sample tubes shown in Figure 4B illustrates the intra-islet signal intensity variation and the inter-islet difference in contrast due to glucose stimulation in the presence of Mn.

Conclusion:

Preliminary results demonstrate the feasibility of obtaining high-resolution MR images and activation maps of isolated human pancreatic islets. T1 & T2 characterization of human islets under control conditions were successfully achieved. The concentration of Mn required for image enhancement has been shown not to inhibit human islet function and provide data on intra- and inter-islet characteristics. Beyond proof-of-concept, careful MR characterization of isolated human islets has been performed which should result in further optimization of the imaging technique when applied in vivo. The availability of a non-invasive technique capable of monitoring the functionality of islets/ β -cells in vivo will enhance the understanding of diabetes and facilitate its treatment.

References:

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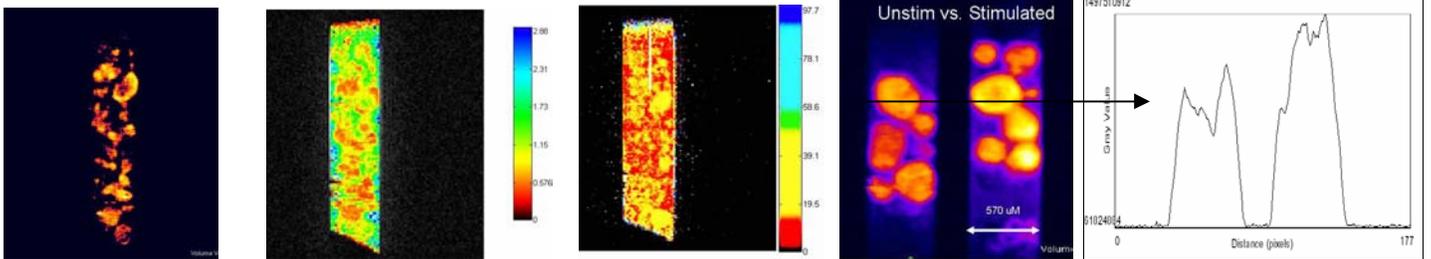


Figure 1: Spin density MR image of human pancreatic islets. 2D high resolution coronal image of islets in a capillary tube.

Figure 2: T1 map of isolated human islets with matrix size 128 x 128, TE=7.95 ms, TR ranges from 300 to 3000 msec, T1 values=500-700 msec.

Figure 3: T2 map of isolated human islets with matrix size 128 x 128, TE=7.95 ms, No. of echos=32, TR=3000 msec, T2 values=19-39 msec.

Figure 4:(A) High resolution 2D image of control (left) & stimulated human islets. Islets were exposed to 35 μ M Mn & 2 mM (left) & 16.7 mM (right) glucose. (B) Line profile of the signal intensity across the two tube samples illustrates intra- and inter-islet characteristics.