TWO SITE WATER EXCHANGE ANALYSIS OF PANCREATIC T1 RELAXATION REVEALS THE KINETICS AND MECHANISM OF BETA CELL LABELING WITH MANGANESE: IMPLICATIONS FOR IMAGING BETA CELL MASS IN DIABETES

P. Antkowiak[1], M. Vandsburger[2], and F. Epstein[2]

[1]University of Virginia, Charlottesville, Virginia, United States, [2]University of Virginia

Introduction: Pancreatic β cells are fully responsible for producing insulin, which is critical for maintaining blood glucose homeostasis. The loss of pancreatic β cells is central to type 1 diabetes mellitus (T1DM), and β cells also play an important role in type 2 diabetes. For the assessment of disease progression and the evaluation of new therapies, the accurate estimation of functional β cell mass by noninvasive imaging is a vital but unrealized goal. Mn^{2+} ions, which are similar to calcium ions (Ca^{2+}) in atomic weight and charge, ostensibly enter pancreatic β cells through voltage-gated calcium channels. In normal subjects, like Ca^{2+}, the flux of Mn^{2+} into β cells is increased in response to a sensed elevation in blood glucose. In addition, Mn^{2+} enhances MR images by shortening the spin-lattice relaxation time (T1) of nearby water protons. We previously developed quantitative β-cell imaging by making use of the property that Mn^{2+} preferentially labels these cells[1]. This property dictates that the infusion of Mn^{2+} leads to a compartment-dependent concentration of the T1-shortening contrast agent, with higher concentration in the intracellular compartment and lower concentration in the extracellular compartment. Rather than monoexponential T1 relaxation, this two-compartment system is expected to exhibit a biexponential relaxation of the water longitudinal magnetization. Accounting for the diffusion of water between compartments, we showed that parameter optimization of a two-site water exchange (2SX) model of the measured T1-relaxation of this system could be used to estimate the intracellular fraction, and that the intracellular fraction was reflective of β cell mass. Similarly, we showed that the intracellular T1 calculated from the 2SX model could be used as an index of β cell labeling by Mn^{2+}. In those studies, we detected significant differences in those two parameters when comparing non-diabetic mice to a T1DM mouse model. The purposes of the present study were to define the kinetics and confirm the mechanism of Mn^{2+} labeling of glucose-stimulated β cells *in vivo*. Specifically, to define the kinetics of β cell labeling with Mn^{2+}, we measured the intracellular T1 and intracellular fraction over time after contrast agent administration in normal mice. Similarly, to show that Mn^{2+} influx into β cells is secondary to Ca^{2+} channel activation, we measured the time course of those parameters in mice given a pharmacological Ca^{2+} channel blocker.

Methods: Our overall approach was to measure pancreatic T1 relaxation using Look-Locker MRI after glucose-stimulation and Mn^{2+} injection, and perform parameter optimization of a two site water exchange model to minimize the difference between the model and the measured T1 relaxation curve. A total of n = 9 mice were imaged on a 7T Clinscan system (Bruker, Germany), including 5 untreated mice and 4 mice injected with the Ca^{2+} channel blocker nifedipine. Prior to imaging, mice were fasted >1 hour. All mice received an intravenous bolus injection of 100µl glucose (1.5 mg/g) 2 minutes prior to an intraperitoneal injection of 50µl $MnCl_2$ (0.1 µmol/g). To block Ca^{2+} channels, 4 mice were intraperitoneally injected with 60 µL of 5mg/mL nifedipine 15 minutes prior to glucose and $MnCl_2$ injections. The pancreas was localized with coronal and axial images. Look-Locker images of pancreatic T1 relaxation (Fig. 1) were acquired at various time points after Mn^{2+} injection ranging from 30 minutes to 24 hours. The Look-Locker sequence used a non-selective 180° inversion pulse followed by a train of gradient echoes separated by delay times between 20-50 msec, depending on time after $MnCl_2$ injection. Specific parameters were as follows: time between inversions = 5500 msec, TE = 1.9msec, flip angle = 3°, slice thickness = 1mm, FOV = 35mm x 25mm, number of images = 100, and number of averages = 3. After image acquisition, T1 relaxation curves were generated by drawing a region of interest in the pancreas, and a two site exchange T1 relaxation model was fit to the measured curves as previously described[1]. Two site exchange analysis of this system resolved the measured T1 relaxation curve into 2 compartments: a compartment with a short T1 that represents β cells and possibly other pancreatic cells that significantly internalize Mn^{2+}, and a compartment with a longer T1 that represents the interstitial space and pancreatic parenchyma that enhances to a lesser degree with Mn^{2+}. The intracellular T1 and intracellular fraction time courses are reported.

Results: All data are shown as mean ± standard deviation. For both untreated and nifedipine-treated mice, the time course of intracellular T1 (an index of the intracellular Mn^{2+} concentration) after glucose and $MnCl_2$ injection is shown in Figure 2A. In untreated normal mice (solid line), 3 distinct phases of Mn^{2+} kinetics are observed: (a) Mn^{2+} uptake (wash-in) by glucose-stimulated β cells from 30 minutes to 2 hours after injection, (b) a fast Mn^{2+} depletion phase as Mn^{2+} likely washes out of β cells, from 2-4 hours and (c) a slow washout/plateau phase from 4 hours to 24 hours. In mice treated with nifedipine (dashed line), the Mn^{2+} wash-in phase was reduced, as evidenced by increased intracellular T1s from 30m to 2h. The fast Mn^{2+} depletion phase (2h to 4h) was also absent, but the slow washout/plateau phase was identical to that of untreated mice. The 1 hour time point after glucose and Mn^{2+} injection represents the time at which β cells are maximally labeled, since intracellular T1 in untreated mice is shortest at that time. These Mn^{2+} labeling kinetics are also reflected in the intracellular fraction data (Figure 2B). In untreated mice, for the time points corresponding to significant β cell labeling (30 minutes to 2 hours), the intracellular fraction was relatively constant at 3.6 – 3.8%. The intracellular fraction in nifedipine-treated mice (Figure 2B, dashed line) remained fairly level around 2.5% at all time points, reflecting the impact of blocking β cell Ca^{2+} channels as well as revealing the background intracellular fraction due to non-specific Mn-labeling of other cells.

Discussion: We performed studies to elucidate both the kinetics and mechanism of *in vivo* cell labeling in the context of Mn-enhanced pancreatic β cells. The experiments here extend work we previously presented in a mouse model of T1DM[1], which developed quantitative Mn-enhanced MRI of β cell mass, but did not address enhancement kinetics or confirm the role of Ca^{2+} channels. The present data suggest that Mn^{2+} accumulates in β cells for up to 2 hours after $MnCl_2$ injection and leaks out of β cells thereafter. From 4 hours after injection onward (the Mn^{2+} plateau phase), intracellular Mn^{2+} likely remains in cells other than β-cells, as evidenced by similar intracellular T1s (Figure 2A) and intracellular fractions (Figure 2B) in untreated mice and mice treated with nifedipine. Further corroborating the hypothesis that the residual 2.5% intracellular fraction is due to non-β cells, the intracellular fraction in diabetic mice with essentially complete β cell destruction confirmed by pancreatic insulin measurements was 2.6%[1]. We confirmed that the mechanism of β cell enhancement is due to Mn^{2+} entry through glucose-stimulated β-cell Ca^{2+} channels, since mice treated with the Ca^{2+} channel blocker nifedipine have increased intracellular T1 (indicative of lower Mn^{2+} concentration) relative to untreated mice. Maximal β cell labeling with Mn^{2+} one hour after injection identifies an optimal time point for β cell imaging in future studies in mouse models of diabetes.

References: 1). Antkowiak et al. "Toward quantitation of pancreatic beta cell mass using a two-site exchange analysis of manganese-enhanced MR images." Proc 17th ISMRM. Abstract #476.

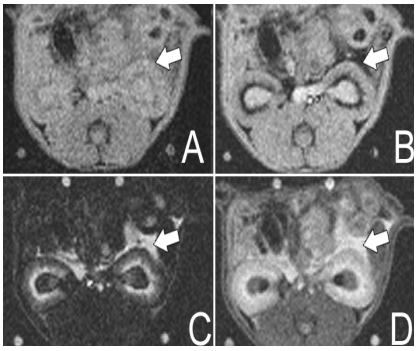

Figure 1: Look-Locker images of pancreas *(white arrow)* 24 h after glucose and $MnCl_2$ injection. Images (A) immediately after inversion, (B) at null time of pancreas, (C) at null time of surrounding tissues, (D) with pancreas at equilibrium.

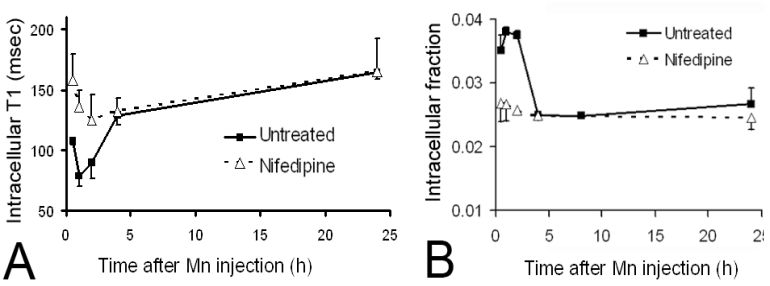

Figure 2: Intracellular T1 time course (A) and intracellular fraction (B) of Mn-enhanced pancreatic β cells in untreated mice (*solid line*) and nifedipine-treated mice (*dashed line*)