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. Antkowiak1, M. Vandsburger2, and F. Epstein2
1University of Virginia, Charlottesville, Virginia, United States, 2University 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. Mn²⁺ ions, which are similar to calcium ions (Ca²⁺) in atomic weight and charge, ostensibly enter pancreatic β cells through voltage-gated calcium channels. In normal subjects, like Ca²⁺, the influx of Mn²⁺ into β cells is increased in response to a sensed elevation in blood glucose. In addition, Mn²⁺ 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 Mn²⁺ preferentially labels these cells⁰. This property dictates that the infusion of Mn²⁺ 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 Mn²⁺. In those studies, we detected significant discrepancies 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 Mn²⁺ labeling of glucose-stimulated β cells in vivo. Specifically, to define the kinetics of β cell labeling with Mn²⁺, we measured the intracellular T1 and intracellular fraction over time after contrast agent administration in normal mice. Similarly, to show that Mn²⁺ influx into β cells is secondary to Ca²⁺ channel activation, we measured the time course of those parameters in mice given a pharmacological Ca²⁺ channel blocker.

Methods: Our overall approach was to measure pancreatic T1 relaxation using Look-Locker MRI after glucose-stimulation and Mn²⁺ 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 Clinsean system (Bruker, Germany), including 5 untreated mice and 4 mice injected with the Ca²⁺ channel blocker nifedipine. Prior to imaging, mice were fasted >1 hour. All mice received an intravenous bolus injection of 100μl of 100 mg/mL MnCl₂ 2 g/ml 2 minutes prior to an intraperitoneal injection of 50μl MnCl₂ (0.1 mmol/g). To block Ca²⁺ influx, 4 mice were intraperitoneally injected with 60 μL of 5 mg/mL nifedipine 15 minutes prior to glucose and MnCl₂ 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 MnCl₂ 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 MnCl₂ injection. Specific parameters were as follows: time between inversions = 5500 msec, TE = 1.9 msec, flip angle = 3°, slice thickness = 1 mm, field of view = 35 mm x 25 mm, matrix size = 256 x 256, number of averages = 100, and number of acquisitions = 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 described¹. 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 Mn²⁺, and a compartment with a longer T1 that represents the interstitial space and pancreatic parenchyma that enhances to a lesser degree with Mn²⁺. 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 Mn²⁺ concentration) after glucose and MnCl₂ injection is shown in Figure 2A. In untreated normal mice (solid line), 3 distinct phases of Mn²⁺ kinetics are observed: (a) Mn²⁺ uptake (wash-in) by glucose-stimulated β cells from 30 minutes to 2 hours after injection, (b) a fast Mn²⁺ depletion phase as Mn²⁺ 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 Mn²⁺ wash-in phase was reduced, as evidenced by increased intracellular T1s from 30min to 2h. The fast Mn²⁺ 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 MnCl₂ injection represents the time at which β cells are maximally labeled, since intracellular T1 in untreated mice is shortest at that time. These Mn²⁺ 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 Ca²⁺ 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 T1DM¹, which developed quantitative Mn-enhanced MRI of β cell mass, but did not address enhancement kinetics or confirm the role of Ca²⁺ channels. The present data suggest that Mn²⁺ accumulates in β cells for up to 2 hours after MnCl₂ injection and leaks out of β cells thereafter. From 4 hours after injection onward (the Mn²⁺ plateau phase), intracellular Mn²⁺ 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%⁵. We confirmed that the mechanism of β cell enhancement is due to Mn²⁺ entry through glucose-stimulated β cell Ca²⁺ channels, since mice treated with the Ca²⁺ channel blocker nifedipine have increased intracellular T1 (indicative of lower Mn²⁺ concentration) relative to untreated mice. Maximal β cell labeling with Mn²⁺ one hour after injection identifies an optimal time point for β cell imaging in future studies in mouse models of diabetes.