Look-Locker MRI measurements of relaxation rate after manganese labeling of pancreatic β cells detect increments in disease progression in a mouse model of type 1 diabetes

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Introduction: Type 1 diabetes (T1D) is a metabolic disorder characterized by an inability to maintain blood glucose homeostasis, and it occurs due to the autoimmune destruction of β cells residing in the pancreatic islets of Langerhans. Currently there is no gold standard method for noninvasively detecting changes in functional β cell mass (BCM), which would be important for assessing disease progression, following therapeutic response, and evaluating the viability of transplanted islets. Manganese (Mn^{2+})-enhanced MRI may represent an attractive solution. Mn^{2+} enters β cells through voltage-gated calcium channels (VGCCs) and increases the NMR longitudinal relaxation rate R1. Mn^{2+}-enhanced MRI has previously been used to detect a gross decrease in BCM in mice with streptozotocin-induced severe T1D[1]; however, the sensitivity of Mn^{2+}-enhanced MRI to detect gradual changes in functional BCM that occur during the progression of T1D remains unknown.

Purpose: The purpose of this study was to test the hypothesis that quantitative measurements of the Mn^{2+}-enhanced pancreatic relaxation rate R1 would reflect decremental changes in functional BCM. To that end, we applied Mn^{2+}-enhanced Look-Locker R1 mapping of the pancreas to cytoxan-accelerated BDC2.5 T-cell receptor transgenic mice on a non-obese diabetic (NOD) background, a T1D model which rapidly and predictably progresses toward T1D within 8 days after cytoxan injection[2].

Methods: We performed Mn^{2+}-enhanced Look-Locker MRI in n=26 cytoxan-accelerated NOD-BDC2.5 transgenic mice and in n=24 of their transgene-negative (Tg-) littermates who do not develop T1D within this time frame after cytoxan treatment. To initiate T1D progression, 200mg/kg cytoxan was injected i.p. Imaging studies were performed before cytoxan injection (day 0) and on days 3-7 after injection. MRI was performed on a 7T Clinscan system (Bruker, Etlingen, Germany) using a 30mm inner-diameter mouse whole body RF coil. Mice were injected with glucose (1.5 g/kg) to stimulate β-cell VGCCs and $MnCl_2$ (0.1 μmol/kg) to label β cells. Look-Locker MRI was performed 1 hour after glucose + $MnCl_2$ injection to quantify R1 in the pancreas. The Look-Locker pulse sequence used a non-selective 180° inversion pulse followed by a train of gradient echoes separated by delay times between 30-50 ms. Specific parameters were as follows: time between inversions = 5500 ms, TE =1.9 ms, flip angle = 3°, slice thickness = 1 mm, FOV = 35 mm x 25 mm, number of images = 100, number of averages = 3, and 500 x 500 μm^2 pixel size. A region of interest was drawn around the pancreas to generate a pancreatic T1 relaxation curve which was fit for R1. Pixel-wise R1 maps were also calculated. Statistical testing was performed using two-way ANOVA.

Results: All mice displayed pancreatic Mn^{2+} enhancement, and example pancreatic R1 maps are depicted in Figure 1 for Tg+ and Tg- mice. The measured pancreatic R1s are shown in Figure 2 for Tg+ and Tg- mice on experimental days 0 and 3-7. Pancreatic R1 remained constant after cytoxan injection in Tg- mice and decreased progressively in Tg+ mice, mirroring their T1D disease progression and the confirmed time-course of loss of BCM[2]. Two-way ANOVA revealed significant differences in pancreatic R1 for same-day Tg+ mice vs. Tg- mice for all days except day 0 (*$p < .01$). Pancreatic R1 in day 0 Tg+ mice was statistically different from Tg+ mouse on all other experimental days (#$p < .05$), and pancreatic R1 in day 3 Tg+ mice was significantly different from day 7 Tg+ mice (&$p < .05$).

Discussion: The constant pancreatic R1 in Tg- mice after cytoxan injection was indicative of their retained functional BCM, while the incrementally decreasing pancreatic R1 in Tg+ mice reflected their declining functional BCM during that time. These results support the hypothesis that Look-Locker imaging of the Mn^{2+}-enhanced pancreas has the sensitivity to detect decreases in functional BCM that occur during the progression of T1D prior to the onset of frank diabetes.

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Citations: 1). Antkowiak et al. *Am J Physiol Endocrinol Metab*. 2009; 296(3):E573-8.
2). André-Schmutz et al. *Eur J Immunol.* 1999; 29:245-255.

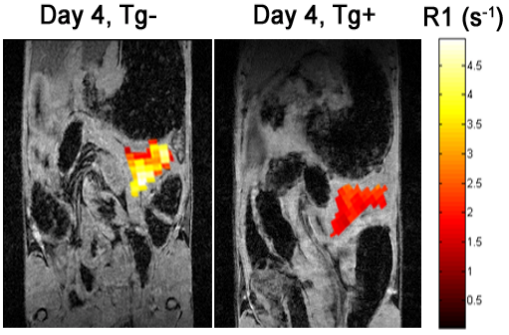

Figure 1: Pancreatic R1 maps of Tg- and Tg+ mice on day 4 after cytoxan injection.

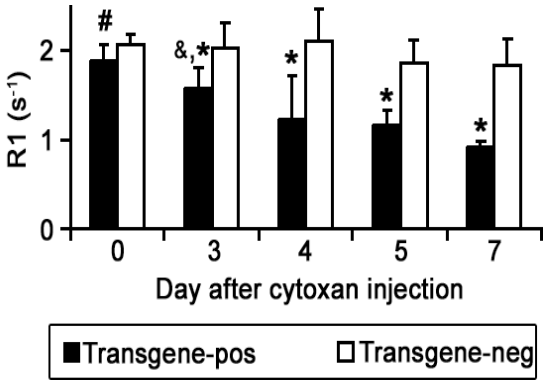

Figure 2: Pancreatic R1 comparison in cytoxan-accelerated NOD-BDC2.5 mice.