MRI with magnetic nanoparticles serves as a biomarker for the inflammation associated with the early, insulitic phase of Type I Diabetes

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Introduction

Type 1 diabetes mellitus (T1D) is an irreversible, chronic disease, that results from autoimmune destruction of the insulin producing beta cells of the pancreatic islets, a true understanding of its immunopathogenesis in humans has remained elusive. No noninvasive means of monitoring the local pancreatic changes associated with diabetes exists, and since T1D is not usually diagnosed until very late in the disease course, most of the events have already played out and options for therapy are limited. Previous techniques used to quantify insulitis (to measure the degree of inflammation and islet destruction) and beta cell mass ((BCM) – a measure to gauge the amount of residual beta cells), are invasive and impractical for most human studies. Imaging may therefore play an important role in quantifying insulitis and BCM. Radionuclide based techniques (e.g. Single Photon Emission Computed Tomography, Positron Emission Tomography) have been developed that are applicable in animal models of diabetes, but have not been translatable to human clinical trials. As novel immunomodulatory therapies evolve in order to prevent beta cell destruction, a noninvasive, accurate means of visualizing, and quantifying BCM and insulitis becomes critical.

We have developed and translated a technique based on the properties of MRI enhanced with magnetic nanoparticles (MNP) to allow indirect quantification and visualization of insulitis in animal models of DM. We validated this MNP-MRI technique by correlating our findings with the standard invasive techniques used to study diabetes, including histology and flow cytometry of pancreas and pancreatic inflammatory infiltrate samples. In rodent models, initiation of insulitis is accompanied by pancreatic microvasculature changes typical of inflammation – in particular, vessel leakiness. These alterations can be detected using long-circulating, phagotropic nanoparticles, which extravasate from the leaky vessels into the surrounding tissue and are engulfed by infiltrating cells, particularly macrophages. MRI quantification of nanoparticle accumulation by T2 quantification was capable of distinguishing major disease landmarks, of reading out disease aggressiveness, and of signaling, early on, responses to immunomodulatory agents -- all without exposure to ionizing radiation. The following work outlines our translation of this methodology to a human clinical trial involving 22 subjects.

Methods

Study participants. N=22 subjects (10 recently diagnosed with T1D, and 12 age matched controls, all age 18 years or older) were enrolled and provided informed consent in this IRB approved study. Subjects in the recent-onset T1D group were within six months of disease diagnosis. The protocols were approved by Joslin Diabetes Center Committee on Human Studies and Massachusetts General Hospital Institutional Review Board.
Magnetic resonance imaging and analyses. The MNP used in this study was ferumoxtran-10 (Combidex, AMAG Pharmaceuticals Inc., Lexington, MA, USA). The MNP was diluted in 100 cc of normal saline and infused at a dose of 2.6 mg of iron per kilogram of body weight over a period of 30 minutes. All imaging was performed on a Siemens 1.5T systems equipped with TIM technology using an 8 channel phased array torso body coil was used for the experimental cohort. The protocol included a modified turbo spin echo (SE) T2 weighted sequence TE/TR ((48, 86, 144)/ 2200ms)) for T2 quantification. A volume interpolated 3D gradient echo sequence was performed for 3D volume estimates of the pancreas (256x192 encoding matrix, asymmetric field of view 30 x 24, TE/TR 2.4/5ms, and voxel size 1.17 x 1.17 x 2.5mm). Image analysis was performed using OsiriX (OsiriX Imaging Software, Geneva, Switzerland) software with custom-made plug-ins for monoexponential fit of T2. Since ferumoxtran-10 is a negative T2 contrast agent, we used changes in T2 measurements as a surrogate for vascular leak/nanoparticle accumulation. We imaged subjects 48 hours following MNP infusion, as this was the optimum point based on our animal models that would capture macrophage accumulation, and minimize retained vascular MNP. We quantified changes in T2 as a surrogate correlate of MNP accumulation, leakiness and insulitis. All images were masked prior to analysis with the readers unaware of the disease status of the study subjects. ROIs for analysis were defined manually on the pancreas or paraspinous muscles on 3 consecutive slices, with only the central slice used for measurement to minimize volume averaging from adjacent tissues. All results were compared to autoantibodies for insulin, GAD, and IA2, which were performed via radioimmunoassays. An unpaired two-tailed t-test with Welch's correction for unequal variance or Mann-Whitney U test was used for comparisons depending on whether distributions conformed to normality as assessed by the Shapiro-Wilk normality test using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). $P \leq 0.05$ was considered statistically significant.

Results

Figure 1a is an illustrative 3D reconstruction for a patient and a control individual before and 48 hours after MNP infusion, showing T2 pseudocolor in the pancreas region overlaid on anatomic T1 images. At baseline, there was no subjective difference in parametric T2 maps between subject and control, with relatively uniform T2 values, while at 48hrs., the patient had evidence of more MNP accumulation, as indicated by a lower T2 (more red coloration) in the region of the pancreas at the 48-hour time point. The delta T2 (Figure 1b) was calculated by subtracting the 48-hour post-contrast T2 value from the pre-contrast T2 value, from matched regions. Scatter plots (Figure 1b) show that there was a significant difference in T2 within a region of interest within the pancreas of patients with recent-onset T1D vs controls: a delta T2 of 14.1 ± 4.7 msec for the former versus 7.1 ± 4.9 msec for the latter (P<0.005). In contrast, there was no difference in T2 within a region of interest in paraspinous muscle: delta T2s of 1.28 ± 0.78 msec and 1.23 ± 0.90 msec, respectively (P=0.784). We did not find a correlation between the delta T2 in patients and daily insulin requirement, time since diagnosis, age, HbA1c, or autoAb titers.

Conclusion

These data demonstrate the successful translation and implementation of a hypothesis underlying the potential pathogenesis associated with T1D, and reveal a potential quantitative approach to distinguishing and monitoring patients in the early, insulitic phase of T1D. As has been demonstrated in previous animal work, the change in T2 may be a surrogate marker of nanoparticle leakage through abnormal vasculature, coupled with uptake by macrophages, findings, which have been noted in humans with T1D histologically. Although provocative and preliminary, these results and their particular biological features require further and more comprehensive investigation.
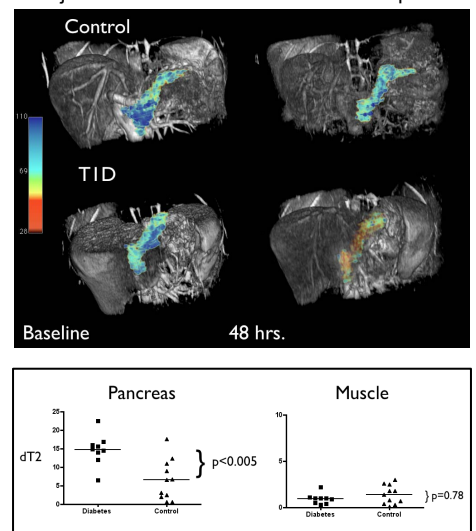

Figure 1a) Immediate and delayed (48hrs post MNP) imaging volumetric parametric T2 maps of the pancreas are shown merged with anatomic imaging. The images on the top are from a control, while the bottom row are from a subject with recent onset diabetes. The red coloration indicates higher R2 which corresponds with increased probe accumulation within the pancreas. 1b) Plots of delta T2 for subjects with T1D vs. control demonstrating a statistically significant difference in the pancreas, and no difference within the muscle.

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