Non-invasive Magnetic Resonance Imaging of Microvascular Changes in Type 1 Diabetes

Zdravka Medarova, Ph.D.\textsuperscript{1}
Gerardo Castillo, Ph.D.\textsuperscript{2}
Guangping Dai, Ph.D.\textsuperscript{1}
Elijah Bolotin, Ph.D.\textsuperscript{2}
Alexei Bogdanov, Ph.D.\textsuperscript{3}
Anna Moore, Ph.D.\textsuperscript{1}\textsuperscript{*}

\textsuperscript{1} Molecular Imaging Laboratory, MGH/MIT/HMS Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital/Harvard Medical School, Boston MA
\textsuperscript{2} PharmaIN, Ltd. (www.pharmain.com), Seattle WA, 98122, USA.
\textsuperscript{3} Department of Radiology, University of Massachusetts Medical School, Worcester, MA

Running Title: Imaging the Vaculature in Type 1 Diabetes

\textsuperscript{*} Correspondence should be addressed to: Anna Moore, Ph.D., Molecular Imaging Laboratory, MGH/MIT/HMS Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital/Harvard Medical School, Rm. 2301, Bldg.149, 13th St., Charlestown, Massachusetts 02129
E-mail: amoore@helix.mgh.harvard.edu

Received for publication 18 June 2007 and accepted in revised form 1 August 2007.

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.
Abstract

Objective: The pathogenesis of type 1 diabetes (T1D) involves autoimmune lymphocytic destruction of insulin-producing beta-cells and metabolic dysregulation. An early biomarker of pancreatic islet damage is islet microvascular dysfunction. Alterations in vascular volume, flow, and permeability have been reported in numerous models of T1D. Consequently, the ability to non-invasively monitor the dynamics of pancreatic microvasculature would aid in early diagnosis and permit the assessment, design, and optimization of individualized therapeutic intervention strategies.

Research Design and Methods: Here, we employed the long-circulating paramagnetic contrast agent, PGC-GdDTPA-F (protected graft copolymer covalently linked to gadolinium-diethylenetriaminepentaacetic acid residues and labeled with fluorescein), for the noninvasive semi-quantitative evaluation of vascular changes in a streptozotocin (STZ)-induced mouse model of T1D. Diabetic animals and non-diabetic controls were monitored by magnetic resonance imaging (MRI) after injection of PGC-GdDTPA-F.

Results: Our findings demonstrated a significantly greater accumulation of PGC-GdDTPA-F in the pancreata of diabetic animals, as compared to controls. MRI permitted the in vivo semi-quantitative assessment and direct visualization of the differential distribution of PGC-GdDTPA-F in diabetic and control pancreata. Ex vivo histology revealed extensive distribution of PGC-GdDTPA-F within the vascular compartment of the pancreas, as well as considerable leakage of the probe into the islet interstitium. By contrast, in non-diabetic controls, PGC-GdDTPA-F was largely restricted to the pancreatic vasculature at the islet periphery.

Conclusions: Based on these observations we conclude that in the STZ model of T1D, changes in vascular volume and permeability associated with early stages of the disease, can be monitored non-invasively and semi-quantitatively by MRI.
Introduction

The rapid rise in the prevalence of diabetes to the estimated level of 230 million sufferers worldwide during the last 20 years has global implications and requires paradigm-shifting approaches to diagnosis, treatment, monitoring and prevention. One approach to the early detection of type 1 diabetes would involve monitoring of changes associated with the pancreatic vasculature. The pancreatic islet is a highly vascularized structure, receiving 10-20% of the blood flow to the pancreas. Typically, islets have one or two afferent arterioles, which give off numerous capillaries to form a glomerular-like network. The development of type 1 diabetes is invariably associated with the early onset of local vascular abnormalities. During the course of type 1 diabetes, the endothelial cell layer, which normally represents a barrier to blood leukocytes, allows T cells to home to, enter and destroy the islets. Changes in vascular permeability in islet blood vessels during the development of Type 1 diabetes have been described in both rats (3; 4) and in mice (5-7). De Papae et al. (3) reported an increase in the permeability of islet capillaries and post-capillary venules at the onset of diabetes in a spontaneous rat model of type 1 diabetes. An increase in islet vascular permeability has also been observed in the diabetes prone BB rat model (4) and in alloxan-induced murine diabetes (6). Notably, this increase represented the first sign of any morphological abnormality in islet function. In addition, an exciting study in an alloxan-induced rat model showed that fractional islet vascular perfusion reached a remarkable 50% compared to 10% in non-diabetic controls, indicating redirection of pancreatic blood flow through the islets (8). Vascular leakiness has also been observed in the streptozotocin (STZ)-induced diabetes model. A single high dose of the beta-cell toxin, which is the model used in our study, caused a significant increase in islet capillary permeability (5; 7; 9) detected as early as 1 hr after STZ administration (9). Importantly, increased islet microvascular permeability was seen before the animals had become diabetic and before signs of pancreatic insulitis (5). These studies suggest a strong link between the increase in vascular flow and permeability and type 1 diabetes. Furthermore, it appears that changes in the islet microvasculature precede other symptoms of the disease, such as advanced inflammation, hyperglycemia, and morphological abnormalities. Consequently, the local microvasculature represents an excellent diagnostic biomarker for the earliest stages of islet dysfunction. However, despite the important role of the microvasculature in the pathogenesis of type 1 diabetes, the extent and time course of pancreatic microvascular changes are still unknown.

All of the above-cited studies on islet vasculature are generally limited to histological post-mortem findings, which suffer from the need for tissue processing and the inability to study the dynamics of blood flow. In this context non-invasive imaging seems to be the most appropriate approach to studying the dynamics of the islet vasculature in vivo. Previous studies utilizing superparamagnetic iron oxides for monitoring the blood pool in a model of diabetes did not permit the direct visualization of the vasculature through angiography due to the negative nature of the T2 contrast agent used in this study (10; 11). Here, we describe a novel approach for the characterization of
pancreatic vascular changes in a model of type 1 diabetes using high-resolution in vivo magnetic resonance imaging (MRI) in combination with a novel nanocarrier delivery system. We utilized a nano-sized protected graft co-polymer (12) (FITC-labeled PGC-GdDTPA, hereafter designated PGC-GdDTPA-F) as a blood pool imaging agent for the delivery of a T1 positive paramagnetic contrast agent (Gd-DTPA) to image the vascular compartment. PGC-GdDTPA has a large hydrodynamic diameter corresponding to a globular protein of 1500 kDa and carries protective groups of polyethylene glycol (PEG) for minimal uptake by the reticuloendothelial system. It selectively distributes within the vascular bed without any initial leakage from blood vessels, and can be used to enhance both the arterial and venous vascular compartments with submillimeter resolution (13; 14). The application of PGC-GdDTPA as a blood pool agent has been studied in detail in models of tumor vascular dysfunction (13; 15-17) and angiogenesis (14).

In the presence of inflammation, PGC-GdDTPA accumulates extensively in areas of high capillary permeability and increased blood flow, including outside the vascular compartment, due to leakage across the hyperpermeable vascular endothelium (12). This effect and its application in the context of MRI and nuclear imaging have been applied in a model of soft-tissue bacterial infection (18).

Here, we describe the application of PGC-GdDTPA in a model of type 1 diabetes. This is the first report of non-invasive blood-volume imaging for the semi-quantitative in vivo definition of pancreatic microvasculature dynamics in type 1 diabetes. We believe that this study provides valuable information on islet vasculature during diabetes progression that could serve not only as a scientific research tool but also as a practical way to stage and monitor islet inflammation and vascular dysfunction in a future clinical scenario.

**Research Design and Methods**

**Imaging agent.**

Protected graft copolymer bearing covalently linked gadolinium-diethylenetriaminepentaacetic acid residues and conjugated to the fluorescent dye FITC (PGC-GdDTPA-F) was obtained from PharmaIN, Ltd (Seattle, WA).

**Animals and Treatment.**

Five-wk old female Balb/c mice (n = 6, weight ~ 20g) were rendered diabetic by i.p. injection of 200mg/kg of the beta-cell toxin streptozotocin (STZ). On the next day, diabetic animals were imaged before as well as 1, 17h, and 40 h after i.v. injection of PGC-GdDTPA-F (0.2mmol Gd/kg). Age matched non-diabetic animals injected with PGC-GdDTPA-F and imaged at the same time points served as controls (n = 4).

Fasting blood glucose levels were determined 24 hrs after STZ administration using Glucometer Elite Testing System (Bayer Diagnostics, Tarrytown, NY).

All animal experiments were performed in compliance with institutional guidelines approved by the Subcommittee on Research Animal Care at the Massachusetts General Hospital and in accordance with the ‘Principles of laboratory animal care’ (NIH publication no. 85–23, revised 1985).

**In vivo MR Imaging**

MR imaging was performed on a 9.4T Bruker horizontal bore scanner (Billerica, MA) equipped with a home-built RF transmit and receive 3 x 4-cm
elliptical surface coil and using ParaVision 3.0 Software. For in vivo imaging of diabetic and control mice we obtained T1 maps, T1 weighted images and low- and high-resolution 3D-angiograms. Details on image sequences and image analysis are presented in Supplemental Materials on-line (available at http://diabetes.diabetesjournals.org).

PGC-GdDTPA-F localization in the pancreas by histology.

PGC-GdDTPA-F was synthesized to carry a fluorescent label (FITC). Fluorescence microscopy on pancreata from experimental and control animals was performed in two channels (FITC for PGC-GdDTPA-F and DAPI for nucleus). Consecutive sections were stained with hematoxylin & eosin (H&E) and analyzed by light microscopy. Additional tissue sections were stained for insulin and analyzed by fluorescence microscopy. Details on the staining procedures are presented in Supplemental Materials on-line.

Results

To evaluate microvascular changes in diabetic pancreas we utilized the long-circulating paramagnetic T1 contrast agent, PGC-GdDTPA-F. PGC-GdDTPA-F was administered to STZ-induced diabetic mice (FBG = 287.8±186.9 mg/dL; range: 24-450 mg/dL) and non-diabetic controls (FBG = 114.0±22.1 mg/dL; range: 94-141 mg/dL). Our results demonstrated sufficient accumulation of the agent in the pancreas for detection by MRI. On T1-weighted images obtained 17 hrs after injection of the contrast agent into STZ-induced diabetic animals, the pancreas was clearly enhanced compared to pre-injection images, due to the T1-shortening effect of Gd-DTPA (Fig. 1A).

Quantitative time-course analysis of T1 relaxation times was based on inversion-recovery T1 maps (Fig. 1B). These studies indicated accumulation of PGC-GdDTPA-F in the pancreata of both diabetic animals and non-diabetic controls, as early as 1hr post-diabetic, reflective of presence of the contrast agent in the blood pool. The peak of contrast agent bioavailability in the pancreas was reached 17 hrs after injection, followed by a gradual wash-out by 40 hrs (Fig. 1C).

While we observed the expected vascular enhancement in both animal groups, the levels of PGC-GdDTPA-F in the pancreata of diabetic animals were significantly higher than in non-diabetic controls (p < 0.05), consistent with increased blood volume and vascular permeability. This difference was reflected by a lower T1 in diabetic mice, compared to controls and was seen at 1hr (diabetic T1 = 385.9 ± 30.1 ms; non-diabetic T1 = 501.3 ± 43.3 ms) and 17 hrs (diabetic T1 = 193.7± 3.5 ms; non-diabetic T1 = 342.5± 52.8 ms) after injection of PGC-GdDTPA-F (Fig. 1C).

To monitor the whole-body vascular distribution of PGC-GdDTPA-F, we performed low-resolution 3D-angiography of the same animals at the specified time-points. As seen in Figure 2, using a 0.4 x 0.4 x 0.4mm.pixel\(^{-1}\) resolution, we could see significant enhancement in the upper left abdomen, consistent with the location of the pancreas, in diabetic animals but not in non-diabetic controls. The time-course of this enhancement closely matched our T2 map analysis. It became apparent at 1 hr post-injection of PGC-GdDTPA-F, increased at 17 hrs, and became negligible by 40 hrs after contrast agent delivery (Fig. 2).

For more precise identification of the origin of this signal, we imaged
diabetic mice and controls by high-resolution 3D-angiography. Brightly-enhancing arteries and less-enhancing veins could be distinguished at the 0.1 x 0.1 x 0.1mm.pixels⁻¹ resolution. The hepatic vasculature was clearly identifiable in both experimental and control animals, as a result of contrast agent availability in the blood pool. Notably, we observed a clear and remarkable enhancement in the area of the pancreas in diabetic animals due to the vascular leakage of the agent as early as 1 hr after injection, followed by a wash-out from the pancreas by 40 hrs (Fig. 3 and Suppl. Movie 1 and 2). No such enhancement in the pancreas was observed in control animals. As expected based on the long circulation half-life of PGC-GdDTPA-F (14 hrs in rodents (12)), residual levels of contrast agent in the larger hepatic arteries caused the enhancement of these vessels to persist even at the 40-hr time point.

Finally, to independently validate our findings, we correlated our imaging conclusions with gold-standard histological measurements of the tissue distribution of the contrast agent. On H&E sections, we could identify pancreatic islets adjacent to islet-feeding pancreatic blood vessels (Figure 4A). In STZ-induced diabetic mice, however, pancreatic islets were less abundant and islet morphology appeared abnormal (Figure 4A). The detrimental effect of STZ on islet architecture was further confirmed by staining for insulin (Supplemental Figure 1). Fluorescence microscopy demonstrated a significant accumulation of PGC-GdDTPA-F in the pancreata of diabetic animals. There was bright green fluorescence, associated with the presence of PGC-GdDTPA-F, in blood vessels feeding pancreatic islets. Fluorescence intensity was markedly enhanced in experimental vs. control animals, consistent with increased vascular volume (Figure 4B, Supplemental Figure 2). Diffuse green fluorescence associated with the islet interstitium and present exclusively in tissue sections from diabetic but not control animals was consistent with extravascular leakage of the contrast agent. In control animals, fluorescence derived from PGC-GdDTPA-F was restricted to the islet periphery (Figure 4C).

Discussion
Accumulating evidence suggests that the vascular endothelium is of crucial importance for the development of inflammatory response in the pancreas. In models of type 1 diabetes, modifications of the pancreatic microvasculature accompany the initiation and progression of disease. Vascular swelling and increased blood flow precede insulitis in nonobese diabetic (NOD) mice and in streptozotocin-induced diabetes (5; 7; 9; 19; 20). Consequently, pancreatic islet vascular dysfunction is a crucial element of the pathology of type 1 diabetes and, therefore, represents an early and potentially predictive biomarker for the loss of beta cell mass. In addition, vascular changes have also been reported in type 2 diabetes. Augmented islet blood flow has been demonstrated after short-term modest hyperglycemia, as well as in several animal models of Type 2 diabetes, including the Goto-Kakizaki (GK) rat (21) and the obese hyperglycemic mouse (22).

Overall, pancreatic vascular dysfunction seems to play a critical role in both types of diabetes and represents a complex multifactorial system that cannot be studied without a comprehensive systematic approach. In vivo MRI has been widely used to characterize vascular events in the brain and in various types of
tumors (23-25). Here we apply this methodology to study the islet vasculature. To the best of our knowledge, this represents the first application of magnetic resonance angiography to visualize islet vascular dysfunction using paramagnetic blood pool imaging agent. Differential accumulation of PGC-GdDTPA-F in the pancreatic area detected by this technique was clearly identifiable in STZ-induced diabetic animals but not in healthy controls. In our experiments, we utilized the exaggerated single-high-dose STZ model of diabetes because it provides a solid framework for the establishment of proof-of-principle for our imaging method. The next logical step in our studies, however, is to test the feasibility of this approach in the multiple-low-dose STZ model, NOD mice as well as in models of type 2 diabetes. These models more closely resemble the human pathology and, therefore, such studies would help assess the sensitivity of this method and its feasibility in a potential future clinical scenario. Furthermore, since the observed effects are the result of two separate events (increased vascular volume and permeability), further studies would be necessary to unravel the relative input of each. We believe that this imaging strategy represents a valuable research tool for studying; 1) the time-course of the disease, 2) the role of the vasculature in the insulitic process and islet cell death relevant to type 1 diabetes, and 3) the vascular response to metabolic dysregulation in both type 1 and type 2 diabetes. In a therapeutic scenario, the described methodology has implications for 1) the early diagnosis of diabetes, 2) the monitoring of events relevant to islet transplantation and other diabetes therapies, and 3) the design of image-guided individualized treatment strategies.

**Acknowledgements**

The authors would like to acknowledge John Moore and Pamela Pantazopoulos (Athinoula A. Martinos Center for Biomedical Imaging, MGH) for excellent technical support with animal surgery. This work has been supported in part by NIDDK NIH Grant DK069727 to E.B.
Imaging the Vacuature in Type 1 Diabetes

References
Figure Legends

**Figure 1.** T1 weighted MR imaging of PGC-GdDTPA-F accumulation in the diabetic pancreas. A: Transverse T1-weighted MR images of an STZ-diabetic mouse obtained before and 17 hrs after injection of PGC-GdDTPA-F. The area of the pancreas is outlined. Note the increase in signal intensity after injection of the contrast agent due to local accumulation of the agent. K: kidney, S: spleen. B: Coronal T1 maps of an STZ-diabetic mouse obtained before, as well as 1, 17, and 40 hrs after injection of PGC-GdDTPA-F. The area of the pancreas is color-coded based on its T1 relaxation time. V: stomach, S: spleen, P: pancreas. C: Quantitative analysis of T1 relaxation times of the pancreas of STZ-diabetic and control mice. There was a drop in the T1 of the pancreas at 1 hr after injection of PGC-GdDTPA-F. This drop was most significant at 17 hrs after injection and was followed by a gradual increase, consistent with contrast agent wash-out. T1 map analysis demonstrated a significant difference in the T1 of the pancreas between diabetic and control animals at 1 and 17 hrs after injection of PGC-GdDTPA-F, reflecting a higher accumulation of the contrast agent in diabetic pancreata. **, p < 0.05.

**Figure 2.** Low-resolution 3D MR angiography of of STZ-diabetic mice (top) and non-diabetic controls (bottom) obtained 1, 17, and 40 hrs after injection of PGC-GdDTPA-F. The pancreas is enhanced in diabetic animals at 1hr post-injection (arrows), followed by a wash-out by 40 hrs. No clear enhancement of the pancreas is seen in non-diabetic controls. L, left; R, right; H, head; T, tail.

**Figure 3.** High-resolution 3D MR angiography of STZ-diabetic mice (top) and non-diabetic controls (bottom) obtained 1 and 40 hrs after injection of PGC-GdDTPA-F. The hepatic vasculature is outlined following injection of the contrast agent (arrowheads). In diabetic animals, the pancreas is associated with bright diffuse enhancement at 1hr post-injection (arrow), consistent with increased vascular volume and leakage into the interstitium. This is followed by a wash-out by 40 hrs. Non-diabetic animals showed no enhancement in the area of the pancreas. V: stomach, K: kidney.

**Figure 4.** Histology of pancreata derived from STZ-diabetic and non-diabetic control mice. A: Pancreatic islets adjacent to blood vessels could be identified in H&E sections. B: Bright green fluorescence could be seen in islet-feeding blood vessels. Fluorescence intensity was markedly higher in diabetic than control animals, suggesting greater vascular volume in diabetic mice. C: Diffuse green fluorescence associated with the islet interstitium was seen in diabetic but not control mice indicating extra-vascular leakage of PGC-GdDTPA-F. By contrast, in non-diabetic controls, green fluorescence was restricted to the islet periphery. A and B, magnification bar, 50 µm; C, magnification bar, 20 µm.
Figure 1
Figure 2
Figure 3

Imaging the Vaculature in Type 1 Diabetes

1h

40h

diabetic

non-diabetic

V

K

V

K

V

K

V

K
Figure 4

A. H&E

B. PGC-GdDTPA-F

C. PGC-GdDTPA-F