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Imaging the pancreatic vasculature in diabetes models

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Abstract

Background—Vascular parameters, such as vascular volume, flow, and permeability, are important disease biomarkers for both type 1 and type 2 diabetes. Therefore, it is essential to develop approaches to monitor the changes in pancreatic microvasculature non-invasively.

Methods—Here, we describe the application of the long-circulating, paramagnetic T1 contrast agent, protected Graft Copolymer bearing covalently linked gadolinium diethylenetriaminepentaacetic acid residues and labelled with fluorescein (PGC-GdDTPA-F) for the non-invasive semi-quantitative evaluation of vascular changes in diabetic models using magnetic resonance imaging.

Results—We observed a significantly higher accumulation of protected graft copolymer bearing covalently linked gadolinium diethylenetriaminepentaacetic acid residues and labelled with fluorescein in the pancreata of BBDR rats induced to develop diabetes, as compared to non-diabetic controls at 1 h post-injection. No differences were seen in the blood pool, kidney, or muscle, indicating that the effect is specific to the diabetic pancreas. Fluorescence microscopy revealed a marked increase in contrast agent availability in the pancreas with the development of the pathology. Similar changes were noted in the homozygous *Lepr^{db}* mouse model of type 2 diabetes. This effect appeared to result both from the increase of vascular volume and permeability.

Conclusions—High-molecular weight paramagnetic blood volume contrast agents are valuable for the *in vivo* definition of pancreatic microvasculature dynamics by magnetic resonance imaging. The increase in vascular volume and permeability, associated with diabetic inflammation, can be monitored non-invasively and semi-quantitatively by magnetic resonance

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Conflict of interest

Zdravka Medarova, Marytheresa Ifediba, Guangping Dai and Mohanraja Kumar performed the *in vivo* experiments, MRI scanning and histology; Elijah Bolotin, Gerardo Castillo and Alexei Bogdanov synthesized the probe; Dale L. Greiner produced the BBDR animal model and participated in experimental design; Anna Moore conceived the idea of the project. Anna Moore and Zdravka Medarova wrote the manuscript.

imaging in diabetic BBDR rats. This imaging strategy represents a valuable research tool for better understanding of the pathologic process.

Keywords

imaging; inflammation; vasculature; diabetes; protected graft copolymer

Introduction

In terms of development, type 1 and type 2 diabetes share a common trait. Both distinct pathologies include the early onset of local vascular dysfunction and suggest a systemic approach to the disease with an emphasis on microenvironmental changes of the microvasculature.

In type 1 diabetes, the increase in vascular permeability, migration of exogenous mononuclear cells into the endocrine pancreas and the destruction of vascular endothelial cells in an islet antigen-dependent manner suggest the presence of an inflammatory response and vascular permeability defects [1–5]. In type 2 diabetes, a process of microangiopathy takes place that results in islet blood flow alterations. The increased blood flow during the early phase of diabetes development ultimately leads to a loss of islet architecture [6].

These properties of the altered islet vasculature could be exploited for the early diagnosis of the disease. We have previously shown by *in vivo* magnetic resonance imaging (MRI) that the long-circulating blood pool agent PGC [7], labelled with gadolinium, accumulates in the areas of inflammation and altered capillary permeability in the islets of streptozotocin-induced diabetic mice via the enhanced permeability and retention effect [8].

Here, we extend these studies to more clinically relevant models of diabetes. *In vivo* MRI of BBDR rats induced to develop autoimmune type 1 diabetes by depletion of regulatory ART2⁺ T cells [9,10] demonstrated that there are distinct changes in the vascular permeability of the islet blood vessels during the development of disease in these animals, which was confirmed by histology. Furthermore, histological examination of pancreatic tissue from homozygous *Lepr^{db}* mice with type 2 diabetes after PGC-GdDTPA-F injection showed a similar trend. We believe that these studies could provide further insight into the anatomical and hemodynamic changes of the diabetic vasculature.

Materials and methods

Imaging agent

PGC-GdDTPA and PGC-GdDTPA conjugated to fluorescein isothiocyanate (PGC-GdDTPA-F) was obtained from PharmaIN, Corp. (Seattle, WA, USA).

Animals and treatment

Autoimmune diabetic BBDR rats (insulinitis and diabetes) obtained from Biomedical Research Models, Inc (Worcester, MA, USA) were generated by injecting 50 µg of the purified DS4.23 anti-ART2a monoclonal antibody, intraperitoneally five times per week and 1 mg/gm of body weight of polyinosinic-polycytidylic acid three times per week. Close to 100% of BBDR animals became diabetic by 14 days after treatment initiation [9,10]. Diabetic BBDR rats were maintained on insulin pellets until imaged to prevent death from acute ketoacidosis. Non-induced BBDR rats were used as controls (*n*=6).

Animals were imaged before as well as 1 and 24 h after intravenous injection of PGC-GdDTPA-F at a dose of 0.2 mmol gadolinium per kilogram.

Type 2 diabetes was studied in 10-week-old B6.BKS-*Lepr^{db}* (abbreviated *Lepr^{db}*) diabetic mice and healthy background controls (C57BLKS/J) injected with the same dose of the probe ($n=4$). Both strains were obtained from The Jackson Laboratory.

Fasting blood glucose levels were determined using Glucometer Elite Testing System (Bayer Diagnostics, Tarrytown, NY, USA).

All animal experiments were performed in compliance with institutional guidelines approved by the Subcommittee on Research Animal Care at the Massachusetts General Hospital and by the Animal Care and Use Committee at the University of Massachusetts Medical School in accordance with the 'Principles of laboratory animal care' (NIH publication no. 85-23, revised 1985).

***In vivo* MRI**

MRI was performed on a 9.4T Bruker horizontal bore scanner (Billerica, MA, USA) equipped with a home-built RF transmit and receive 3×4-cm elliptical surface coil using ParaVision 3.0 Software. Three dimensional-angiograms were obtained using a gradient echo imaging sequence comprising first-order flow compensation. The following imaging parameters were utilized: TE = 2.43 ms, TR = 40 ms, FOV = 38.4 × 50.0 × 38.4 mm³, spatial resolution = 0.3 = 0.312 × 0.3 mm/pixel, matrix size 128 × 160 × 128, and a total imaging time of 13 min 32 s. Image reconstruction was performed using a maximum intensity projection algorithm. For quantitative analysis, colour-coded maps were constructed using Marevisi 3.5 software (Institute for Biodiagnostics, National Research Council, Canada). The pancreas image was manually segmented and subjected to region-of-interest (ROI) analysis for the determination of pancreas-associated signal intensity. The inferior vena cava, muscle tissue, and ipsilateral kidney were also segmented out and subjected to ROI analysis of signal intensity for the purposes of signal normalization.

Histology

Pancreata from experimental and control animals were removed, immediately snap frozen, and cut into 5 µm sections. For PGC-GdDTPA-F localization in tissue, tissue sections were fixed for 20 min in ice-cold acetone, washed with PBS and mounted with a 4,6 diamidino-2-phenylindole-containing mounting medium (Vectashield, Vector Laboratories, Burlingame, CA, USA) for nuclear staining. Additional tissue sections were stained for the vascular marker PECAM-1. Sections were incubated with a mouse anti-rat PECAM-1-phycoerythrin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Fluorescence microscopy was performed in two channels (fluorescein isothiocyanate for PGC-GdDTPAF, red for PECAM-1, and 4,6 diamidino-2-phenylindole for nucleus) using a Nikon Eclipse 50i fluorescence microscope equipped with an appropriate filter set (Chroma Technology Corporation, Rockingham, VT, USA). Images were acquired using a CCD camera with near infrared sensitivity (SPOT 7.4 Slider RTKE; Diagnostic Instruments, Sterling Heights, MI, USA) and analysed using SPOT 4.0 advanced version software (Diagnostic Instruments).

Consecutive sections were stained with haematoxylin and eosin and analysed by light microscopy.

Statistical analysis

All data were represented as means ± standard deviation. Statistical analysis was performed using two-tailed Student's *t* test. A *p*-value 0.05 was considered statistically significant.

Results

Steady-state MRI was employed in order to assess the *in vivo* bioavailability of the blood pool agent PGC-GdDTPA-F. Since this is a large-molecular weight, long-circulating construct with a blood half-life of 14 h in rats [7], it permitted the visualization of larger blood vessels and the detection of tissues characterized by increased vascular permeability, using the selected MRI protocols.

We observed a strong increase in magnetic resonance signal intensity associated with the pancreatic ROI in the diabetic animals that was not present in the healthy controls in three-dimensional TOF angiograms (Figure 1A and B). The enhancement was present 1 h after the injection of PGC-GdDTPA-F and appeared to originate from blood vessels feeding the pancreas. This effect could be due to phenomena such as vasodilation, local vascular hyperpermeability, or both.

Quantitative evaluation of the pancreatic ROI relative to regions of interest involving the blood pool, muscle, or kidney tissues revealed a significantly higher accumulation of PGC-GdDTPA-F in the pancreata of diabetic animals, as compared to non-diabetic controls at 1 h post-injection. No differences were seen in the blood pool, kidney, or muscle, indicating that the effect is specific to the diabetic pancreas (Figure 2). By 24 h after injection of the contrast agent, no differences in magnetic resonance signal enhancement between diabetic and control animals were observed.

Closer examination of the kinetics of PGC-GdDTPA-F accumulation in the pancreas suggests that while in the healthy animals there is a steady increase in signal intensity even at 24 h, in the diabetic animals there is a trend towards a plateau of the signal. This could reflect a continued build-up of the contrast agent in the pancreata of healthy animals through intact vasculature in contrast to a slow interstitial leakage in their diabetic counterparts (Figure 2).

These observations were validated using *ex vivo* histology. Since PGC-GdDTPA-F carries a fluorescein label, it can be detected in tissue sections using fluorescence microscopy. In healthy animals, fluorescence from the agent was restricted to larger blood vessels within the pancreas. Surprisingly, in the diabetic rats higher levels of fluorescence were visible throughout the tissue including both its endocrine and exocrine components (Figure 3A).

To determine the extent of PGC-GdDTPA-F extravasation we co-stained the pancreatic tissue sections with the vascular marker, PECAM-1. As seen in Figure 3B, the distribution of the contrast agent was largely restricted to the islet microvasculature in the healthy rats. In contrast, there was visible leakage of PGC into the islet interstitium, suggesting a relative increase in vascular permeability (Figure 3B).

Similar findings were observed in the Leprdb mouse model of type 2 diabetes (Figure 4). There was a pronounced leakage of PGC-GdDTPA-F in the diabetic islets compared to the healthy controls in which the probe was confined only to the vasculature.

Discussion

Despite significant interest in the islet vascular system in diabetes, there have been no longitudinal quantitative *in vivo* studies to date investigating the local circulation in the pancreatic islets during the development of type 1 and type 2 diabetes. The vast majority of the studies are performed either on histological specimens or using microspheres [11–15]. These techniques do not allow for correlation between capillary area and blood flow or for dynamic tracking of the changing vasculature during diabetes progression. Therefore, a systematic *in vivo* imaging approach is highly desirable to understand the heterogeneity of

the vasculature during diabetes progression, with particular emphasis on the correlation of vascular permeability, blood flow, and other vascular parameters with inflammation and glycemic status.

Recent advances in imaging techniques have enabled the non-invasive assessment of tissue vasculature. These techniques include X-ray angiography, X-ray computed tomography [16,17], positron emission tomography [18,19], single photon emission computed tomography, and MRI.

Among these techniques, MRI has the benefit of exquisite soft-tissue contrast and high spatial resolution (voxel sizes of 20 μm^3 are now technically achievable) [20–22], which is important for microvasculature imaging in small animals. In addition, the remarkable flexibility of MRI is a significant advantage, in that this single modality can provide several spatially coincident microvascular characteristics within a single session.

Our previous studies have shown that using PGC-GdDTPA-F, it is possible to obtain accurate assessment of tissue blood volume under steady-state conditions in models of tumour vascular dysfunction [23,24] and angiogenesis [25,26]. Here, we extend the application of this contrast agent to models of type 1 and type 2 diabetes to document the increase in vascular volume and permeability associated with these diabetic conditions. These results suggest that the vascular changes in the pancreas of diabetic animals allowed for accumulation of PGC-GdDTPA-F following intravenous administration, a feature that could help us gain insight into the underlying causes, mechanisms, and dynamics of vascular dysfunction and exploit it for delivering therapeutics.

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References

1. De Papae M, Coriveau M, Tannous W, Seemayer T, Colle E. Increased vascular permeability in pancreas of diabetic rats: detection with high resolution protein-A gold cytochemistry. *Diabetologia*. 1992; 35:1118–1124. [PubMed: 1478363]
2. Majno G, Joris I, Handler E, Desemone J, Mordes J, AA R. A pancreatic venular defect in the BB/Wor rat. *Am J Pathol*. 1987; 128:210–214. [PubMed: 3618725]
3. Sandler S, Jansson L. Vascular permeability of pancreatic islets after administration of streptozotocin. *Virchows Arch A [Pathol Anat]*. 1985; 407:359–367.
4. Jansson L, Sandler S. Alloxan-induced diabetes in the mouse: time course of pancreatic B-cell destruction as reflected in an increased vascular permeability. *Virchows Arch A [Pathol Anat]*. 1986; 410:17–21.
5. Beppu H, Maruta K, Kurner T, Kolb H. Diabetogenic action of streptozocin: essential role of membrane permeability. *Acta Endocrinol (Copenh)*. 1987; 114:90–95. [PubMed: 2949474]
6. Ehses J, Calderari S, Irminger J, et al. Islet inflammation in type 2 diabetes (T2D): from endothelial to β -cell dys-function. *Curr Immunol Rev*. 2007; 3:216–232.
7. Bogdanov AJ, Weissleder R, Frank H, et al. A new macromolecule as a contrast agent for MR angiography: preparation, properties, and animal studies. *Radiology*. 1993; 187:701–706. [PubMed: 8497616]
8. Medarova Z, Castillo G, Dai G, Bolotin E, Bogdanov AJ, Moore A. Noninvasive magnetic resonance imaging of microvascular changes in type 1 diabetes. *Diabetes*. 2007; 56:2677–2682. [PubMed: 17682091]

9. Mordes, JP.; Poussier, P.; Rossini, A.; Blankenhorn, E.; Greiner, D. Rat models of type 1 diabetes: genetics, environment, and autoimmunity. In: Shafrir, E., editor. In *Animal Models of Diabetes: Frontiers in Research*. 2nd edition. CRC Press Taylor & Francis Group; Boca Raton, FL: 2007. p. 1-39.
10. Mordes, J.; Serreze, D.; Greiner, D.; Rossini, A. Animal models of autoimmune diabetes mellitus. In: LeRoith, D.; Taylor, S.; Olefsky, J., editors. In *Diabetes Mellitus. A Fundamental and Clinical Text*. Lippincott Williams & Wilkins; Philadelphia, NY: 2004. p. 591-610.
11. Carlsson P, Sandler S, Jansson L. Pancreatic islet blood perfusion in the nonobese diabetic mouse: diabetes-prone female mice exhibit a higher blood flow compared with male mice in the prediabetic phase. *Endocrinology*. 1998; 139:3534–3541. [PubMed: 9681505]
12. Jansson L, Bodin B, Carlsson P. Changes in graft blood flow early after syngeneic rat pancreas-duodenum transplantation. *Ups J Med Sci*. 2005; 110:57–67. [PubMed: 15801686]
13. Sandler S, Jansson L. Blood flow measurements in autotransplanted pancreatic islets of the rat. Impairment of the blood perfusion of the graft during hyperglycemia. *J Clin Invest*. 1987; 80:17–21. [PubMed: 3110210]
14. Carlsson PO, Bodin B, Andersson A, Jansson L. Carbon monoxide and pancreatic islet blood flow in the rat: inhibition of haem oxygenase does not affect islet blood perfusion. *Scand J Clin Lab Invest*. 2006; 66:543–548. [PubMed: 17101545]
15. Svensson A, Ostenson C, Efendic S, Jansson L. Effects of glucagon-like peptide-1 (7–36)amide on pancreatic islet and intestinal blood perfusion in Wistar rats and diabetic GK rats. *Clin Sci*. 2006; 112:345–351. [PubMed: 17087662]
16. McNeel DG, Eickhoff J, Lee FT, et al. Phase I trial of a monoclonal antibody specific for alphavbeta3 integrin (MEDI-522) in patients with advanced malignancies, including an assessment of effect on tumor perfusion. *Clin Cancer Res*. 2005; 11:7851–7860. [PubMed: 16278408]
17. Kindlmann GL, Weinstein DM, Jones GM, Johnson CR, Capecchi MR, Keller C. Practical vessel imaging by computed tomography in live transgenic mouse models for human tumors. *Mol Imaging*. 2005; 4:417–424. [PubMed: 16285903]
18. Denoyer D, Perek N, Le Jeune N, Dubois F. Spectrum of radiopharmaceuticals in nuclear oncology. *Curr Cancer Drug Targets*. 2006; 6:181–196. [PubMed: 16712456]
19. Chen X. Multimodality imaging of tumor integrin alphavbeta3 expression. *Mini Rev Med Chem*. 2006; 6:227–234. [PubMed: 16472190]
20. Jacobs RE, Fraser SE. Magnetic resonance microscopy of embryonic cell lineages and movements. *Science*. 1994; 263:681–684. [PubMed: 7508143]
21. Johnson GA, Benveniste H, Black RD, Hedlund LW, Maronpot RR, Smith BR. Histology by magnetic resonance microscopy. *Magn Reson Q*. 1993; 9:1–30. [PubMed: 8512830]
22. Smith BR, Johnson GA, Groman EV, Linney E. Magnetic resonance microscopy of mouse embryos. *Proc Natl Acad Sci USA*. 1994; 91:3530–3533. [PubMed: 8170941]
23. Donahue KM, Weisskoff RM, Chesler DA, et al. Improving MR quantification of regional blood volume with intravascular T1 contrast agents: accuracy, precision, and water exchange. *Magn Reson Med*. 1996; 36:858–867. [PubMed: 8946351]
24. Kim YR, Savellano MD, Weissleder R, Bogdanov A Jr. Steady-state and dynamic contrast MR imaging of human prostate cancer xenograft tumors: a comparative study. *Technol Cancer Res Treat*. 2002; 1:489–495. [PubMed: 12625776]
25. Kim Y, Yudina A, Figueiredo J, et al. Detection of early antiangiogenic effects in human colon adenocarcinoma xenografts: in vivo changes of tumor blood volume in response to experimental VEGFR tyrosine kinase inhibitor. *Cancer Res*. 2005; 65:9253–9260. [PubMed: 16230386]
26. Lewin M, Bredow S, Sergeev N, Marecos E, Bogdanov AJ, Weissleder R. In vivo assessment of vascular endothelial growth factor-induced angiogenesis. *Int J Cancer*. 1999; 83:798–802. [PubMed: 10597197]

Figure 1.

T1-weighted magnetic resonance imaging of PGCGdDTPA-F accumulation in the pancreas of diabetic and non-diabetic control BBDR rats. (A) Colour-coded three-dimensional angiograms of diabetic and non-diabetic control BBDR rats obtained 1 and 24 h after injection of protected graft copolymer bearing covalently linked gadolinium diethylenetriaminepentaacetic acid residues and labelled with fluorescein. The area of the pancreas is outlined. Note the enhancement of blood vessels of the pancreas (arrows). IVC: inferior vena cava, S: stomach; K: kidney. Colour-coding is based on signal intensity. (B) maximum intensity projections derived from three-dimensional angiograms of diabetic and non-diabetic control rats obtained 1 and 24 h after injection of protected graft copolymer bearing covalently linked gadolinium diethylenetriaminepentaacetic acid residues and labelled with fluorescein. The area of the pancreas is outlined. In the diabetic animals, blood vessels feeding the pancreas can be visualized based on their strong enhancement. This effect was not seen in the non-diabetic controls

Figure 2.

Quantitative analysis of signal intensity derived from three-dimensional angiography. In diabetic BBDR rats (squares), there is an increase in the signal intensity of the pancreas at 1 h after the injection of protected graft copolymer bearing covalently linked gadolinium diethylenetriaminepentaacetic acid residues and labelled with fluorescein. This increase was followed by a gradual decrease, consistent with a slow contrast agent wash-out. There is a significant difference in the signal intensity of the pancreas between diabetic and control non-diabetic animals (diamonds) at 1 h after injection of protected graft copolymer bearing covalently linked gadolinium diethylenetriaminepentaacetic acid residues and labelled with fluorescein, reflecting a higher accumulation of the contrast agent in diabetic pancreata. ****p < 0.05**. There were no differences in the signal intensity of blood (inferior vena cava), muscle, or kidney, indicating that the effect was specific to the pancreas

Figure 3.

(A) Histology of pancreata derived from diabetic and non-diabetic control BBDR rats 1 h after the injection of protected graft copolymer bearing covalently linked gadolinium diethylenetriaminepentaacetic acid residues and labelled with fluorescein (PGC-GdDTPA-F). In the haematoxylin and eosin-stained sections, pancreatic islets adjacent to blood vessels can be identified (outlined). Under fluorescence, signal can be seen in the larger pancreatic blood vessels of the healthy animals. Overall fluorescence intensity is markedly higher in diabetic than control animals and extends to the endocrine (outlined) as well as exocrine pancreas suggesting a global effect on vascular volume and/or permeability in the diabetic rats. (B) Histological analysis of PGC-GdDTPA-F intra- *versus* extravascular distribution in pancreata derived from diabetic and non-diabetic control BBDR rats 1 h after the injection of PGC-GdDTPA-F. Diffuse green fluorescence associated with the islet interstitium is seen in diabetic rats indicating extravascular leakage of PGC-GdDTPA-F. In contrast, in non-diabetic controls, green fluorescence is restricted to the intravascular space. Islets are outlined. Magnification bar, 50 μ m

Figure 4. B6.BKS-*Lepr^{db}* diabetic mice show enhanced leakage of protected graft copolymer bearing covalently linked gadolinium diethylenetriaminepentaacetic acid residues and labelled with fluorescein in pancreatic islets (A and B). In healthy animals the probe is confined to the vasculature with no leakage (C and D). Magnification: **10×** (A and C); **40×** (B and D)