ORIGINAL ARTICLE

Imaging Pancreas in Healthy and Diabetic Rodent Model Using [^{18}F]Fallypride Positron Emission Tomography/Computed Tomography

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Abstract

Background: A noninvasive method of monitoring the loss of islet cells can provide an earlier and improved diagnosis for therapeutics development of preclinical phases of diabetes. The use of [^{18}F]fallypride, a dopamine D2/D3 receptor radiotracer, has been developed as a surrogate marker to evaluate loss of pancreatic islet cells in a rodent model of type 1 diabetes.

Materials and Methods: Healthy Sprague–Dawley rats were administered [^{18}F]fallypride and imaged for 2 h in a positron emission tomography (PET)/computed tomography (CT) scan. Diabetes was then induced in the same rats by administration of streptozotocin, and a PET/CT scan was performed 4 days after establishing diabetes. Pancreata of a separate set of rats were evaluated by insulin immunostaining for loss of islet cells by streptozotocin.

Results: Blood glucose levels of 125 mg/dL and 550 mg/dL were established for those rats without and with diabetes, respectively. [^{18}F]Fallypride uptake in the pancreas of both groups of rats was rapid, but the rats with diabetes showed a significantly lower uptake (less than 50%). The specific binding ratio was decreased by 77% in the diabetic rats.

Conclusions: [^{18}F]Fallypride can be a useful surrogate marker for monitoring changes in pancreatic islet cells, thus providing a noninvasive method to evaluate efficacy of therapeutics.

Introduction

DEVELOPMENT OF TYPE 1 DIABETES mellitus leading to elevated blood glucose levels is a result of a decrease in insulin-producing cells in pancreatic islets. Technological advances in noninvasive imaging approaches to monitor islet cell loss are therefore necessary in preclinical phases of diabetes.¹ This would enable evaluation of emerging therapeutic approaches.²

Efforts have been made to discover a noninvasive method of imaging β -cells in the pancreas in rodent models of diabetes.³ Our goal is to develop a noninvasive method for imaging rodent islet cells using dopamine D2/D3 receptor imaging with [^{18}F]fallypride positron emission tomography (PET)/computed tomography (CT).⁴ [^{18}F]Fallypride has shown specific binding to islets in vitro and to pancreas ex vivo and in vivo.⁴ The objective of this preliminary report was to extend in vivo imaging of pancreas in a streptozotocin (STZ) rodent model of type 1 diabetes mellitus.

Materials and Methods

Radioactivity was counted using a Capintec (Ramsey, NJ) dose calibrator, and low-level counting was done using a well-counter. Slices of the rat pancreas were prepared using the Leica Microsystems (Bannockburn, IL) CM1850 cryotome. Pancreatic sections were immunostained for insulin as previously described.⁴ All animal studies were approved by the Institutional Animal Health Care and Use Committee of University of California–Irvine.

The synthesis of [^{18}F]fallypride was performed using previously reported methods.⁵ [^{18}F]Fallypride was typically obtained in specific activity greater than 2,000 Ci/mmol in approximately 10 mCi batches for imaging studies. The final sterile 0.9% NaCl solution of [^{18}F]fallypride, with a pH value in the range of 6–7, was dispensed for in vitro and in vivo studies.

Healthy male Sprague–Dawley rats (8 weeks old; weighing 250 g; $n = 6$) were used for the study, which included two

PET/CT scans. A baseline scan was first carried out before treatment with STZ. The Inveon PET and CT scanners (Siemens Medical Solutions USA, Inc., Malvern, PA) were placed in the docked mode for combined PET/CT experiments. Before the start of the imaging study, rats were kept fasting in a quiet place for more than 12 h. In preparation for the scans, the rats were anesthetized with isoflurane and then maintained under anesthesia during the scan (4% induction, 2.5% maintenance). After positioning on the scanner bed, rats were injected with 0.7–1 mCi of [^{18}F]fallypride via the tail vein. PET studies typically lasted 2 h. Three rats were sacrificed, and pancreata were isolated for immunostaining. The remaining three rats were used for STZ treatment. For chemical destruction of pancreatic β -cells, rats were administered STZ at a dose of 55 mg/kg.⁶ Rats were classified as having diabetes when nonfasting serum blood glucose levels rose above 350 mg/dL for 3 consecutive days.

The images were reconstructed using Fourier rebinning and two-dimensional filtered backprojection (ramp filter and cutoff at the Nyquist frequency), with an image matrix of $128 \times 128 \times 159$, resulting in a pixel size of 0.77 mm and a slice thickness of 0.796 mm. All dynamic images were corrected for radioactive decay.

Animals were positioned in the CT scanner. Abdominal CT scanning was first performed without contrast agent preinjection for 30 min. The acquired CT images were spatially transformed automatically to match the PET image and were used for attenuation correction of the PET data and identification of animal organs. Reconstructed images were analyzed with the ASIPro and PMOD software packages. The standard uptake value (SUV), equal to (^{18}F]fallypride radioactivity in pancreas/injected radioactivity) $\times$ body weight, was computed and averaged across subjects.

Results

STZ preferentially accumulates in pancreatic β -cells via the GLUT2 glucose transporter. A single high dose (55 mg/kg) of STZ was enough to cause hyperglycemia in rats (Fig. 1A). Blood glucose was monitored daily and showed induced hyperglycemia in as little as 1 day after STZ administration. Typically, blood glucose levels increase and remained elevated over time. Destruction of islet cells correlates with increased blood glucose levels after STZ administration. The pancreas of rats with diabetes ($n=3$; average random blood glucose, 550 mg/dL) was harvested 7 days after the onset of hyperglycemia (>350 mg/dL). Age-matched rats without diabetes ($n=3$; average random blood glucose, 125 mg/dL) were used as controls. Insulin staining showed a substantial loss of islets (Fig. 1B) in STZ-treated rats, compared with the healthy rats ($P<0.05$). A decrease of $>90\%$ islet to pancreas ratio was observed in sample pancreas sections from STZ-treated rats compared with controls.

After intravenous administration, uptake of [^{18}F]fallypride was seen in various regions in the abdomen, including the liver, stomach, kidneys, pancreas, and other organs. Initial uptake in the pancreas and exhibited a gradual clearance (Fig. 2A). Consistent with our previous results,⁴ these data indicate that [^{18}F]fallypride uptake occurs in the pancreas in vivo and that the tracer binds to dopamine D2/D3 receptors.

SUVs were plotted over the course of the scan. All baseline and all diabetes data were averaged into one dataset. The av-

FIG. 1. Destruction of β -cells correlates with increased blood glucose levels after streptozotocin administration. **(A)** Blood glucose levels rose after streptozotocin administration. Asterisks indicated levels in the fasting state on days when a positron emission tomography scan was acquired. **(B)** Representative pancreatic islets showing insulin staining (inset) in Sprague–Dawley rats without diabetes (control) and rats with streptozotocin-induced diabetes. Image J software was used to calculate the islet to pancreas ratio (92% decrease). Color images available online at www.liebertonline.com/dia

erage SUVs were plotted and compared before and after diabetes induction with STZ. Control pancreas showed an initial SUV of >3 , and after about an hour the value came down to 2. Comparatively, the SUV in the diabetes state is nearly half (<1) for much of the last hour (Fig. 2A). This result is comparable to in vivo studies done with [^{18}F]fallypride and competition with haloperidol.⁴ Variability of tracer delivery to the pancreas occurred initially after injection in both datasets; however, the variability reduced over time.

The specific binding ratio (SBR) was calculated as the activity in the pancreas minus the activity in the back muscle, divided by the activity in the back muscle. SBR was calculated using time activity data from 90 to 120 min. SBR was significantly reduced by 77% with STZ treatment ($P<0.03$) (Fig. 2B).

Discussion

In STZ-treated diabetic rats, a significant loss of islet cells (>70 – 80%) in the pancreas has been reported.⁶ An expected reduction in [^{18}F]fallypride binding to pancreas sections of STZ-treated diabetic rats was observed and paralleled the

FIG. 2. Reduced standard uptake value (SUV) and specific binding ratio (SBR) in streptozotocin (STZ)-treated rats. **(A)** The average SUVs were plotted and compared before and after diabetes induction with STZ. Variability of tracer delivery to the pancreas occurred initially after injection in both datasets; however, the variability decreased over time. $n=3$ per group. **(B)** The SBR was calculated using time activity data from 90 to 120 min. SBR was calculated as (activity in the pancreas – activity in errectile spinae muscle)/activity in errectile spinae muscle. SBR was reduced 73% with STZ treatment ($P=0.03$). $n=3$ per group. Color images available online at www.liebertonline.com/dia

loss of islets by insulin immunostaining. The extent of [^{18}F]fallypride reduction in the pancreas is consistent with homogenate assays performed using [^3H]YM-09151-2.⁷ The reduction in [^{18}F]fallypride binding in the STZ-treated rats validates the usefulness of this method in a rodent model of type 1 diabetes mellitus.⁴

In vivo imaging of pancreas tissue before and after induction of diabetes showed a significant reduction after about 1 h of scanning. Variability of SUV in the first 15 min is probably due to inflammatory effects of STZ among animals. With STZ treatment, we see a significantly reduced SBR in the pancreas of 77% during the last 90–120 min of the scan. By this time the concentrations of tracer had equilibrated and are bound to dopamine D2/D3 receptors if present, and the bolus amount seen in earlier frames had washed out with slight nonspecific binding because of blood flow in the pancreas. The >70% reduction in the diabetes state correlates well with the in vitro data showing 56% reduction of [^{18}F]fallypride binding to fresh frozen pancreatic sections.⁴

Uptake of [^{11}C]DTBZ in the pancreas of Lewis rats with STZ-induced diabetes, compared with controls, showed decreased radioligand uptake in these rats.⁸ On the other hand, using subjects with long-standing type 1 diabetes and healthy controls, the pancreatic functional binding capacity overestimates β -cell mass in the T1D subjects. This may be due to a higher nonspecific binding in the pancreas than in the reference tissue (renal cortex).⁹

[^{18}F]Fallypride has a physical half-life of 110 min (due to fluorine-18). This half-life makes clinical application possible. [^{18}F]Fallypride has been used in several human brain studies and has been found to be safe for imaging studies. Application of [^{18}F]fallypride for studies of the dopamine receptors in human pancreas in vivo needs to be demonstrated. Uptake of [^{18}F]fallypride in adjacent organs such as the liver and stomach is a concern because detection of the low levels of dopamine receptors in the pancreas may be compromised.

In conclusion, reduction of [^{18}F]fallypride in a rat type 1 diabetes mellitus model correlates to the destruction of islet cells and the elevated blood glucose levels that are typical of subjects with diabetes. [^{18}F]Fallypride could be a useful noninvasive diagnostic method to monitor diabetes progression or treatment response; however, larger in vivo studies are needed for validation.

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Author Disclosure Statement

No competing financial interests exist.

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