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Positron emission tomography study on pancreatic somatostatin receptors in normal and diabetic rats with ^{68}Ga-DOTA-octreotide: A potential PET tracer for beta cell mass measurement

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia, and the loss or dysfunction of pancreatic beta cells has been reported before the appearance of clinical symptoms and hyperglycemia. To evaluate beta cell mass (BCM) for improving the detection and treatment of DM at earlier stages, we focused on somatostatin receptors that are highly expressed in the pancreatic beta cells, and developed a positron emission tomography (PET) probe derived from octreotide, a metabolically stable somatostatin analog. Octreotide was conjugated with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), a chelating agent, and labeled with ^{68}Gallium (^{68}Ga). After intravenous injection of ^{68}Ga-DOTA-octreotide, a 90-min emission scan of the abdomen was performed in normal and DM model rats. The PET studies showed that ^{68}Ga-DOTA-octreotide radioactivity was highly accumulated in the pancreas of normal rats and that the pancreatic accumulation was significantly reduced in the rats administered with an excess amount of unlabeled octreotide or after treatment with streptozotocin, which was used for the chemical induction of DM in rats. These results were in good agreement with the ex vivo biodistribution data. These results indicated that the pancreatic accumulation of ^{68}Ga-DOTA-octreotide represented specific binding to the somatostatin receptors and reflected BCM. Therefore, PET imaging with ^{68}Ga-DOTA-octreotide could be a potential tool for evaluating BCM.

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1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease that is characterized by high blood glucose level caused by defects in insulin secretion or action [1]. Several studies suggested that the number of pancreatic beta cells decreased in type 2 DM as well as in type 1 DM [2–4]. Such a decrease of the beta cell mass (BCM) has been shown to occur before the appearance of clinical symptoms, such as abnormal blood glucose level [3,4]. These observations indicate that BCM in the pancreas is a promising biomarker for the early-phase diagnosis and prognosis of DM. The diagnosis of DM is currently made by plasma glucose concentration, and the diagnostic methods are based on metabolic responses, such as oral glucose tolerance test [5]. However, blood insulin concentration does not directly reflect BCM. To date, biopsy is the only way to precisely measure BCM but it is highly invasive and not suitable for repeated testing. Improvement in the clinically applicable techniques for the evaluation of BCM is desirable for therapeutic evaluation and good control of DM and its complications.

In the last several decades, noninvasive molecular imaging techniques, such as positron emission tomography (PET), have allowed quantitative investigations of tissue distribution and dynamic changes of targeted molecules or cells in vivo because of their high sensitivity and spatiotemporal resolution. To date, several PET probes for BCM measurement have been developed [6,7]. Antidiabetic drugs, such as repaglinide and glyburide, have been radiolabeled with carbon-11 or fluorine-18 [8,9]. Sweet et al. [10] have screened several molecules that are associated with pancreatic beta cells and radiolabeled fluorodithizone and glibenclamide. Dihydrotetrabenazine (DTBZ), a ligand of the vesicular

Abbreviations: BCM, beta cell mass; STZ, streptozotocin; DTBZ, dihydrotetrabenazine; SSTR, somatostatin receptor; DOTA-OC, ^{68}Ga-DOTA-octreotide; FORE, Fourier rebinning; FBP, filtered back-projection; MAP, maximum a posteriori; VOI, volumetric region of interest; ID, injected dose; GLP-1, glucagon-like peptide-1; AC, adenylyl cyclase.

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monoamine transporter 2, and its derivatives have been developed as PET probes to measure BCM [11,12]. However, none of these ligands have been proven to be the ideal candidate for measurement of BCM in clinical applications.

To develop new PET probes for BCM, we focused on somatostatin receptors (SSTRs) that are highly expressed in pancreatic beta cells [13]. In the pancreas, somatostatin acts on beta cells and other endocrine cells and is considered an important regulator of insulin and other pancreatic endocrine hormones secretion [14]. In the rodent islets of Langerhans that consist of endocrine cells, the insulin-secreting beta cells are the majority (60–70%) of the cell population and abundantly express SSTRs. Therefore, the expression of SSTRs is a potential biomarker for the measurement of beta cells.

In the present study, we conjugated octreotide, a metabolically stable somatostatin analog with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), a chelating agent, labeled DOTA-octreotide with ^{68}Gallium (^{68}Ga), and investigated the possibility of evaluating BCM in normal and DM model rats.

2. Methods

2.1. Synthesis of ^{68}Ga-DOTA-octreotide (DOTA-OC)

DOTA-octreotide was synthesized with the standard 9-fluorenyl-methyloxycarbonyl (Fmoc) protocol [15]. The conjugation of DOTA was performed on the basis of the method described by Hasegawa et al. [16]. In brief, side-chain protected octreotide was synthesized on a polyethylene glycol-polystyrene resin by the standard Fmoc protocol. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (2.0 mg, 40 μmol) and N-hydroxysuccinimide (1.2 mg, 40 μmol) were added to a suspension of DOTA (4.2 mg, 10 μmol) in dimethyl sulfoxide (DMSO; 1.0 mL) and stirred for 60 min. This mixture and *N*,*N*-diisopropylethylamine (63 μL, 40 μmol) were then added to the resin. After 60 min, the resin was washed several times with dimethylformamide (DMF). This reaction was repeated several times until the Kaiser test showed a negative result. The resin was washed with methanol (3 × 3 mL) to remove residual DMF, and then dried under a vacuum. The peptide conjugate was cleaved from the resin by stirring it with a mixture of trifluoroacetic acid (TFA)/water/ethanedithiol/triisopropylsilane (94:2.5:2.5:1) for 2 h, which was followed by precipitation in cold dimethyl ether. High-performance liquid chromatography (HPLC) purification of DOTA-[Cys^{2,7}(SH)]-octreotide was conducted on a 5C18 ARII column (4.6 × 250 mm; Nacalai Tesque, Inc., Kyoto, Japan) with two solvent systems and the gradient elution method at a flow rate of 1 mL/min. Solvent A was 0.1% TFA in water, and solvent B was 0.1% TFA in acetonitrile. Purified DOTA-[Cys^{2,7}(SH)]-octreotide (approximately 15% yield) was obtained. A disulfide bond was formed by the air oxidation method. The peptide was dissolved in phosphate-buffered saline (3 mL), and DMSO (0.1 mL) was added. After 15 h, DOTA-octreotide was purified under the same conditions as those described above. Purified DOTA-octreotide was obtained as a white powder after lyophilization. The compound was measured by matrix-assisted laser desorption/ionization-time of flight mass spectroscopy. The calculated mass for (M+H)+ was 1406.6. The found mass for (M+H)+ was 1407.3. The peptide was quantified using an amino-acid analysis after hydrolysis in constant-boiling 6 N HCl at 110°C for 24 h.

Next, radiolabeling of ^{68}Ga to DOTA was conducted using the method previously described by Zhernosekov et al. [17]. In brief, a titanium dioxide-based and commercially available ^{68}Germanium (^{68}Ge)/^{68}Ga generator was eluted with 0.1 N HCl, and ^{68}Ga was purified using a cation exchange column (AG 50 W-X8 resin, 400 Mesh, Bio-Rad Laboratories, Inc., Hercules, CA, USA). ^{68}Ga was eluted with 3 mL of a 98% acetone–0.05 M HCl solution. After elution, the acetone in the eluent was eliminated under a nitrogen stream. The DOTA-octreotide that was dissolved in 1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer was added to the eluent, and the pH of this solution was adjusted to 3.5 using 1 M HEPES buffer. The reaction was conducted for 15 min at 140°C by microwave irradiation, and the reaction mixture was then passed over a C18 column cartridge (Sep-Pak C18, Waters Corporation, Milford, MA, USA), washed with 4 mL water, and eluted with 400 μL ethanol. Finally, a purified solution of ^{68}Ga-DOTA-octreotide (DOTA-OC) was condensed under a nitrogen stream. The residue was dissolved in saline solution before the animal experiments.

2.2. Animal preparation

Male Sprague-Dawley rats (8–9 weeks old) weighing approximately 250 g were purchased from Japan SLC, Inc. (Hamamatsu, Japan). They were kept in a light- and temperature-controlled environment with food and water available ad libitum. Before the PET scans, an injection port (Insuflon, Unomedical, Engmosen, Denmark) was inserted into the tail vein for intravenous bolus injections of the PET probe. Anesthetization was maintained with a mixture of 1.5% isoflurane and nitrous oxide/oxygen (7:3). Anesthetized rats were positioned in the PET scanner gantry with a thermosensing probe inserted into the rectum to monitor their body temperature. During the experiment, their body temperature was maintained at 37°C with a temperature controller (CMA150, CMA/Microdialysis, Stockholm, Sweden).

In addition, DM model rats were prepared by administration of streptozotocin (STZ, Wako Pure Chemical Industries, Ltd., Osaka, Japan), which is the most frequently used drug for the chemical induction of type 1 DM [18] and known to selectively destroy pancreatic beta cells. STZ (70 mg/kg, n = 3) or vehicle (n = 3) was intraperitoneally administered to normal Sprague–Dawley rats. The rats showed higher blood sugar levels (457 ± 16.9 mg/dL) 15 days after STZ treatment than vehicle-treated rats.

All experimental protocols were approved by the Ethics Committee on Animal Care and Use of RIKEN and were performed in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985).

2.3. PET studies

All PET scans were conducted using microPET Focus220 (Siemens Co., Ltd., Knoxville, TN, USA). While under 1.5% isoflurane anesthesia, a rat was placed in the PET scanner and a 30-min transmission scan with a rotating ^{68}Ge–^{68}Ga point source was performed for attenuation correction. At the start of the emission scan, the PET probe was intravenously injected through the injection port inserted in the tail vein, and an emission scan was performed over the abdomen for 90 min after the injection. The injection dose was 25.7 ± 2.77 MBq/body. The 90-min emission scan was in three-dimensional list-mode and was sorted into 41-frame dynamic sinograms according to the following sequence: 6 × 10 s, 6 × 30 s, 11 × 60 s, 15 × 180 s, and 3 × 600 s.

To evaluate the specific binding of DOTA-OC to SSTRs, a blocking PET study was conducted with unlabeled octreotide (Bachem, Bubendorf, Switzerland). Naive octreotide (1 mg; approximately 1000-fold the amount of radiolabeled octreotide) was dissolved in 300 μL saline and administered through the tail vein 1 min before the injection of DOTA-OC (n = 3). For the vehicle solution, the same amount of saline was administered at the same time point (n = 3).

The PET images were reconstructed using microPET manager 2.4.1.1 (Siemens) by Fourier Rebinning (FORE) and standard

two-dimensional-filtered back-projection (FBP) using a Ramp filter with a cutoff at the Nyquist frequency or by the maximum a posteriori (MAP) with Point Spread Function for the definition of volumetric regions of interest (VOIs). VOIs representing the liver and pancreas were delineated on static MAP images from 10 to 90 min after injections of DOTA-OC using the PMOD software ver. 3.2 (PMOD Technologies Ltd.), and the VOIs were placed on dynamic FBP images to obtain time-activity curves. The percent injected dose (% ID) was calculated by normalization with the animal weight and the injected dose.

2.4. Ex vivo biodistribution study

After the PET scans, the rats were euthanized 120 min after the PET probe injection. After perfusion with saline to remove blood from the blood vessels, the organs and tissues were harvested. The radioactivity in each sample was measured by a gamma counter (Wallac 1470, PerkinElmer, Waltham, MA, USA) and corrected for time decay from the point of PET probe administration.

2.5. Data analysis

Data were calculated as the means ± standard errors of the mean for three to four determinations. A Student's two-tailed unpaired *t*-test was used to identify significant differences between the groups. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Accumulation of DOTA-OC in the normal pancreas

Representative PET images of the abdominal region after bolus injections of DOTA-OC are shown in Fig. 1A–C. High accumulation of DOTA-OC was observed in the urinary bladder and kidney (Fig. 1A–C). Accumulation of DOTA-OC was apparent in the normal pancreas, while weak radioactivity was detected in the liver. The time-activity curves for the pancreas and liver of normal rats ($n = 3$) are shown in Fig. 2. The DOTA-OC radioactivity in the pancreas showed a rapid increase within 1 min following radiotracer administration and then gradually increased and reached 0.99 ± 0.24% ID at the end of the PET scans. In contrast, DOTA-OC radioactivity in the liver reached a peak at 15 s after the bolus injection and decreased rapidly thereafter reaching 0.17 ± 0.08% ID at the end of the PET scans. The accumulation of DOTA-OC was much higher in the kidney and urinary bladder, which are the organs related to excretion. The radioactivity of the kidney reached 1.64 ± 0.58% ID and that of the urinary bladder reached 12.8 ± 0.63% ID at the end of the PET scans (data not shown).

3.2. Blocking of the pancreatic uptake by unlabeled octreotide

To clarify whether the accumulation of DOTA-OC in the tissues indicated the specific binding to SSTRs, blocking studies were performed using unlabeled octreotide. Fig. 1D–F show representative PET images of DOTA-OC following the injection of unlabeled octreotide (1000 times excess amount of DOTA-OC). The accumulation of DOTA-OC was observed in the urinary bladder, kidney, and liver, but it was not obviously detected in the supposed location of the pancreas. The ex vivo biodistribution data ($n = 3$ for each group) showed that the pancreatic accumulation of DOTA-OC was significantly decreased in the unlabeled octreotide-treated group. The levels of radioactivity decreased to 3.49% (blocking/vehicle) of that in the vehicle-treated group (Fig. 3).

3.3. Reduced pancreatic uptake of DOTA-OC in STZ-treated DM model rats

To further examine whether DOTA-OC radioactivity in the pancreas represented changes in BCM, we performed PET studies with DOTA-OC in the STZ-induced DM model rats ($n = 3$). The PET images of these rats exhibited lower accumulation in the pancreas than that in normal rats (Fig. 1G–I), and the ex vivo biodistribution data indicated that the pancreatic accumulation of DOTA-OC decreased to 47.3% (STZ/normal) of normal rats (Fig. 3). The levels of radioactivity in the pancreas significantly decreased in the STZ-treated DM rats ($p < 0.05$, two-tailed unpaired *t*-test).

4. Discussion

In the present study, we demonstrated that PET with DOTA-OC enabled evaluation of BCM in normal and DM model rats, and DOTA-OC could be a potential candidate for the diagnostic and therapeutic monitoring of DM in clinical applications. Using in vivo PET and ex vivo biodistribution studies, we clearly demonstrated that DOTA-OC radioactivity accumulated in the pancreas of normal rats (Figs. 1A–C and 3) and that the pancreatic accumulation was dramatically decreased by an excess amount of unlabeled octreotide (1000-fold the amount of injected radiolabeled octreotide), which indicated that the pancreatic accumulation of DOTA-OC was caused by specific binding to SSTRs in the pancreas (Figs. 1D–F and 3). Moreover, SSTRs have been reported to be highly expressed in pancreatic beta cells [14]. Taken together, these observations suggest that PET with DOTA-OC is an efficient tool for evaluating BCM.

To date, several PET probes have been developed for BCM measurement [6,7]. Although they have good affinity or specificity to pancreatic beta cells in vitro, only a few PET probes have shown feasibility in in vivo imaging. For instance, [^{11}C]DTBZ and [^{18}F]FP-(+)-DTBZ, which are specific PET probes for vesicular monoamine transporter type 2, have been proposed for pancreatic BCM imaging, but a high accumulation of radioactivity in the liver may cause an overestimation of BCM [19]. Moreover, nonspecific binding to pancreatic exocrine cells has been reported [20]. In contrast, DOTA-OC accumulated much more in the pancreas than in the liver and other digestive organs around the pancreas, although the influence of renal accumulation needs to be considered. Since the hepatic accumulation of DOTA-OC is lower than that of DTBZ-derived PET probes, it could be a better tool for estimating pancreatic islet grafts in islet transplantation in the liver [21,22].

In the insulin secretory pathway, the most important transducer of ligand activation is the G-protein-coupled system, which includes glucagon-like peptide-1 (GLP-1) receptors and SSTRs [23]. The former stimulates adenylyl cyclase (AC), and the latter inhibits AC. In this sense, the GLP-1 receptors and SSTRs might represent two sides of the same coin. Recently, several long-lasting GLP-1 analogs have been approved as therapeutic drugs for DM, and its derivatives have also been developed as PET probes for BCM measurement [24–26]. However, none of them provided high-contrast imaging in vivo. These observations indicated that ^{68}Ga-labeled somatostatin analog could be a potential PET tracer for BCM measurement.

In the present study, we investigated the correlation between the pancreatic accumulation of DOTA-OC and the changes in BCM in STZ-induced DM model rats. STZ is known to selectively destroy pancreatic beta cells and is used to create type 1 DM model rats [18]. Kakkar et al. [27] reported that the insulin immunoreactivity was decreased approximately 50% in the pancreas of the STZ-induced DM model rats 3 weeks after a single dose injection of STZ. Consistent with these observations, our PET and ex vivo

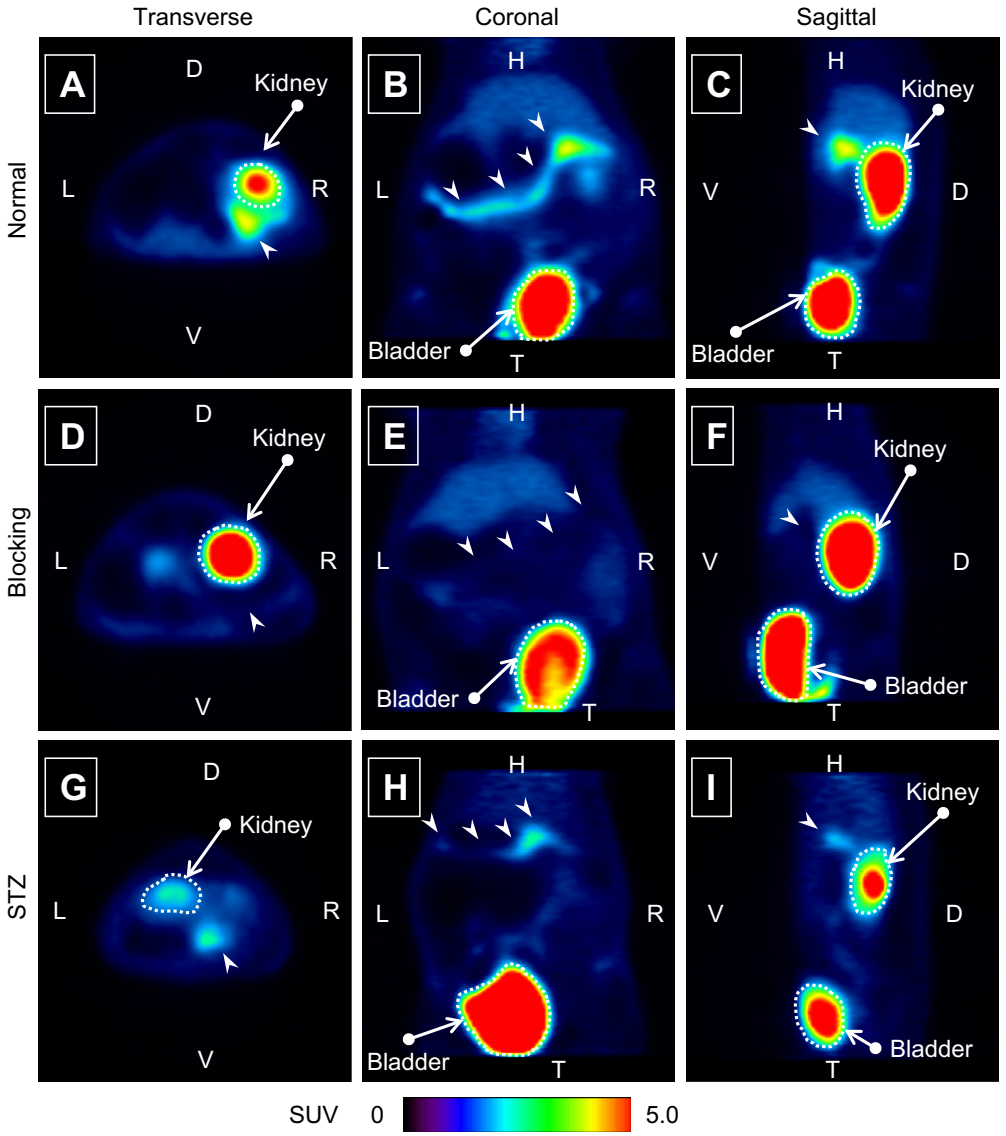

Fig. 1. PET images with DOTA-OC: (A–C) normal rat, (D–F) unlabeled octreotide-treated rat, (G–I) STZ-induced DM rat. Maximum a posteriori (MAP)-reconstructed static images captured 10–90 min after DOTA-OC injections. The white arrowheads show the pancreas. Abbreviations: D = dorsal, V = ventral, L = left, R = right, H = head, T = tail.

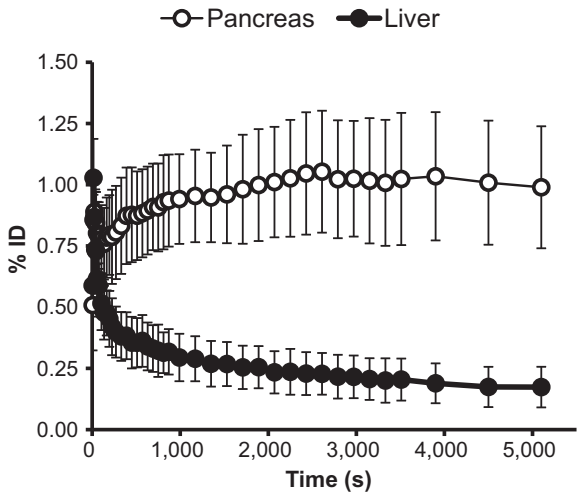

Fig. 2. Time-activity curves of the pancreas and liver of normal rats with DOTA-OC. The data are presented as means ± standard errors of the mean.

biodistribution studies showed that pancreatic accumulation of DOTA-OC in the STZ-induced model rats significantly decreased to 47.3% of that in normal healthy rats (Figs. 1G–I and 3). Singhal et al. [28] reported a similar result and have shown that [^{18}F]FP-(+)-DTBZ radioactivity in the pancreas decreased to the same extent in the STZ-induced DM model rats. These observations indicated that the pancreatic accumulation of DOTA-OC may reflect changes in BCM in vivo.

Several radiolabeled somatostatin analogs have already been reported for use in the imaging and therapy of neuroendocrine tumors which are known to express SSTRs [29]. ^{111}In-DTPA-octreotide (Octreoscan) is a commercially available agent for scintigraphy and single photon emission tomography and is one of the most commonly used agents for detection and localization of neuroendocrine tumors and other SSTR-expressing tumors. Recently, other somatostatin analogs labeled with gamma-emitting radionuclides, such as ^{68}Ga-DOTA-Tyr^3-octreotide and ^{68}Ga-DOTA-Tyr^3-octreotate, have also been proposed and tested on the patients with neuroendocrine tumors [30,31]. Although mild uptake of radiolabeled somatostatin analogs by the normal pancreas was noted by some investigators [32,33], those studies on

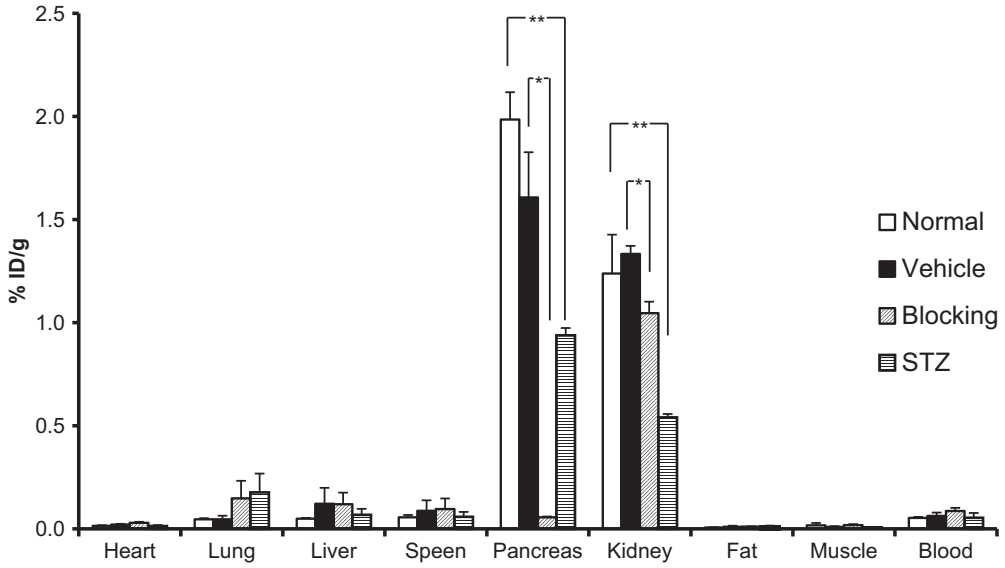

Fig. 3. The ex vivo biodistribution data of the PET studies. The data are presented as means ± standard errors of the mean. The pancreatic accumulation in unlabeled octreotide-treated rats was blocked to 96.5% of the vehicle-treated rats ($^{*}p < 0.01$) and that in STZ-induced DM rats was decreased to 47.3% of normal rats ($^{**}p < 0.01$).

SSTR-expressing tumors did not intend to measure BCM or to improve early detection of DM. The results of the present preclinical study warrant clinical research on the evaluation of pancreatic BCM with PET using DOTA-OC.

In the present study, the renal accumulation of DOTA-OC also decreased significantly in STZ-induced DM model rats ($p < 0.05$, two-tailed unpaired *t*-test). Hall-Craggs et al. [34] reported acute renal failure and squamous tubular metaplasia following treatment with STZ. These observations suggest that the reduction of renal accumulation of DOTA-OC in STZ-induced model rats resulted from dysfunction of the kidneys.

In conclusion, we demonstrated that DOTA-OC highly accumulated in the pancreas of normal rats by specifically binding to SSTRs of the pancreatic endocrine cells. PET images and ex vivo biodistribution data of STZ-induced DM model rats indicated that the pancreatic accumulation of DOTA-OC reflected BCM. Therefore, DOTA-OC could be a potential PET tracer for evaluating BCM.

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References

[1] A.C. Powers, Diabetes mellitus, in: E. Braunwald, A.S. Fauci, D.L. Kasper, S.L. Hauser, D.L. Longo, J.L. Jameson (Eds.), Harrison's Principles of Internal Medicine, McGraw-Hill, New York, 2001, pp. 2109–2116.
[2] M.Y. Donath, P.A. Halban, Decreased beta-cell mass in diabetes: significance, mechanisms and therapeutic implications, Diabetologia 47 (2004) 581–589.
[3] A.E. Butler, J. Janson, S. Bonner-Weir, et al., Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes, Diabetes 52 (2003) 102–110.
[4] C.J. Rhodes, Type 2 diabetes-a matter of beta-cell life and death?, Science 307 (2005) 380–384
[5] B. Goke, What are the potential benefits of clinical beta-cell imaging in diabetes mellitus?, Curr Pharm. Des. 16 (2010) 1547–1549.
[6] F. Souza, M. Freeby, K. Hultman, et al., Current progress in non-invasive imaging of beta cell mass of the endocrine pancreas, Curr. Med. Chem. 13 (2006) 2761–2773.
[7] F. Saudek, C.H. Brogren, S. Manohar, Imaging the beta-cell mass: why and how, Rev. Diabet. Stud. 5 (2008) 6–12.
[8] B. Wangler, C. Beck, C.Y. Shiue, et al., Synthesis and in vitro evaluation of (S)-2-([^{11}C]methoxy)-4-[3-methyl-1-(2-piperidine-1-yl-phenyl)-butyl-carbamoyl]-benzoic acid ([^{11}C]methoxy-repaglinide): a potential beta-cell imaging agent, Bioorg. Med. Chem. Lett. 14 (2004) 5205–5209.
[9] A. Schmitz, C.Y. Shiue, Q. Feng, et al., Synthesis and evaluation of fluorine-18 labeled glyburide analogs as beta-cell imaging agents, Nucl. Med. Biol. 31 (2004) 483–491.
[10] I.R. Sweet, D.L. Cook, A. Lernmark, et al., Systematic screening of potential beta-cell imaging agents, Biochem. Biophys. Res. Commun. 314 (2004) 976–983.
[11] N.R. Simpson, F. Souza, P. Witkowski, et al., Visualizing pancreatic beta-cell mass with [^{11}C]DTBZ, Nucl. Med. Biol. 33 (2006) 855–864.
[12] M.P. Kung, C. Hou, B.P. Lieberman, et al., In vivo imaging of beta-cell mass in rats using ^{18}F-FP-(+)-DTBZ: a potential PET ligand for studying diabetes mellitus, J. Nucl. Med. 49 (2008) 1171–1176.
[13] Y.C. Patel, Somatostatin and its receptor family, Front. Neuroendocrinol. 20 (1999) 157–198.
[14] E. Ludvigsen, Somatostatin receptor expression and biological functions in endocrine pancreatic cells: review based on a doctoral thesis, Ups. J. Med. Sci. 112 (2007) 1–20.
[15] W.C. Chan, P.D. White, Basic procedures, in: W.C. Chan, P.D. White (Eds.), Fmoc Solid Phase Peptide Synthesis: A Practical Approach, Oxford University Press, New York, 2000, pp. 41–76.
[16] K. Hasegawa, Y. Kanayama, M. Nishimura, et al., A facile method for solid-phase conjugation of peptide with unprotected DOTA and synthesis of ^{68}Ga-DOTA-somatostatin Peptide science 2008, in: Proceedings of the Japanese Peptide Symposium, Vol. 45, 2009, pp. 417–418.
[17] K.P. Zhernosekov, D.V. Filosofov, R.P. Baum, et al., Processing of generator-produced ^{68}Ga for medical application, J. Nucl. Med. 48 (2007) 1741–1748.
[18] T. Szkudelski, The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas, Physiol. Res. 50 (2001) 537–546.
[19] R. Goland, M. Freeby, R. Parsey, et al., ^{11}C-dihydrotetrabenazine PET of the pancreas in subjects with long-standing type 1 diabetes and in healthy controls, J. Nucl. Med. 50 (2009) 382–389.
[20] V. Fagerholm, K.K. Mikkola, T. Ishizu, et al., Assessment of islet specificity of dihydrotetrabenazine radiotracer binding in rat pancreas and human pancreas, J. Nucl. Med. 51 (2010) 1439–1446.
[21] A.M. Shapiro, J.R. Lakey, E.A. Ryan, et al., Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen, N. Engl. J. Med. 343 (2000) 230–238.
[22] B.W. Paty, S. Bonner-Weir, M.R. Laughlin, et al., Toward development of imaging modalities for islets after transplantation: insights from the National Institutes of Health Workshop on beta cell imaging, Transplantation 77 (2004) 1133–1137.
[23] M.E. Doyle, J.M. Egan, Pharmacological agents that directly modulate insulin secretion, Pharmacol. Rev. 55 (2003) 105–131.
[24] E. Mukai, K. Toyoda, H. Kimura, et al., GLP-1 receptor antagonist as a potential probe for pancreatic beta-cell imaging, Biochem. Biophys. Res. Commun. 389 (2009) 523–526.
[25] Y. Wang, K. Lim, M. Normandin, et al., Synthesis and evaluation of [^{18}F]exendin (9–39) as a potential biomarker to measure pancreatic beta-cell mass, Nucl. Med. Biol. 39 (2012) 167–176.

[26] B.M. Connolly, A. Vanko, P. McQuade, et al., Ex vivo imaging of pancreatic beta cells using a radiolabeled GLP-1 receptor agonist, Mol. Imaging Biol. 14 (2012) 79–87.
[27] R. Kakkar, S.V. Mantha, J. Radhi, et al., Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes, Clin. Sci. (Lond.) 94 (1998) 623–632.
[28] T. Singhal, Y.S. Ding, D. Weinzimmer, et al., Pancreatic beta cell mass PET imaging and quantification with [^{11}C]DTBZ and [^{18}F]FP-(+)-DTBZ in rodent models of diabetes, Mol. Imaging Biol. 13 (2011) 973–984.
[29] D.J. Kwekkeboom, B.L. Kam, M. van Essen, et al., Somatostatin-receptor-based imaging and therapy of gastroenteropancreatic neuroendocrine tumors, Endocr. Relat. Cancer 17 (2010) R53–73.
[30] M. Gabriel, C. Decristoforo, D. Kendler, et al., ^{68}Ga-DOTA-Tyr3-octreotide PET in neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and CT, J. Nucl. Med. 48 (2007) 508–518.
[31] A.R. Haug, R. Cindea-Drimus, C.J. Auernhammer, et al., The role of ^{68}Ga-DOTATATE PET/CT in suspected neuroendocrine tumors, J. Nucl. Med. 53 (2012) 1686–1692.
[32] C. Boy, T.A. Heusner, T.D. Poeppel, et al., ^{68}Ga-DOTATOC PET/CT and somatostatin receptor (sst1–sst5) expression in normal human tissue: correlation of sst2 mRNA and SUVmax, Eur. J. Nucl. Med. Mol. Imaging3 38 (2011) 1224–1236.
[33] M. Shastry, I. Kayani, D. Wild, et al., Distribution pattern of ^{68}Ga-DOTATATE in disease-free patients, Nucl. Med. Commun. 31 (2010) 1025–1032.
[34] M. Hall-Craggs, D.E. Brenner, R.D. Vigorito, et al., Acute renal failure and renal tubular squamous metaplasia following treatment with streptozotocin, Hum. Pathol. 13 (1982) 597–601.