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Evaluation of Cu-64 and Ga-68 Radiolabeled Glucagon-Like Peptide-1 Receptor Agonists as PET Tracers for Pancreatic β cell Imaging

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Abstract

Purpose—Copper-64 (Cu-64) and Gallium-68 (Ga-68) radiolabeled DO3A and NODA conjugates of exendin-4 were used for preclinical imaging of pancreatic β cells via targeting of glucagon-like peptide-1 receptor (GLP-1R).

Procedures—DO3A-VS- and NODA-VS-tagged Cys⁴⁰exendin-4 (DO3A-VS-Cys⁴⁰-exendin-4 and NODA-VS-Cys⁴⁰-exendin-4, respectively) were labeled with Cu-64 and Ga-68 using standard techniques. Biodistribution and dynamic positron emission tomography (PET) were carried out in normal Sprague–Dawley (SD) rats. *Ex vivo* autoradiography imaging was conducted with freshly frozen pancreatic thin sections.

Results—DO3A-VS- and NODA-VS-Cys⁴⁰-exendin-4 analogues were labeled with Cu-64 and Ga-68 to a specific activity of 518.7 ± 3.7 Ci/mmol (19.19 ± 0.14 TBq/mmol) and radiochemical yield above 98 %. Biodistribution data demonstrated pancreatic uptake of 0.11 ± 0.02 %ID/g for [⁶⁴Cu]DO3A-VS-, 0.14 ± 0.02 %ID/g for [⁶⁴Cu]NODA-VS-, 0.11 ± 0.03 for [⁶⁸Ga]DO3A-VS-, and 0.26 ± 0.03 for [⁶⁸Ga]NODA-VS-Cys⁴⁰-exendin-4. Excess exendin-4 and exendin-(9–39)-amide displaced all four Cu-64 and Ga-68 labeled exendin-4 derivatives in blocking studies.

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Conflict of Interest. David A. Griffith, Chris Limberakis, David A. Tess, Chen Jianqing, and Rikki Waterhouse are employees of Pfizer Inc.

Conclusions— $[^{64}\text{Cu}]/[^{68}\text{Ga}]\text{DO3A-VS-Cys}^{40}$ - and $[^{64}\text{Cu}]/[^{68}\text{Ga}]\text{NODA-VS-Cys}^{40}$ -exendin-4 can be used as PET imaging agents specific for GLP-1R expressed on β cells. Here, we report the first evidence of pancreatic uptake visualized with exendin-4 derivative in a rat animal model via *in vivo* dynamic PET imaging.

Keywords

GLP-1; GLP-1R; Pancreatic β cells; β cell mass; Exendin-4; PET; Cu-64; Ga-68

Introduction

Diabetes mellitus (DM) is one of the most common chronic illnesses and the seventh leading cause of death in the USA [1]. Hyperglycemia, the main symptom of DM, results from insulin resistance and consequent increased insulin levels leading to eventual decline in pancreatic β cell mass (BCM) [2]. Until recently, the established method to quantify BCM requires an autopsy [3, 4]. Another drawback of the current postmortem technique is that the pancreas is one of the first organs to undergo autolysis [5] and thus the counts observed during autopsy may not be an accurate measure of β cell function prior to death. An *in vivo* method to quantify BCM, such as imaging using positron emission tomography (PET) or single photon emission computed tomography radio-ligands, would be valuable as a measure to determine disease progression in diabetic patients.

Glucagon-like peptide-1 (GLP-1) is a 30 amino acid hormone produced by intestinal enteroendocrine L cells that binds to the glucagon-like peptide-1 receptor (GLP-1R), a 463 amino acid class B G protein-coupled receptor known to be present in pancreatic β cells [6–8]. GLP-1 has been identified as an important mediator of the endocrine cycle by inducing the release of insulin in a glucose-dependent manner and has also been known to promote β cell proliferation in mammalian biological systems [9]. As the expression of GLP-1R in the pancreas is restricted to β cells, it is a good marker of β cell mass [10–12]. Therefore, a PET radioligand based on a GLP-1 agonist that targets the GLP-1R was sought as a potential non-invasive imaging agent for BCM.

The rapid *in vivo* degradation of GLP-1 by dipeptidylpeptidase IV (DPP-IV or CD 26) makes native GLP-1 an unreliable and short-lived compound for use as a PET tracer [13]. Therefore, more stable GLP-1R ligands such as exendin-4 have been used to produce PET tracers.

Exendin-4 is a 39-amino acid GLP-1R agonist, originally isolated from the venom of the lizard *Heloderma suspectum* (Gila monster) [14], which is more metabolically stable than GLP-1. Synthetic exendin-4 is currently commercially available as Exenatide and is used as a treatment for DM. Exendin-(9–39)-amide is a truncated exendin-4 analogue that has also been investigated as an imaging agent as it is a potent GLP-1R antagonist [15, 16]. GLP-1 [12, 15, 17] and exendin-3 [18] analogues have also been investigated as possible peptide choices for imaging agents. The earliest attempt to radiolabel a GLP-1R agonist was in 1993 where exendin-4 was iodinated with I-125 by Göke et al. [15]. Over the years, various GLP-1R agonists/antagonists have been radiolabeled with I-125 [12, 15, 17, 19], In-111 [20–22], Tc-99 m [23], F-18 [24, 25], Cu-64 [10, 26–28], and Ga-68 [27, 29] to study BCM

and also tumors of β cell origin. With the availability of medical cyclotrons and $^{68}\text{Ge}/^{68}\text{Ga}$ generators, there has been an increased interest in GLP-1 PET tracers [10, 27, 29]. Most of the above work has highlighted the challenges associated with imaging BCM using GLP-1 analogues in small animals [10, 29, 30]. The first successful visualization of rodent pancreata with dynamic PET imaging was recently reported using a Cu-64-labeled GLP-1-based imaging agent [28]. Herein, we report the first use of exendin-4-derived radioligands to image rodent pancreata *in vivo*.

In most cases, the metal chelate conjugation of GLP-1 analogues has been performed with the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) macrocycle [10, 26, 29]. While the non-macrocyclic diethylene triamine pentaacetic acid (DTPA) [22] chelator has also been reported, the DOTA chelator is used more extensively since the latter has been shown to provide Cu-64 complexes with higher *in vivo* stability. Previous bifunctional chelator studies (BFC) have demonstrated 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) might potentially be a better chelator for some radio-metals [31, 32]. These studies investigated the effects of small structural changes on enhanced binding with Cu-64 and Ga-68 in *in vivo* systems [32–35]. In the present study, we sought to compare the pancreatic uptake of DOTA and NOTA derivatives of exendin-4 (DO3A-VS-Cys⁴⁰-exendin-4 and NODA-VSCys⁴⁰-exendin-4; Fig. 1), using two different PET isotopes, one short lived (Ga-68, $t_{1/2}$ =68 min) and one with a relatively longer half-life (Cu-64, $t_{1/2}$ =12.7 h), in healthy rats.

Materials and Methods

General

All solvents and reagents were ultra-pure or trace metal grade and obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. Water (18 M Ω -cm resistivity) was obtained from a Millipore Integral 5 Milli-Q water system (Billerica, MA). The synthesis of DO3A-VS-Cys⁴⁰-exendin-4 and NODA-VSCys⁴⁰-exendin-4 are provided in the electronic supplementary materials. The unlabeled exenatide peptides were purchased from Tocris Biosciences (Bristol, UK). Whatman 3 M silica gel thin-layer chromatography (TLC) plates were purchased from Fisher Scientific (Pittsburgh, PA).

Radiochemistry

Cu-64 ($t_{1/2}$ =12.7 h, β^+ =17 %, β^- =39 %, EC=43 %, E_{max} =0.656 MeV) was produced by a (p, n) reaction on enriched Ni-64 on a CS-15 biomedical cyclotron (Cyclotron Corporation, Berkeley, CA) at Mallinckrodt Institute of Radiology, Washington University School of Medicine and purified with an automated system using standard procedures [36, 37]. A stock solution of $^{64}\text{CuCl}_2$ was diluted with a 10-fold excess of 0.1 M ammonium acetate (NH_4OAc), pH 7 for radiolabeling.

Ga-68 ($t_{1/2}$ =68 min; β^+ =89 %, E_{max} =1.92 MeV) was obtained from a commercial $^{68}\text{Ge}/^{68}\text{Ga}$ generator (Eckert & Ziegler) system. Ten to 12 ml of 0.1 M HCl was used to elute $^{68}\text{GaCl}_3$ (1 ml/min) through a Strata X-C column [38]. The Strata X-C columns (Phenomenex, 30 mg/ml) were used without preconditioning. After eluting with 0.1 M HCL, the resin was

dried with N₂ gas and the purified Ga-68 was obtained by desorbing the resin with 500 µl of 0.02 M HCl/98 % acetone [38].

Typical labeling of DO3A-VS- and NODA-VS-Cys⁴⁰-exendin-4 with Cu-64 was achieved by adding 10 µg of peptides to 37 MBq (1 mCi) ⁶⁴CuCl₂ in 200 µl of 0.1 M NH₄OAc (pH 7). The reactions were incubated on a mixer with 800 rpm agitation at 37°C for 1 h. For Ga-68, the same amount of peptide and activity was used in 200 µl of 1 M HEPES (pH 7) and incubated at 60 °C for 15 min. Radiolabelled compounds were analyzed by TLC and high-performance liquid chromatography (HPLC). Radio-TLCs were developed with PBS and analyzed using a Bioscan 200 imaging scanner (Bioscan, Inc., Washington, DC). Radio-HPLC analysis were performed using Kinetex (Phenomenex) and XTerra (Waters) C-18 columns (5 µm, 4.6×150 mm I.D.) with a mobile phase of A: water (0.1 % TFA) and B: acetonitrile (0.1 % TFA), using a gradient of 30–80 % acetonitrile over 20 min with a 1 ml/min flow rate.

Serum Stability Studies

Ten microliters (~100 µCi) of Cu-64/Ga-68-labeled peptides (DO3A-VS- and NODA-VS-Cys⁴⁰-exendin-4) was added to 90 µl of rat serum (Sigma-Aldrich) and incubated at 37 °C with agitation (500 rpm). Aliquots were removed at each time point and analyzed using radio-TLC and HPLC. For HPLC, analysis used an additional guard column, (Phenomenex SecurityGuard 3.00 mm I.D.) for the protection of the C-18 column. Stability of Ga-68 complexes was evaluated up to 3 h (0.5, 1, 2, and 3 h) and Cu-64 complexes up to 24 h (0.5, 1, 2, 4, and 24 h time points). All reactions were conducted in triplicate.

Biodistribution Studies

All animal experiments were performed in compliance with the Guidelines for Care and Use of Research Animals established by the Division of Comparative Medicine and the Animal Studies Committee of Washington University School of Medicine. Bio-distribution studies were conducted in normal Sprague–Dawley (SD) male rats (Charles River Laboratories) of age 72–75 days weighing 350±40 g. For the imaging and biodistribution studies, the injection and blocking dose were diluted in saline solution (0.9 % NaCl, *w/v*) such that the amount of NH₄OAc was 10 % or less (*v/v*). The compounds were evaluated in rats (*n*=3) which were injected via the tail vein with 100 µl of 0.925–1.85 MBq (25– 50 µCi) of each radiotracer. Rats were anesthetized with 1–2% isoflurane and euthanized by cervical dislocation. Organs of interest were harvested, and the amount of radioactivity in each organ was determined by gamma counting. The data were corrected for radioactive decay, and percent injected dose per gram (%ID/g) of tissue was calculated. All samples were standardized against a sample of the injected dose of radiolabeled analogues.

An initial pilot biodistribution was conducted with both [⁶⁴Cu]DO3A-VS-Cys⁴⁰-exendin-4 and [⁶⁴Cu]NODA-VS-Cys⁴⁰-exendin-4 to determine an optimal imaging time point. This biodistribution was performed 10, 60, and 240 min after radiotracer injection. Based on this pilot study, subsequent biodistribution with all four conjugates were conducted at 1 h. Blocking studies were conducted using tail vein injections of 2.9 mg/kg body weight (adapted from Connolly et al. [10]) unlabeled exendin-4 and exendin-(9–39)-amide 10 min

prior to PET ligand administration. Post-imaging biodistribution studies were also performed immediately after small animal PET/X-ray computed tomography (CT). Rodents were euthanized, and organs of interest were collected, weighted, and radioactivity counted on a Beckman Gamma 8000 counter containing a NaI crystal (Beckman Instruments, Inc., Irvine, CA). Immediately after counting, the pancreata were subjected to autoradiography studies.

PET/CT Imaging Studies

Small animal PET/CT imaging studies were conducted in normal SD male rats. 1.85–3.70 MBq (50–100 μ Ci; 0.5–1.0 μ g) of each compound was administered via tail vein injection. Blocking studies were conducted by pre-injection with unlabeled exendin-4 analogues (exendin-4 and exendin-(9–39)-amide), 2.9 mg/kg body weight, intravenously, 10 min prior to radioligand injection. Rats were anesthetized with 1–2 % isoflurane/oxygen and imaged on an Inveon small animal PET/CT scanner (Siemens Medical Solutions). Dynamic images were collected for 1 h and reconstructed with the maximum a posteriori probability (MAP) algorithm followed by CT co-registration with the Inveon Research Workstation image display software (Siemens Medical Solutions, Knoxville, TN).

Regions of interest (ROI) were selected from PET images with the CT anatomical guidelines, and the associated radioactivity was measured using Inveon Research Workstation software. The pancreas was identified in the CT images by following the edges of the stomach, spleen, and small intestines [39]. Standard uptake values (SUV) were calculated as $\text{nCi/cc} \times \text{animal weight/injected dose}$.

Ex vivo Autoradiography Studies

Harvested fresh pancreata were immediately frozen by immersing in liquid nitrogen. Sectioning was carried out using a whole body cryo-microtome (Vibratome 8850). The tissues were adhered to the metal block holder using Cryo-M-Bed embedding compound (A-M systems) and frozen at -30°C on a Vibratome Cold Snap. These were cut into 20–40 μm sections and attached to adhesive glass slides (CFSA 1X, Leica Bio Systems) and were covered by adhesive tape (CryoJane Tape Transfer System). Prepared sections were exposed to a phosphor imaging plate (GE Healthcare Life Sciences). After exposing for 1, 4, and 12 h, the plates were scanned using phosphor imager plate scanner (Storm 840). The resulting images were processed using ImageQuant 5.2 (Molecular Dynamics) and ImageJ (v1.48, public domain) software.

Statistical Analysis

Quantitative data were processed by Prism 6 (GraphPad Software, v 6.03, La Jolla, CA) and expressed as $\text{Mean} \pm \text{SD}$. Statistical analysis performed using one-way analysis of variance and Student's *t* test. Differences at the 95 % confidence level ($p < 0.05$) were considered statistically significant.

Results

Cu-64, obtained as $^{64}\text{CuCl}_2$, and Ga-68, obtained as $^{68}\text{GaCl}_3$, were used to radiolabel potent GLP-1R agonist DO3A-VS and NODA-VS conjugates of exendin-4 (cAMP $\text{EC}_{50}=27$ and 26 pM, respectively [40]) using the conditions stated above. Quality control assays were conducted using ITLC and/or HPLC. Radiochemical purities of $98.5\pm1.8\%$ and $99.1\pm0.8\%$ for the ^{64}Cu]DO3A-VS and NODA-VS analogues, respectively, and $97.6\pm4.1\%$ and $99.8\pm0.6\%$ for the ^{68}Ga]DO3A-VS and NODA-VS analogues, respectively, were obtained. For all complexes, the HPLC retention times were observed to be 6.1 ± 0.1 min using 4.6×150 mm C18 column without a guard column (6.9 ± 0.1 min with a guard column). We maintained the specific activity of $100.0\pm1.6\text{ }\mu\text{Ci}/\mu\text{g}$ ($518.7\pm3.7\text{ Ci}/\text{mmol}$, $19.19\pm0.14\text{ TBq}/\text{mmol}$) for all four complexes by using the same amount of radioactivity and mass. The radiolabeled compounds were obtained with high radiochemical purity and specific activity suitable for *in vivo* imaging and were used directly for preclinical applications without further purification.

In vitro stability studies for the Cu-64- and Ga-68-labeled DO3A-VS- and NODA-VS-Cys⁴⁰ exendin-4 analogues were determined by measuring the radioactive fraction of the parent compound at incubation intervals in rat serum at 37°C. As can be seen in Fig. 2 (complete data listed in Table S1), essentially no unbound metal was observed after the labeling. The ^{64}Cu]DO3A-VS and NODA-VS complexes were $83.1\pm0.6\%$ and $85.7\pm1.4\%$ intact in serum at the 24 h. At 3 h, the Ga-68-labeled compounds were $91.9\pm0.3\%$ and $92.9\pm1.0\%$ intact. *In vitro* stability of the peptide was also monitored using HPLC ($\lambda=254$, 266, and 280 nm) and observed to be 985 % intact at 24 h in rat serum. These results indicated that both Ga-68 and Cu-64 complexes remained intact over the time of evaluation.

Results of the pilot study with the Cu-64-radiolabeled complexes in selected organs at 10, 60, and 240 min after tracer administration are shown in Fig. 3 (Table S2). These data show that the highest pancreatic uptakes for both complexes were at 1 h, and thus, this time point was used for all additional studies. The main difference between the ^{64}Cu]DO3A-VS and NODA-VS analogues was the uptake in kidneys (53.1 ± 5.3 for DO3A-VS vs. $35.3\pm3.0\%$ ID/g for NODA-VS, Fig. 3c) and liver (0.48 ± 0.1 for DO3A-VS vs. $0.64\pm0.1\%$ ID/g for NODA-VS, Fig. 3d). Based on the closer proximity of the liver versus the kidney to the pancreas, the ^{64}Cu]DO3A-VS-Cys⁴⁰-exendin-4 compound (with lower liver retention) was identified as the preferred imaging agent compared to ^{64}Cu]NODA-VS-Cys⁴⁰-exendin-4.

In order to establish the *in vivo* specificity of ^{64}Cu]DO3A-VS-Cys⁴⁰-exendin-4, blocking studies were performed with an excess of unlabeled GLP-1R agonist exendin-4 and antagonist exendin-(9–39)-amide. These peptides were administered at 2.9 mg/kg to assess GLP-1R-specific uptake of ^{64}Cu]DO3A-VS-Cys⁴⁰-exendin-4 (Fig. 4, Table S3). Exendin-4 and exendin-(9–39)-amide reduced the radiolabeled signal by approximately 55 and 45 %, respectively, in the pancreas (with uptakes of 0.051 ± 0.01 and $0.062\pm0.01\%$ ID/g), confirming the specificity of the tracer for the GLP-1R. Decreased radiopharmaceutical uptake was also noted in the kidney, lung, and stomach which is consistent with the reported presence of GLP-1R in rat kidney, lung, brain, and stomach [15].

The pancreatic uptakes of all four conjugates at 1 h after injection are shown in Fig. 5. Pancreatic uptakes were 0.11 ± 0.02 %ID/g for [^{64}Cu]DO3A-VS-, 0.14 ± 0.02 %ID/g for [^{64}Cu]NODA-VS-, 0.11 ± 0.03 for [^{68}Ga]DO3A-VS-, and 0.26 ± 0.03 for [^{68}Ga]NODA-VS-Cys⁴⁰-exendin-4. A summary of the overall biodistribution results with [^{68}Ga]NODA-VS-/DO3A-VS- and [^{64}Cu]NODA-VSCys⁴⁰-exendin-4 can be found in supplementary data (Fig. S1, Table S4), and [^{64}Cu]DO3A-VS-Cys⁴⁰-exendin-4 is listed in Table S3. Exendin-4 blocked pancreatic uptake of radiolabeled DO3A-VS-/NODA-VS-Cys⁴⁰-exendin-4 analogues by 48–88 %. The reduced uptakes were 0.051 ± 0.01 , 0.043 ± 0.01 , 0.043 ± 0.01 , and 0.031 ± 0.01 for [^{64}Cu]DO3A-VS-/NODA-VS- and [^{68}Ga]DO3A-VS-/NODA-VS-Cys⁴⁰-exendin-4, respectively. [^{68}Ga]NODAVS-Cys⁴⁰-exendin-4 exhibited the highest pancreatic uptake of all four conjugates examined and was also blocked to the greatest extent by the presence of excess unlabeled exendin-4. These data demonstrate the specific binding of [^{64}Cu]/[^{68}Ga]DO3A-VS- and [^{64}Cu]/[^{68}Ga]NODA-VS-Cys⁴⁰-exendin-4 to GLP-1R.

Ex vivo autoradiography studies on excised rat pancreas slices were conducted to localize the accumulation and retention of labeled analogues. Similar to results from previous studies [10], focal areas of radiopharmaceutical uptake were observed in the pancreas consistent with specific uptake by pancreatic β cells. With [^{64}Cu]DO3AVS/NODA-VS analogues, a clear delineation of pancreatic islets was observed (Fig. 6). The signal intensity observed in [^{64}Cu]DO3A-VS- and NODA-VS-Cys⁴⁰-exendin-4 pancreatic sections (9.9×10^7 and 5.2×10^7 counts/mm², respectively) was blocked by the excess of unlabeled exendin-4 and exendin-(9–39)-amide (97.7, 97.4, 96.2, and 94.2 %) in the β cells (Table 1). The [^{68}Ga]DO3A-VS-/NODA-VS- exendin-4 derivatives showed less contrast (Fig. S2) when compared to [^{64}Cu]DO3A-VS/NODA-VS radioligands, likely due to the short half-life of this isotope which resulted in low amounts of activity present at the time of imaging. The signal intensities for the [^{68}Ga]DO3A-VS- and NODA-VSCys⁴⁰-exendin-4 derivatives without blocking, 1.4×10^6 and 8.8×10^5 cts/mm², were reduced to 1.4×10^5 and 1.5×10^5 cts/mm², respectively. These data correspond with the trend observed in pancreatic uptake observed in biodistribution studies summarized in Fig. 5.

The distribution of the radiolabeled exendin-4 analogues in normal SD rats was demonstrated via PET imaging. The maximum intensity projection (MIP) images of summed PET dynamic data are shown in Fig. 7. [^{64}Cu]DO3A-VSCys⁴⁰-exendin-4 and [^{68}Ga]NODA-VS-Cys⁴⁰-exendin-4 up-take and the extent of blocking by exendin-4 were compared. Although the kidney and lung uptakes were higher than the uptake from the pancreas, we were able to distinguish the pancreatic uptake in these rat images using the sagittal projection with a PET threshold of 17,300–52,800 Bq/ml (1427–1468 nCi/cc). The excess of unlabeled exendin-4 demonstrated the *in vivo* specific binding of the $^{64}\text{Cu}/^{68}\text{Ga}$ radioligands to the pancreatic GLP-1R. Radio-ligand uptake in the lungs and stomach was also completely reduced as observed in the biodistribution studies. This confirms the distribution of GLP-1 receptors in the rat lung and stomach. To our knowledge, this is the first example of exendin-4 pancreatic uptake visualized in a rat model using Cu-64 and Ga-68 radiolabeled analogues for *in vivo* imaging.

Discussion

GLP-1R agonist PET ligands have shown promise for the *in vivo* determination of BCM. Several DOTA and DO3A functionalized exendin-4 analogues labeled with radionuclides have been reported [10, 26, 29]. However, the pancreas was not imaged in small animal models due to high kidney uptake and the low contrast between target tissue and background [27, 30]. In this report, we successfully imaged pancreatic β cells in normal SD male rats using DO3A and NODA functionalized exendin-4 analogues radiolabeled with Cu-64 and Ga-68 PET isotopes.

Exendin-4 has ~10-fold greater binding affinity for GLP-1R than the truncated antagonist exendin-(9–39)-amide [15]. A radiopharmaceutical based on the agonist exendin-4 is likely to be the optimal choice to measure β cell mass due to superior ligand affinity and the potential for radiotracer accumulation in the β cell by receptor internalization following agonist stimulation. In this study, a GLP-1R agonist and antagonist were both used as blocking agents to demonstrate the GLP-1R specificity of the exendin-4 based PET ligands.

In this work, we have evaluated [^{68}Ga]/[^{64}Cu]DO3A-VS and NODA-VS-conjugated exendin-4 derivatives for their potential as BCM imaging agents. In healthy rats, pancreata showed similar uptake for both [^{64}Cu]DO3A-VS- and [^{64}Cu]NODA-VS-tagged exendin-4 analogues. However, the DO3A-VS analogue proved more promising imaging agent when coupled with the Cu-64 isotope due to lower uptake in liver, resulting in a higher signal to noise ratio. [^{68}Ga]NODA-VS-Cys⁴⁰-exendin-4 exhibited the highest pancreatic accumulation with 0.26 ± 0.03 % ID/g as well as highest signal reduction (8-fold) in blocking studies with exendin-4.

Recent publications have described the challenges of imaging β cells using GLP derivatives in small animal models [27, 30]. Several researchers have opted for models employing larger animals due to the ease of organ localization. Although pig and canine pancreata have larger volumes, the rat is a desirable model for imaging due to greater β cell density [41]. However, the most challenging aspect of imaging small animals is the higher uptake observed in the kidney which may obscure the pancreas. In order to visualize pancreas despite the high renal uptake, a PET threshold of 17,300–52,800 Bq/ml (1427–1468 nCi/cc) was utilized to highlight the contrast of pancreatic uptake. As shown in Fig. 7, the sagittal images clearly indicate the distinctive difference between the kidney uptake and the pancreatic uptake.

Conclusions

[^{64}Cu]/[^{68}Ga]DO3A-VS-Cys⁴⁰-exendin-4 and NODA-VS-Cys⁴⁰-exendin-4 radiopharmaceuticals were prepared with 998 % purity and 518.7 ± 3.7 Ci/mmol (19.19 ± 0.14 TBq/mmol) specific activity. The radiometal conjugates were very stable in rat serum *in vitro*. Biodistribution studies confirmed that all compounds had peak pancreas uptake at 1-h post injection. Blocking studies confirmed that the uptake of the radioligands by the pancreas was due to specific GLP-1R binding. Pancreatic uptake of the radio-ligands was imaged *in vivo* in rats and visualized with sagittal projection and the threshold of 17,300–52,800 Bq/ml (1427–1468 nCi/cc). [^{68}Ga]NODA-VS-Cys⁴⁰-exendin-4 had the highest

accumulation and specific binding to the GLP-1R of the four conjugates examined. These Cu-64- and Ga-68-labeled DO3A-VS/NODA-VS-tagged exendin-4 analogues are PET imaging agents specific for GLP-1R which have potential utility for the quantification of BCM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Hoyert DL, Xu J. Deaths: Preliminary Data for 2011. National Vital Statistics Reports. 2012; 61(6)
2. Matveyenko AV, Butler PC. Relationship between beta-cell mass and diabetes onset. Diabetes Obes Metab. 2008; 10:23–31. [PubMed: 18834430]
3. Butler AE, Janson J, Bonner-Weir S, et al. β -Cell deficit and increased β -cell apoptosis in humans with type 2 diabetes. Diabetes. 2003; 52:102–110. [PubMed: 12502499]
4. Meier JJ, Bhushan A, Butler AE, et al. Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? Diabetologia. 2005; 48:2221–2228. [PubMed: 16205882]
5. Shimizu M, Hayashi T, Saitoh Y, et al. Postmortem autolysis in the pancreas: multivariate statistical study. The influence of clinico-pathological conditions. Pancreas. 1990; 5:91–94. [PubMed: 2293713]
6. Mayo KE, Miller LJ, Bataille D, et al. International Union of Pharmacology. XXXV. The glucagon receptor family. Pharmacol Rev. 2003; 55:167–194. [PubMed: 12615957]
7. Kieffer TJ, Habener JF. The glucagon-like peptides. Endocr Rev. 1999; 20:876–913. [PubMed: 10605628]
8. Thorens B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci U S A. 1992; 89:8641–8645. [PubMed: 1326760]
9. Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007; 87:1409–1439. [PubMed: 17928588]
10. Connolly B, Vanko A, McQuade P, et al. *Ex Vivo* imaging of pancreatic beta cells using a radiolabeled GLP-1 receptor agonist. Mol Imaging Biol. 2012; 14:79–87. [PubMed: 21394533]
11. Tornehave D, Kristensen P, Rømer J, et al. Expression of the GLP-1 receptor in mouse, rat, and human pancreas. J Histochem Cytochem. 2008; 56:841–851. [PubMed: 18541709]
12. Göke R, Conlon JM. Receptors for glucagon-like peptide-1(7–36) amide on rat insulinoma-derived cells. J Endocrinol. 1988; 116:357–362. [PubMed: 2832504]
13. Deacon CF. Circulation and degradation of GIP and GLP-1. Horm Metab Res. 2004; 36:761–765. [PubMed: 15655705]
14. Eng J, Kleinman WA, Singh L, et al. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on

- dispersed acini from guinea pig pancreas. *J Biol Chem.* 1992; 267:7402–7405. [PubMed: 1313797]
15. Göke R, Fehmann HC, Linn T, et al. Exendin-4 is a high potency agonist and truncated exendin-(9–39)-amide an antagonist at the glucagon-like peptide 1-(7–36)-amide receptor of insulin-secreting beta-cells. *J Biol Chem.* 1993; 268:19650–19655. [PubMed: 8396143]
 16. Mukai E, Toyoda K, Kimura H, et al. GLP-1 receptor antagonist as a potential probe for pancreatic beta-cell imaging. *Biochem Biophys Res Commun.* 2009; 389:523–526. [PubMed: 19737540]
 17. Körner M, Stockli M, Waser B, Reubi JC. GLP-1 receptor expression in human tumors and human normal tissues: potential for *in vivo* targeting. *J Nucl Med.* 2007; 48:736–743. [PubMed: 17475961]
 18. Gotthardt M, Fischer M, Naehrer I, et al. Use of the incretin hormone glucagon-like peptide-1 (GLP-1) for the detection of insulinomas: initial experimental results. *Eur J Nucl Med Mol Imaging.* 2002; 29:597–606. [PubMed: 11976797]
 19. Singh G, Eng J, Raufman JP. Use of ^{125}I -[Y39]exendin-4 to characterize exendin receptors on dispersed pancreatic acini and gastric chief cells from guinea pig. *Regul Pept.* 1994; 53:47–59. [PubMed: 7800858]
 20. Wild D, Behe M, Wicki A, et al. [Lys 40 (Ahx-DTPA- ^{111}In)NH $_2$]exendin-4, a very promising ligand for glucagon-like peptide-1 (GLP-1) receptor targeting. *J Nucl Med.* 2006; 47:2025–2033. [PubMed: 17138746]
 21. Wild D, Macke H, Christ E, et al. Glucagon-like peptide 1-receptor scans to localize occult insulinomas. *N Engl J Med.* 2008; 359:766–768. [PubMed: 18703486]
 22. Gotthardt M, Lalyko G, van Eerd-Vismale J, et al. A new technique for *in vivo* imaging of specific GLP-1 binding sites: first results in small rodents. *Regul Pept.* 2006; 137:162–167. [PubMed: 16930741]
 23. Wild D, Wicki A, Mansi R, et al. Exendin-4-based radio-pharmaceuticals for glucagonlike peptide-1 receptor PET/CT and SPEC T/CT. *J Nucl Med.* 2010; 51:1059–1067. [PubMed: 20595511]
 24. Gao H, Niu G, Yang M, et al. PET of insulinoma using ^{18}F FBEM-EM3106B, a new GLP-1 analogue. *Mol Pharm.* 2011; 8:1775–1782. [PubMed: 21800885]
 25. Kieseewetter DO, Gao H, Ma Y, et al. ^{18}F -radiolabeled analogs of exendin-4 for PET imaging of GLP-1 in insulinoma. *Eur J Nucl Med Mol Imaging.* 2012; 39:463–473. [PubMed: 22170321]
 26. Wu Z, Todorov I, Li L, et al. *In vivo* imaging of transplanted islets with ^{64}Cu -DO3A-VS-Cys 40 -exendin-4 by targeting GLP-1 receptor. *Bioconj Chem.* 2011; 22:1587–1594. [PubMed: 21692471]
 27. Kirsi M, Cheng-Bin Y, Veronica F, et al. ^{64}Cu - and ^{68}Ga -labelled [Nle 14 , Lys 40 (Ahx-NODAGA)NH $_2$]-exendin-4 for pancreatic beta cell imaging in rats. *Mol Imaging Biol.* 2014; 16:255–263. [PubMed: 24101374]
 28. Manandhar B, Ahn JM. Glucagon-like peptide-1 (GLP-1) analogs: recent advances, new possibilities, and therapeutic implications. *J Med Chem.* 2015; 58:1020–1037. [PubMed: 25349901]
 29. Selvaraju R, Velikyan I, Johansson L, et al. *In vivo* imaging of the glucagonlike peptide 1 receptor in the pancreas with ^{68}Ga -labeled DO3A-exendin-4. *J Nucl Med.* 2013; 54:1458–1463. [PubMed: 23761918]
 30. Blomberg BA, Codreanu I, Cheng G, et al. Beta-cell imaging: call for evidence-based and scientific approach. *Mol Imaging Biol.* 2013; 15:123–130. [PubMed: 23413090]
 31. De Silva RA, Jain S, Lears KA, et al. Copper-64 radiolabeling and biological evaluation of bifunctional chelators for radiopharmaceutical development. *Nucl Med Biol.* 2012; 39:1099–1104. [PubMed: 22743158]
 32. Sin I, Kang CS, Bandara N, et al. Novel hexadentate and pentadentate chelators for ^{64}Cu -based targeted PET imaging. *Bioorg Med Chem.* 2014; 22:2553–2562. [PubMed: 24657050]
 33. Lin M, Welch MJ, Lapi SE. Effects of chelator modifications on ^{68}Ga -labeled [Tyr 3]octreotide conjugates. *Mol Imaging Biol.* 2013; 15:606–613. [PubMed: 23529373]
 34. Maecke HR, Hofmann M, Haberkorn U. ^{68}Ga -labeled peptides in tumor imaging. *J Nucl Med.* 2005; 46(Suppl 1):172S–178S. [PubMed: 15653666]

35. Cai Z, Anderson CJ. Chelators for copper radionuclides in positron emission tomography radiopharmaceuticals. *J Labelled Comp Radiopharm*. 2014; 57:224–230. [PubMed: 24347474]
36. McCarthy DW, Shefer RE, Klinkowstein RE, et al. Efficient production of high specific activity ^{64}Cu using a biomedical cyclotron. *Nucl Med Biol*. 1997; 24:35–43. [PubMed: 9080473]
37. Kume M, Carey PC, Gaehle G, et al. A semi-automated system for the routine production of copper-64. *Appl Radiat Isot*. 2012; 70:1803–1806. [PubMed: 22516717]
38. Lin M, Ranganathan D, Mori T, et al. Long-term evaluation of TiO_2 -based $^{68}\text{Ge}/^{68}\text{Ga}$ generators and optimized automation of [^{68}Ga]DOTATOC radiosynthesis. *Appl Radiat Isot*. 2012; 70:2539–2544. [PubMed: 22897970]
39. Nadey, S.; Hakim, RJ.; Stratta, DG. Pancreas, islet and stem cell transplantation for diabetes. Oxford University Press; Oxford: 2010.
40. Hoang HN, Song K, Hill TA, et al. Short hydrophobic peptides with cyclic constraints are potent glucagon-like peptide-1 receptor (GLP-1R) agonists. *J Med Chem*. 2015; doi: 10.1021/acs.jmedchem.5b00166
41. Steiner DJ, Kim A, Miller K, Hara M. Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. *Islets*. 2010; 2:135–145. [PubMed: 20657742]

Fig. 1.
Chemical structures of **a** DO3A-VS-Cys⁴⁰-exendin-4 and **b** NODA-VS-Cys⁴⁰-exendin-4.

Fig. 2. Results of the *in vitro* serum stability study. **a** [^{68}Ga]DO3A-VS-Cys⁴⁰-exendin-4 and [^{68}Ga]NODA-VSCys⁴⁰-exendin-4 and **b** [^{64}Cu]DO3A-VS-Cys⁴⁰-exendin-4 and [^{64}Cu]NODA-VS-Cys⁴⁰-exendin-4.

Fig. 3.

a Overall biodistribution results of $[^{64}\text{Cu}]\text{DO3A-VS-}$ and $[^{64}\text{Cu}]\text{NODA-VS-}$ analogues given in percent injected dose per gram of tissue for the three time points evaluated (10 min, 1 h, and 4 h); **b** pancreatic uptake; **c** renal uptake 1 h p.i.; **d** hepatic uptake 1 h p.i.

Fig. 4. Blocking studies conducted in normal SD rats ($n=3$) by pre-injection with unlabeled exendin-4 and exendin-(9-39)-amide, intravenously, 10 min prior to [^{64}Cu]DO3A-VS-Cys⁴⁰-exendin-4 injection. Pancreatic uptake is highlighted in the insert.

Fig. 5.

Summary of pancreatic uptake values for *in vivo* blocking studies with exendin-4 injected through the tail vein 10 min prior to the injection of **a** [^{64}Cu]DO3A-VS-Cys 40 -exendin-4, **b** [^{64}Cu]NODA-VS-Cys 40 -exendin-4, **c** [^{68}Ga]DO3A-VS-Cys 40 -exendin-4, and **d** [^{68}Ga]NODA-VS-Cys 40 -exendin-4.

Fig. 6.

Ex vivo autoradiography imaging of pancreatic sections (20–40 μm) from normal SD rats 1-h post i.v. injection of [^{64}Cu]DO3A-VS-Cys⁴⁰-exendin-4 and [^{64}Cu]NODA-VS-Cys⁴⁰-exendin-4. The blocking doses of cold peptides were injected 10 min prior to the radiolabeled compound administration. [^{64}Cu]DO3A-VS- and [^{64}Cu]NODA-VS-Cys⁴⁰-exendin-4 derivatives without block shown in **a** and **d**; [^{64}Cu]DO3A-VS-Cys⁴⁰-exendin-4 with exendin-4 and exendin-(9–39)-amide shown in **b** and **c**; [^{64}Cu]NODA-VS-Cys⁴⁰-exendin-4 with exendin-4 and exendin-(9–39)-amide shown in **e** and **f**, respectively.

Fig. 7. Representative sagittal maximum intensity projections of normal SD rat PET/CT 1-h dynamic scan results following i.v. injection of **a** $[^{64}\text{Cu}]\text{DO3A-VS-Cys}^{40}\text{-exendin-4}$ and **c** $[^{68}\text{Ga}]\text{NODA-VS-Cys}^{40}\text{-exendin-4}$; **b** and **d** are respective images from blocking studies with exendin-4 injected i.v. 10 min prior to the radioligand. (*L* lung, *P* pancreas, *S* stomach, and *K* kidney).

Table 1
The signal intensities and percentage blockade observed in pancreatic sections in *ex vivo* autoradiography studies

	Unblocked (cts/mm ²)	Exendin-4 (cts/mm ²)	% blockade	Exendin-(9-39)-amide (cts/mm ²)	% blockade
[⁶⁴ Cu]DO3A-VS-Cys ⁴⁰ -exendin-4	9.9×10 ⁷	2.3×10 ⁶	97.7	2.6×10 ⁶	97.4
[⁶⁴ Cu]NODA-VS-Cys ⁴⁰ -exendin-4	5.2×10 ⁷	2.0×10 ⁶	96.2	3.0×10 ⁶	94.2
[⁶⁸ Ga]DO3A-VS- Cys ⁴⁰ -exendin-4	1.4×10 ⁶	1.4×10 ⁵	90.0	NP	NP
[⁶⁸ Ga]NODA-VS-Cys ⁴⁰ -exendin-4	8.8×10 ⁵	1.5×10 ⁵	83.0	NP	NP

NP experiment not performed