^{18}F-Labeled exendin(9-39)

[^{18}F]Ex(9-39)

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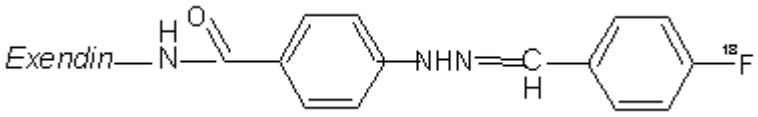
Chemical name:	^{18}F-Labeled exendin(9-39)
Abbreviated name:	[^{18}F]Ex(9-39)
Synonym:	
Agent Category:	Peptide
Target:	Glucagon-like peptide 1 receptor (GLP-1R)
Target Category:	Receptor
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents



Structure of [^{18}F]Ex(9-39) according to Wang et al. (1).

Background

[PubMed]

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Autoimmune processes and other environmental factors that destroy β-cells located in the pancreatic islet cells are known to promote the development of insulin-dependent diabetes mellitus (type 1 diabetes) in individuals genetically predisposed to the disease (2). Due to this destruction, the net mass of the β-cells in the islet cells of the pancreas is reduced, which leads to decreased production of insulin in the individual, and the maintenance of blood glucose at proper physiological levels is impaired. Type 2 diabetes is the most common form of the disease and is primarily caused by insulin resistance as a consequence of low insulin secretion by the β-cells. This form of diabetes can often be corrected with exercise, diet control, and/or medication (3). Upon diagnosis of diabetes, it is important to determine the individual's β-cell mass (BCM) or volume to devise a successful treatment regimen for the condition (4). Changes in the BCM during the onset of diabetes is poorly understood, and an indirect method that measures the amount of stimulated insulin secretion by the pancreas is currently used to quantify the BCM in humans (5). However, the β-cells appear to have a reserved capacity to produce insulin, so the use of insulin secretion as a determinant of BCM is of limited value (5).

As an alternative to determine insulin secretion to quantify BCM, investigators have evaluated the use of noninvasive positron emission tomography (PET) imaging techniques to determine the BCM in rats with the use of ^{11}C- or ^{18}F-labeled dihydrotetrabenazine, which is an antagonist of the vesicular monoamine transporter type 2 (VMAT2) in the islet cells (6). Careful evaluation of results obtained with the VMAT2 antagonists has revealed that a large proportion of these radiolabeled compounds tend to reside in the exocrine pancreas, indicating that these radiotracers are not suitable for the determination of BCM with PET imaging (7). Recently, some G-protein–coupled receptors (e.g., the glucagon-like peptide 1 (GLP 1) receptor (GLP-1R)), which show a selective location in the β-cells compared to the surrounding exocrine pancreatic cells, were identified by database mining and immunohistochemical staining of pancreatic tissue (1, 7, 8). On the basis of these observations and the known involvement of GLP-1R in β-cell function and biology (for details, see Baggio and Drucker (9)), this receptor was identified as a possible target of radiolabeled probes that can be used to quantify the BCM. Although GLP-1 is the natural ligand for the GLP-1R, a major drawback of using this peptide to measure the BCM is that it is rapidly inactivated by dipeptidyl peptidase, a proteolytic enzyme, while in circulation (half-life, ~2 min). As a consequence, investigators identified and have used radiolabeled Exendin-4 or its analogs (Exendin is a peptide of 39 amino acids that has a 54% homology with GLP-1, acts as an agonist of the GLP-1R with a K_d of ~1.4 × 10−^{10} M for this receptor, is not inactivated by proteolytic enzymes, and has a circulation half-life of ~2 h) for the measurement of BCM with noninvasive molecular imaging techniques (1, 7). Connolly et al. evaluated the use of ^{64}Cu-labeled Lys^{40}(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)NH_2-conjugated Exendin-4 for the *in vivo* imaging and measurement of the BCM in rats; however, because this tracer accumulated in the kidneys of the rodents, the pancreas was masked in the PET images and the BCM in the animals could not be quantified (7).

Radionuclide-labeled Exendin(9-39) (Ex(9-39)), a truncated version of Exendin, which acts as an antagonist of the GLP-1R and exhibits a high affinity for the receptor (K_d, ~3 ×

10–^{9} M) has also been used by investigators for the visualization of the GLP-1R (1). In an effort to develop a GLP-1R imaging agent, investigators labeled Ex(9-39) with [^{18}F]fluoride using *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]FB) (1). However, the ^{18}F-succinimidyl ester reacts with all of the primary amine groups available in a peptide, and the final labeled product is a mixture of the labeled peptide molecules that have [^{18}F]FB attached randomly on the primary amine groups of amino acids that constitute the peptide. Therefore, the exact structure of ^{18}F-labeled FB-conjugated Ex(9-39) is not well defined. In addition, during the labeling reaction the radionuclide may be introduced on an important amine group of the peptide, which can result in the loss of its biological activity. In order to develop a ^{18}F-labeled Ex(9-39) with a defined structure, Wang et al. used a site-specific technique to introduce ^{18}F into the molecule (1). To achieve this, [^{18}F]-4-fluorobenzaldehyde ([^{18}F]FBA) was conjugated with a 6-hydrazinonicotinyl group (6-HYNIC) located on the ε-amine of Lys27 in the Ex(9-39) molecule to generate [^{18}F]Ex(9-39). To evaluate the GLP-1R imaging ability of the labeled peptide, Sprague-Dawley (SD) rats and BioBreeding diabetes-prone (BB-DP) rats were injected with [^{18}F]Ex(9-39), and the time-activity curves for the pancreas, kidney, and the liver of the animals were obtained with PET imaging. Data generated with PET images of the pancreas were then correlated with insulin expressed by β-cells of the organ.

Related Resource Links

Related chapters in MICAD

Clinical trials related to GLP-1R

Gene information regarding human GLP-1R (Gene ID: 2740)

Glucagon-like peptide 1 in Online Mendelian Inheritance in Man Database (OMIM)

Mouse GLP-1R in Kyoto Encyclopedia of Genes and Genomes Database (KEGG)

GLP-1R agonists for the treatment of type II diabetes: Exenatide and Liraglutide

American Diabetes Association

Synthesis

[PubMed]

The site-specific ^{18}F labeling of Ex(9-39) on the 6-HYNIC moiety on the ε-amine of Lys27 has been described by Wang et al. (1). The identity of [^{18}F]Ex(9-39) obtained from the synthesis was confirmed by co-injecting the labeled peptide with non-radiolabeled ^{19}F-Ex(9-39) on an analytical size-exclusion high-performance liquid chromatography (HPLC) column. The radiochemical yield (RY) of the labeling reaction was not reported. The radiochemical purity (RP) of [^{18}F]Ex(9-39) was usually >95% as determined with HPLC, and the specific activity of the labeled peptide was reported to be ~22.2–25.9 TBq/μmol (~600–700mCi/μmol).

The source, synthesis, RY, RP, and specific activity of ^{125}I-labeled Ex(9-39) ([^{125}I]Ex(9-39)) used in an *in vitro* study were not reported (1).

For an *ex vivo* study, Ex(9-39) was labeled with Cy5.5 (Ex(9-39)-Cy5.5), a near-infrared fluorescent dye, using a commercially available kit (1). The dye:peptide ratio of the conjugate was determined to be ~0.4:1.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

To ascertain the β-cell binding selectivity of Ex(9-39), Wang et al. studied the uptake and retention of [^{125}I]Ex(9-39) in INS-1 (832/13) cells (a rat insulinoma β-cell line) and PANC-1 cells (a human pancreatic exocrine cell line) (1) The normalized uptake and retention of the label was significantly higher ($P < 0.01$) in the INS-1 cells (34.1 ± 8.0 and 3.0 ± 1.3, respectively) than in the PANC-1 cells (1.4 ± 0.2 and 0.1 ± 0.0, respectively). This indicated that Ex(9-39) had a binding specificity for the β-cells.

In a binding assay of Exendin-Cy5.5 with INS-1 (832/13) cells, it was shown that the fluorescent peptide bound to these cells with specificity and that the binding was saturable (1).

Ex vivo fluorescence imaging of pancreas and liver tissue obtained from mice given an bolus injection of ~40 nmol Ex(9-39)-Cy5.5 showed that the relative fluorescence intensity of the pancreas was ~5-fold higher than that of the liver (1). This indicated that Ex(9-39) bound preferentially to the pancreatic tissue in these animals.

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{18}F]Ex(9-39) in different organs of SD and BB-DP rats was studied as described elsewhere (1). The SD rats were divided into two groups: a control group ($n = 4$ animals) and a diabetic group (denoted as STZ for streptozotocin; $n = 6$ animals; diabetes in these animals was induced by treatment with STZ, a drug that causes β-cell death). Each rat was injected with ~7 MBq (~0.2 mCi) [^{18}F]Ex(9-39) through the tail vein, and dynamic PET images of the animals (under anesthesia) were acquired for the next 180–240 min. The images were reconstructed with appropriate software, and regions of interest on the images were delineated manually for the pancreas, liver, and kidney cortex of the animals. Regional time-activity curves (TAC) were generated from the dynamic PET images to calculate the standardized uptake value (SUV) units after normalization for injected activity and rodent body weight. After the imaging session was over, the animals were euthanized and the pancreas were extracted to determine insulin content.

A high uptake of radioactivity was observed in the myocardium and kidneys of the control SD rats at 10–30 min postinjection (p.i.) (1). The pancreas of these animals showed a low accumulation of label that was similar to the amount in the surrounding tissues, which have a low expression of the GLP-1R. The SUV of the pancreas from animals in all three groups (SD, STZ, and BB-DP) showed a similar and rapid increase in accumulation of label during the initial 10 min p.i.; accumulation peaked at 50 min p.i. and plateaued thereafter. In addition, there was no correlation between the SUV of the pancreas and the postmortem insulin content of these organs. From these observations, the investigators concluded that [^{18}F]Ex(9-39) probably did not bind specifically to the GLP-1R in the pancreas (1).

From these studies, it was concluded that ^{18}F-labeling of Ex(9-39) on the HYNIC modified Lys27 of the peptide generated a radiolabeled probe that did not bind specifically to the GLP-1R (1). Therefore, this tracer is not suitable for the determination of BCM in rodents.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available

Human Studies

[PubMed]

No publication is currently available

Supplemental Information

[Disclaimers]

No information is currently available

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References

1. Wang Y., Lim K., Normandin M., Zhao X., Cline G.W., Ding Y.S. *Synthesis and evaluation of [(18)F]exendin (9-39) as a potential biomarker to measure pancreatic beta-cell mass.* Nucl Med Biol. 2012;39(2):167–176. PubMed PMID: 22033026.
2. Concannon P., Rich S.S., Nepom G.T. *Genetics of type 1A diabetes.* N Engl J Med. 2009;360(16):1646–54. PubMed PMID: 19369670.
3. Krentz A.J., Bailey C.J. *Oral antidiabetic agents: current role in type 2 diabetes mellitus.* Drugs. 2005;65(3):385–411. PubMed PMID: 15669880.
4. Ritzel R.A. *Therapeutic approaches based on beta-cell mass preservation and/or regeneration.* Front Biosci. 2009;14:1835–50. PubMed PMID: 19273167.
5. Harris P.E., Ferrara C., Barba P., Polito T., Freeby M., Maffei A. *VMAT2 gene expression and function as it applies to imaging beta-cell mass.* J Mol Med (Berl). 2008;86(1):5–16. PubMed PMID: 17665159.
6. Kung M.P., Hou C., Lieberman B.P., Oya S., Ponde D.E., Blankemeyer E., Skovronsky D., Kilbourn M.R., Kung H.F. *In vivo imaging of beta-cell mass in rats using 18F-FP-(+)-DTBZ: a potential PET ligand for studying diabetes mellitus.* J Nucl Med. 2008;49(7):1171–6. PubMed PMID: 18552132.
7. Connolly B.M., Vanko A., McQuade P., Guenther I., Meng X., Rubins D., Waterhouse R., Hargreaves R., Sur C., Hostetler E. *Ex Vivo Imaging of Pancreatic Beta Cells using a Radiolabeled GLP-1 Receptor Agonist.* Mol Imaging Biol. 2012;14(1):79–87. PubMed PMID: 21394533.
8. Cline G.W., Zhao X., Jakowski A.B., Soeller W.C., Treadway J.L. *Islet-selectivity of G-protein coupled receptor ligands evaluated for PET imaging of pancreatic beta-cell mass.* Biochem Biophys Res Commun. 2011;412(3):413–8. PubMed PMID: 21820405.
9. Baggio L.L., Drucker D.J. *Biology of incretins: GLP-1 and GIP.* Gastroenterology. 2007;132(6):2131–57. PubMed PMID: 17498508.