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Evaluation of ^{18}F-labeled exendin(9-39) derivatives targeting glucagon-like peptide-1 receptor for pancreatic β-cell imaging

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ABSTRACT

β-cell mass (BCM) is known to be decreased in subjects with type-2 diabetes (T2D). Quantitative analysis for BCM would be useful for understanding how T2D progresses and how BCM affects treatment efficacy and for earlier diagnosis of T2D and development of new therapeutic strategies. However, a noninvasive method to measure BCM has not yet been developed.

We developed four ^{18}F-labeled exendin(9-39) derivatives for β-cell imaging by PET: [^{18}F]FB9-Ex(9-39), [^{18}F]FB12-Ex(9-39), [^{18}F]FB27-Ex(9-39), and [^{18}F]FB40-Ex(9-39). Affinity to the glucagon-like peptide-1 receptor (GLP-1R) was evaluated with dispersed islet cells of ddY mice. Uptake of exendin(9-39) derivatives in the pancreas as well as in other organs was evaluated by a biodistribution study. Small-animal PET study was performed after injecting [^{18}F]FB40-Ex(9-39).

FB40-Ex(9-39) showed moderate affinity to the GLP-1R. Among all of the derivatives, [^{18}F]FB40-Ex(9-39) resulted in the highest uptake of radioactivity in the pancreas 30 min after injection. Moreover, it showed significantly less radioactivity accumulated in the liver and kidney, resulting in an overall increase in the pancreas-to-organ ratio. In the PET imaging study, pancreas was visualized at 30 min after injection of [^{18}F]FB40-Ex(9-39).

[^{18}F]FB40-Ex(9-39) met the basic requirements for an imaging probe for GLP-1R in pancreatic β-cells. Further enhancement of pancreatic uptake and specific binding to GLP-1R will lead to a clear visualization of pancreatic β-cells.

1. Introduction

Diabetes is a chronic disease in which insulin production and its effect are insufficient for normalizing glucose tolerance. Type-2 diabetes (T2D) accounts for the majority of all diagnosed cases of diabetes in adults. T2D is characterized by both decreased ability to secrete insulin and increased insulin resistance, and it has been reported that β-cell mass (BCM) in T2D is significantly decreased.[1–3] Significant reduction of BCM in T2D patients is thought to be a cause for poor response to existing T2D pharmacotherapy.[4] Furthermore, incretin-related drugs, recently developed agents for T2D, are reported to have proliferative and antiapoptotic effects on pancreatic β-cells in *in vitro* and rodent experiments.[5–7] However, since noninvasive quantification of BCM is not yet possible,

Abbreviations: BCM, β-cell mass; BH-Ex(9-39), Bolton-Hunter labeled exendin (9-39); DMF, *N,N*-dimethylformamide; FB, Fluorobenzoyl; GFP, Green fluorescent protein; GLP-1, Glucagon-like peptide-1; GLP-1R, Glucagon-like peptide-1 receptor; %ID/g, Percent injected dose per gram; MIP, Mouse insulin I gene promoter; MIP-GFP mice, Transgenic mice expressing GFP under the control of mouse insulin promoter I; P/B, Pancreas-to-blood; P/K, Pancreas-to-kidney; P/L, Pancreas-to-liver; PET, Positron emission tomography; RP-HPLC, Reverse-phase high-performance liquid chromatography; SPECT, Single-photon emission computed tomography; [^{18}F]SFB, *N*-succinimidyl-4-[^{18}F]fluorobenzoate; T1D, Type-1 diabetes; T2D, Type-2 diabetes.

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it is unknown how and when a decrease in BCM begins and whether incretin-related drugs actually preserve BCM in humans. Therefore, a noninvasive method for BCM measurement is urgently required to understand the pathogenesis, facilitate early diagnosis, and develop novel therapeutics for diabetes.

Previously, various molecular imaging probes[8–10] have been developed for target molecules expressed in pancreatic β-cell imaging. Among them, glucagon-like peptide-1 receptor (GLP-1R) is a promising target molecule. GLP-1R is reported to be expressed on human pancreatic β-cells at high densities.[11,12] GLP-1R binds specifically with glucagon-like peptide-1 (GLP-1), secreted from intestinal L-cells by the stimulation of nutrients, leading to the promotion of glucose-dependent insulin secretion. GLP-1R agonists are now clinically used in the treatment of T2D.[13–15] Exendin-4 isolated from *Heloderma suspectum* venom is one of the agonistic ligands of GLP-1R, and exendin (9-39) is the antagonistic ligand that is the truncated form of exendin-4.[16] Thus, their derivatives are also expected to be GLP-1R agonists and antagonists. Previously, we reported that [^{125}I]Bolton-Hunter labeled exendin(9-39) ([^{125}I]BH-Ex(9-39)) bind to pancreatic islets via GLP-1R and accumulates on pancreatic β-cells by intravenous administration.[17] We have also investigated the possibility that GLP-1R could be the target molecule for β-cell imaging by using exendin derivatives labeled with ^{111}In, a conventional radionuclide for SPECT.[18,19]

Antagonistic ligand exendin(9-39) is superior to agonistic ligand exendin-4 as it is less prone to induce hypoglycemia because of insulin secretion. There is a report on exendin(9-39) derivative labeled with ^{18}F, a conventional radionuclide for PET.[20] In that report, [^{18}F]exendin(9-39) was synthesized by introducing a labeling group to Lys at position 27 and its potential was evaluated. The authors described that conjugating ^{18}F to other sites of exendin(9-39) may improve the ability for binding to GLP-1R.

Therefore, on the basis of the previous results, we developed four novel derivatives of exendin(9-39) and labeled them with ^{18}F on various conjugating sites as well as Lys at position 27 and evaluated their pharmacokinetics and PET properties.

2. Experimental section

2.1. General

Commercially obtained chemicals and solvents of reagent grade were ≥95% pure and were used without further purification. [^{18}F]Fluoride was produced by a cyclotron (CYPRIS HM-18; Sumitomo Heavy Industries Ltd., Tokyo, Japan) using ^{18}O(p,n)^{18}F nuclear reaction with proton irradiation of an enriched [^{18}O]H_2O target and passed through an anion-exchange solid phase cartridge (Sep-Pak Accell Plus QMA Plus Light Cartridge, Waters Co., Milford, MA, USA). The cartridge was dried by N_2, and [^{18}F]Fluoride was eluted with a mixture of potassium carbonate (1.7 mg, 12 μmol) and Kryptofix 222 (9 mg, 24 μmol) in acetonitrile/water (96:4, 1 mL). To measure radioactivity, a curiemeter (IGC-7, Hitachi Aloka Medical, Ltd., Tokyo, Japan), dose calibrator (Atomlab100+, Biodex Medical Systems, Inc., Shirley, NY, USA, and CRC-15 BETA, Capintec, Inc., Ramsey, NJ, USA), and an automatic γ-counter (Wallac 1480 WIZARD 3," PerkinElmer, Inc., Waltham, MA, USA) were used. Exendin (9-39) labeled with [^{125}I]Bolton-Hunter was purchased from PerkinElmer, Inc. and used as [^{125}I]BH-Ex(9-39).

Fluorobenzoyl-modified exendin(9-39) derivatives and 9-fluorenylmethyl carbamate (Fmoc)-protected exendin(9-39) derivatives as precursors for radiolabeling were provided by KNC Laboratories (Kobe, Japan).

2.2. In vitro binding assay

Dispersed islet cells were used to assess the displacing effect of FB-exendin(9-39) derivatives on GLP-1R binding, as described previously.[21] Pancreatic islets were isolated from male mice (ddY, 6 weeks old) by a collagenase digestion technique.[22] A mixture of 0.05% Trypsin-EDTA (1X), Phenol Red (Life Technologies Co.)/PBS (pH 7.4, containing 0.53-mM EDTA) (20:80) was used to disperse the isolated islet cells. Islet cells were incubated with [^{125}I]BH-Ex (9-39) (3.7 kBq) in buffer (1 mL, 20-mM HEPES, pH 7.4, containing 1-mM magnesium chloride, 1-mg/mL bacitracin, 1-mg/mL BSA) for 1 h at room temperature in the presence of varying concentrations of nonradiolabeled FB-exendin(9-39) derivatives. Binding was terminated by rapid filtration through Whatman GF/C filters (24 mm), followed by washing three times with ice-cold PBS (5 mL). Radioactivity of the filters was measured using an automatic γ-counter. Results were expressed as the percent radioactivity of bound [^{125}I]BH-exendin(9-39) that remained after adding the nonradiolabeled compound. GraphPad Prism version 5.03 for Windows software (GraphPad Software Inc., San Diego, CA, USA) was used to calculate IC_{50} values.

2.3. Radiochemistry

Anhydrous acetonitrile (0.5 mL) was added to the [^{18}F]Fluoride solution. The solvent was removed at 120 °C under argon gas flow. The residue was azeotropically dried with anhydrous acetonitrile (1 mL) at 120 °C under argon gas flow. *t*-Butyl 4-*N,N,N*-trimethylammoniumbenzoate triflate (5 mg, 13 μmol) in anhydrous acetonitrile (1 mL) was added to the reaction vessel containing the [^{18}F]Fluoride. The mixture was heated at 110 °C for 15 min and cooled. A tetrapropylammonium hydroxide solution (1 M in water, 20 μL) was added, and the mixture was heated at 120 °C again for 2 min. *O*-(*N*-succinimidyl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (15 mg, 50 μmol) in acetonitrile (0.1 mL) was added and heated at 90 °C for 2 min. The solution was diluted with 5% (v/v) acetic acid in water (10 mL) and loaded onto an activated Sep-Pak Plus PS-2 cartridge (Waters). The cartridge was washed with water/acetonitrile (80:20, 20 mL) to remove unlabeled ^{18}F, and the [^{18}F]SFB was then eluted with acetonitrile (2.5 mL). The solvent was removed at 90 °C under argon gas flow. A solution of peptide precursors (0.6–0.8 mg, 0.14–0.21 μmol) in acetonitrile/buffer (50-mM borate, pH 7.8, containing 50-mM potassium chloride) (50:50, 40 μL) was added to the reaction vessel containing [^{18}F]SFB. Acetonitrile/triethylamine (98:2) was added to the reaction mixture step by step until the pH of the mixture reached 9.0. The reaction mixture was incubated at room temperature for 60 min. Sephadex G-25 Fine gel chromatography media (GE Healthcare UK, Ltd, Little Chalfont, England) immersed in buffer (50-mM borate, pH 7.8, containing 50-mM potassium chloride) was packed in Mobicol "Classic" (MoBiTec GmbH, Goettingen, Germany) with filters (35-μm pore), and the reaction mixture was loaded onto the packed column. The loaded column was centrifuged at 215*g* for 2 min. DMF (80 μL), and piperidine (40 μL) were added to the eluate from the column and incubated at room temperature for 30 min to remove the Fmoc group. Synthesized [^{18}F]FB-exendin (9-39) derivatives were purified by reverse-phase high-performance liquid chromatography (RP-HPLC). The conditions for purification of [^{18}F]FB-exendin(9-39) derivatives were same as for purification of nonradiolabeled FB-exendin(9-39) derivatives. The eluate was evaporated and [^{18}F]FB-exendin(9-39) derivatives were dissolved in saline and used for biological studies. Analytical RP-HPLC was performed with UV detection in a series with γ-detector US-3000 (Universal Giken Co., Ltd., Kanagawa, Japan). The radiochemical purity of [^{18}F]FB-exendin(9-39) derivatives was determined by analytical radio-RP-HPLC.