Electronic Supplementary Material

Evaluation of pancreatic VMAT2 binding with active and inactive enantiomers of [^{18}F]FP-DTBZ in healthy subjects and patients with type 1 diabetes

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Synthesis of [^{18}F]-FP-(+)-DTBZ and [^{18}F]-FP-(−)-DTBZ

[^{18}F]FP-(+)-DTBZ was synthesized by [^{18}F]fluorination of the mesylate precursor, 9-(3-methanesulfonyloxypropoxy)-1,3,4,6,7,11b-hexahydro-10-methoxy-3-(2-methylpropyl) (2R,3R,11bR)-2H-benzo[a]quinolizin-2-ol. Aqueous [^{18}F]fluoride was produced with a 30 min beam at 35 μA from a GE PETtrace cyclotron (GE; Milwaukee, WI) via the ^{18}O(p,n)^{18}F reaction. Preparation of [^{18}F]FP-(+)-DTBZ was carried out either in a TRACERLab™ FX_{F-N} or a TRACERLab™ FX_{F-N} Pro automated synthesis module (GE Medical Systems).

Aqueous [^{18}F]fluoride produced from the cyclotron was loaded onto a Chromafix 30-PS-HCO_3 separation cartridge and eluted into the graphite reaction vessel with a solution of 7.14 mg Kryptofix K_{222} and 0.74 mg K_2CO_3 in 1 mL of CH_3CN/water (1:0.4, v/v). The solvent was evaporated at 70 ºC at reduced pressure (~33 kPa) under an argon stream for 5 min. A 1 mL aliquot of CH_3CN was added and azeotropic evaporation resumed at 70 ºC for 3 min, then a second 1 mL aliquot of CH_3CN was added and evaporation continued at 100 ºC for another 5 min. The argon flow was stopped and any remaining solvent evaporated at 100 ºC at reduced pressure (~8 kPa) for an additional 5 min leaving a dried residue. After cooling to 60 ºC, the radiolabeling precursor (0.5 ± 0.1 mg) in 0.5 mL mixture of DMF/acetonitrile (3/2, v/v) was added. The reaction vessel is sealed, stirred and heated at 110 ºC for 7 min, then cooled to 50 ºC. The reaction mixture was diluted with H_2O (6.75 mL) and loaded onto a C-18 SepPak Light cartridge. The reaction vessel was rinsed with another portion of H_2O (6.75 mL) and the rinse was also passed through the SepPak Light cartridge. The SepPak cartridge was eluted with 1 mL of EtOH into a receiving vial containing 3 mL of semipreparative HPLC mobile phase. The content in the receiving vial was loaded onto a semi-preparative column (Phenomenex Luna C18(2), 10 μm, 10 x 250 mm), which was eluted at a flow rate of 2.5 mL/min with a mixture of 40% acetonitrile and 60% 0.1M aqueous ammonium formate (v/v) containing sodium ascorbate (5 g/L mobile phase). The eluent was monitored by a UV detector and a radioactivity detector. The product fraction was collected, diluted with H_2O (15 mL) and passed through a second C18 SepPak Light cartridge. The SepPak cartridge was rinsed with 0.5% sodium ascorbate solution (15 mL).

[^{18}F]FP-(+)-DTBZ was eluted off the SepPak with 1 mL of absolute ethanol (USP), followed by 3 mL of 0.5% sodium ascorbate in USP saline, into a product vial containing 7 mL of 0.5% sodium ascorbate in USP saline. This mixture was then passed through a sterile membrane filter (0.22 μm) for terminal sterilization and collected in a sterile vial to afford a formulated solution ready for dispensing and injection. Total synthesis time was about 80 min from end of beam (EOB).

A portion of the final formulated product solution (~1 mL) was used for quality control tests. For determination of chemical purity, radiochemical purity, and specific radioactivity, an amount (5-100 μL) of the solution was withdrawn into an HPLC syringe and the amount of radioactivity (mCi) assayed using a dose calibrator (Capintec). The solution was then analyzed by HPLC (column: Phenomenex Luna C18(2), 5 μm, 100Å, 4.6 x 250 mm; mobile phase: 31% acetonitrile and 69% 0.1 M aqueous ammonium formate solution; flow rate: 2 mL/min; UV detector wavelength: 280 nm). The area of the UV peak associated with FP-(+)-DTBZ was compared to a pre-defined standard mass curve to determine the mass (nmol). The specific activity (MBq/nmol) of [^{18}F]FP-(+)-DTBZ was calculated as the ratio between the amount of radioactivity (MBq) and the amount of mass (nmol). Additional quality control tests were performed before the product batch is released for use.

Synthesis procedures of [^{18}F]FP-(−)-DTBZ were same as those for [^{18}F]FP-(+)-DTBZ, except using the (−)-enantiomer of the radiolabeling precursor.

Determination of Ligand Metabolism in Plasma

Analysis of the unchanged parent compound and its metabolites in arterial plasma samples collected at 5, 10, 30, 60, 90, and 120 min or 3, 7, 15, 30, 60, 90 and 120 min after injection of [^{18}F]FP-(+)-DTBZ or [^{18}F]FP-(−)-DTBZ, respectively, was conducted by using an automatic column-switching high performance liquid chromatography (HPLC) assay detailed in (*1*). In short, up to 5 mL of the filtered plasma mixture with urea was loaded onto the self-packed capture column with Phenomenex Strata-X sorbent with 1% acetonitrile water flowing at 2 mL/min. After 4 min, the capture column was backflushed with the analytical mobile phase designated 60: 40 0.1M ammonium formate: acetonitrile (v/v) flowing at 1.5 mL/min onto a

Phenomenex Luna C18 analytical column (250x4.6mm, 5µm) giving the parent compound retention time at ~10.5 min. The unmetabolized parent fraction was calculated as the ratio of the sum of radioactivity in fractions containing the parent compound to the total amount of radioactivity collected, and fitted with an inverted gamma function. This fraction curve was also corrected by the time-varying extraction efficiency of radioactivity in corresponding filtered plasma sample. The plasma input function was calculated as the product of the total plasma curve and the parent fraction curve.

Table S1 Distribution volume (V_T) values with blood volume correction

Regions	V_T(mL/cm^3)					
	[^{18}F]FP-(+)-DTBZ			[^{18}F]FP-(−)-DTBZ		
	HC	T1DM	*P*-value	HC	T1DM	*P*-value
Pancreas	165±48	95±38	0.04	23±3	17±5	0.06
Spleen	26± 2	29±5	0.21	7±2	8±3	0.91
Renal cortex	23±3	20±4	0.19	10±2	10±2	0.88

Table S2 Non-displaceable distribution volume (V_{ND}) and pancreatic BP_{ND} with blood volume correction

Method	V_{ND}		α			Pancreatic BP_{ND}			
	HC	T1DM	HC	T1DM	All subjects	HC	T1DM	*P*-value	%difference
1. V_T(+,spleen)	26(8%)	29(17%)				5.3(27%)	2.4(53%)	0.01	56%
1. V_T(+,renal cortex)	23(11%)	20(18%)				6.3(33%)	3.9(51%)	0.11	38%
2. V_T(−,pancreas)	23(14%)	17(28%)				6.5(50%)	4.5(40%)	0.31	30%
3. V_T(+,spleen)/α_{spleen}	*68(8%)	*75(17%)	0.33(27%)	0.46(43%)	0.38(39%)	*1.4(38%)	*0.29(167%)	0.01	80%
3. V_T(+,renal cortex)/α_{renal}	*45(11%)	*40(18%)	0.44(22%)	0.59(28%)	0.50(29%)	*2.7(39%)	*1.47(69%)	0.11	45%

V_{ND} values and α ratios were derived from SUV values using Methods 1 and 3. See text for details. Data are expressed as mean (COV%). Group differences in pancreatic SUVR-1 were compared using *t*-test.
*α_{spleen} and α_{renal} from all subjects were used for calculation.

Figure S1

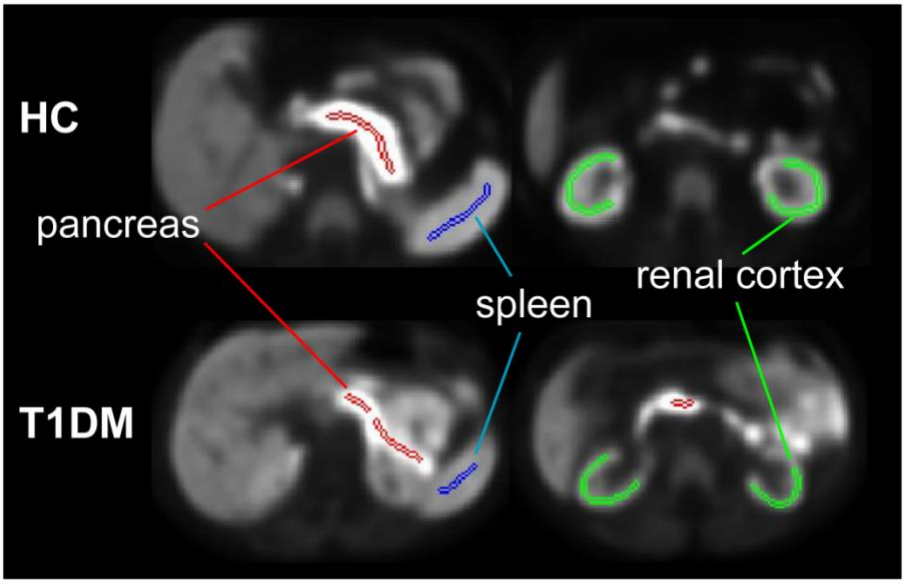

Figure S1: Typical example of ROIs on transaxial PET SUV images. Contours of each region are shown in different colors: pancreas (red), spleen (blue), and renal cortex (green). The contours are not included in the ROI.

Figure S2

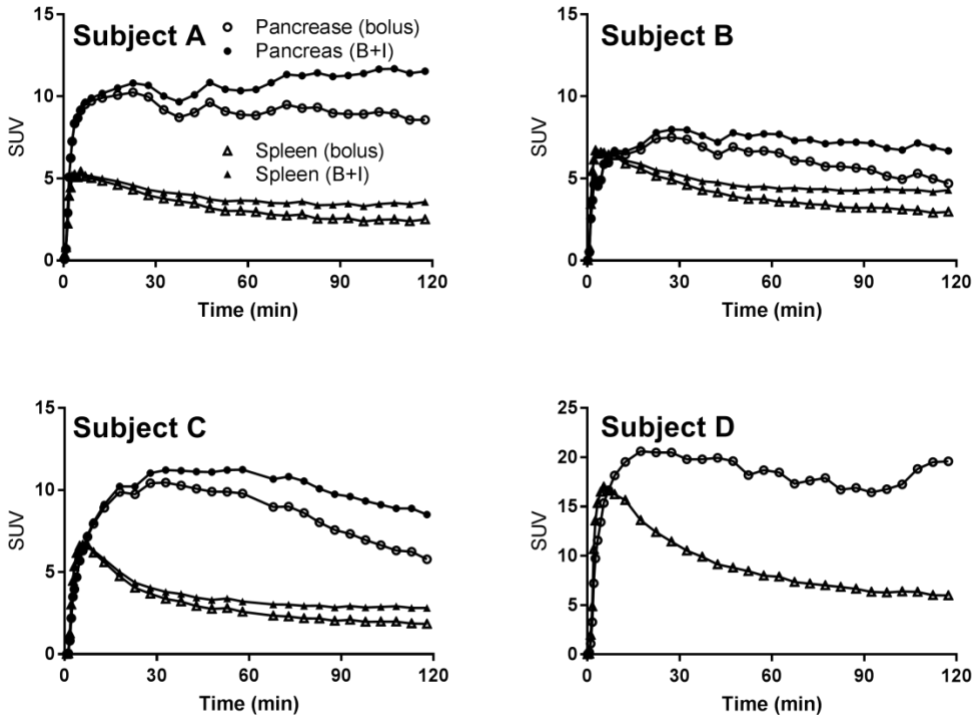

Figure S2: Pancreas and spleen time-activity curves (TACs) of ^{18}F-FP-(+)-DTBZ before and after conversion of T1DM patients form infusion to bolus. Subjects A, B, and C were scanned in a bolus + infusion (B+I) protocol and regional TACs were converted to bolus data. Subject D was scanned after a bolus injection of ^{18}F-FP-(+)-DTBZ.

References

1. Naganawa M, Lin SF, Lim K, et al. Evaluation of pancreatic VMAT2 binding with active and inactive enantiomers of 18F-FP-DTBZ in baboons. *Nucl Med Biol.* 2016;43:743-751.