Nuclear Medicine and Biology 40 (2013) 60–64

Contents lists available at SciVerse ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

PET quantification of pancreatic VMAT 2 binding using (+) and (−) enantiomers of [^{18} F]FP-DTBZ in baboons ☆

Paul E. Harris [a], Michael D. Farwell [b], Masanori Ichise [b,*]

[a] Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY 10032
[b] Department of Radiology, Columbia University College of Physicians and Surgeons, New York, NY 10032

ARTICLE INFO

Article history:
Received 12 July 2012
Received in revised form 6 September 2012
Accepted 18 September 2012

Keywords:
Vesicular monoamine transporter
β-cell mass
PET
[^{18} F]FP-DTBZ

ABSTRACT

Objectives: The exact cause(s) of apparent overestimation of β cell mass (BCM) with vesicular monoamine transporter type 2 (VMAT2) PET imaging in type 1 diabetes (T1D) is unknown. The objectives were to estimate in baboons non-displaceable binding of [^{18} F]fluoropropyl (FP)-(+)-dihydrotetrabenazine (DTBZ) with its inactive enantiomer, [^{18} F]FP-(−)-DTBZ, to validate the use of the reference tissue (renal cortex or spleen) in VMAT2 quantification; and also to compare specific pancreatic VMAT2 binding with that of the striatum in the same baboon brains because high specific binding signal for the pancreas would be desirable for its accurate quantification.

Methods: Baboons (*Papio ursinus*) had multiple dynamic abdominal and brain PET scans each for 2 h with (+) and (−) enantiomers on separate occasions. Data were analyzed by compartmental models to estimate non-displaceable (V_{ND}) and specific (V_S) VMAT2 binding in respective organs.

Results: [^{18} F]FP-DTBZ PET showed excellent target tissue signal and specific VMAT2 binding in the pancreas ($Vs = 41 \pm 11$ mL/cm^3) at nearly 80% that of the striatum. Directly estimated non-displaceable binding in the pancreas ($V_{ND} = 12 \pm 1$ mL/cm^3) was similar to that of the renal cortex, spleen or cerebellum.

Conclusion: ^{18} FFP-DTBZ PET shows excellent specific VMAT2 binding in both the pancreas and striatum in baboons. The renal cortex or spleen as the reference tissue in VMAT2 quantification appears supported. However further studies appear warranted to directly estimate pancreatic non-displaceable binding in humans including T1D patients and also to clarify the cause of the apparent overestimation of BCM in T1D.

1. Introduction

The vesicular monoamine transporter type 2 (VMAT2) is found in the dopaminergic terminals of the brain. It has been well established that positron emission tomography (PET) imaging using radiotracers derived from dihydrotetrabenazine (DTBZ) can be used as a biomarkers of striatal dopaminergic innervation [1]. VMAT2 PET imaging using radiolabeled DTBZ and its analogs has been used in the evaluation of central nervous system disorders such as Parkinson's disease [2], Lewy body dementia [3,4], and Tourette's syndrome [5,6]. VMAT2 is also found on the membrane of putative vesicles that store insulin and dopamine within β-cells of the endocrine pancreas [7]. VMAT2 also appears to have the potential to be used as a PET imaging biomarker of β-cell mass (BCM) [8–11]. Accurate noninvasive BCM measurements may ultimately aid in more clearly defining the disease process and management of patients with type 1 diabetes (T1D) and type 2 diabetes (T2D) [9–11].

We previously evaluated VMAT2 as a potential BCM biomarker by [^{11}C]DTBZ PET imaging in the first proof-of-concept and cross sectional study of healthy human volunteers and patients with long standing T1D [10]. T1D patients were predicted to have little or no BCM based on metabolic measurements. VMAT2 binding potential (BP_{ND}) in the pancreas, which is proportional to the VMAT2 density [12], was reduced only modestly in T1D patients [10]. The presence of significant PET VMAT2 binding signal in the T1D population was unexpected because histological studies suggest near complete depletion of BCM in long standing T1D [13]. Recently, studies using an ^{18} F-labeled VMAT2 ligand, [^{18} F]fluoropropyl (FP)-(+)-DTBZ [14,15], showed more marked reductions by 40% in pancreatic VMAT2 BP_{ND} in long-standing T1D patients compared with healthy controls [11]. However, there was still significant VMAT2 binding signal in the pancreas of T1D patients and the exact cause(s) of this finding is unknown. [^{18} F]FP-(+)-DTBZ ($K_i = 0.11$ nM) has almost 8-fold higher affinity for VMAT2 binding sites than does [^{11}C]DTBZ ($K_i = 0.97$ nM) [14,15]. Although β-cells are low in abundance (1%–2% of the total pancreatic tissue) and dispersed throughout the pancreas, [^{18} F]FP-(+)-DTBZ provided exquisitely excellent target tissue (pancreas) signal-to-noise ratios on PET images with a peak pancreatic time activity of over 20 standardized uptake values (SUV) in humans

☆ Funding: This work was supported by PHS, NIH, NIDDK (5 R01 DK063567).
* Corresponding author. Tel.: +1 212 342 0721.
E-mail address: mi2193@columbia.edu (M. Ichise).

0969-8051/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nucmedbio.2012.09.003

[11]. Together with the advantage of a longer physical half life of ^{18}F (110 min) compared with ^{11}C (20 min), [^{18}F]FP-(+)-DTBZ currently appears to be the tracer of choice for VMAT2 imaging of pancreatic beta cell mass.

Apparent overestimation of BCM with PET VMAT2 binding measurements in these studies [10,11] may be due to the limitations in PET quantification using reversible receptor (transporter) binding radiotracers such as [^{18}F]FP-(+)-DTBZ. The PET signal from the pancreas after a bolus injection of [^{18}F]FP-(+)-DTBZ consists of two major components, non-displaceable (free + nonspecific binding) and displaceable (specific) VMAT2 binding [12]. Specific binding can be estimated from kinetic model-based PET data analysis if the non-displaceable component can also be estimated. VMAT2 PET imaging of the brain allows for the estimation of the non-displaceable binding portion of the striatum using the cerebellar cortex, which is devoid of VMAT2 (called the reference tissue) [16]. However, there is no comparable reference tissue within the pancreas because β cells are scattered throughout the pancreas. Instead, the renal cortex has been used as the reference tissue to estimate non-displaceable binding in the pancreas because it is known to be devoid of VMAT2 [10,11]. However, if non-displaceable binding in the pancreas is significantly greater than in the renal cortex, PET measured BCM using the renal cortex as the reference tissue would be overestimated.

One approach to estimate this non-displaceable binding is to conduct a separate PET experiment in which all VMAT2 sites are blocked by pre-administering a large pharmacologically-active dose of non-radioactive VMAT2 antagonists such as DTBZ. In this VMAT2 blocked condition, all pancreatic [^{18}F]FP-DTBZ signal is due to non-displaceable binding. However, this approach is not desirable, at least in humans due to the potential pharmacological effects of blocking all VMAT2 sites including those in the brain. Alternatively, to directly estimate this nondisplaceable binding within the pancreas, we can perform a second PET experiment using a stereoisomer of [^{18}F]FP-(+)-DTBZ, namely [^{18}F]FP FP-(−)-DTBZ. Binding of DTBZ to VMAT2 is stereospecific. The (−)-enantiomer has practically no affinity for VMAT2 (Ki>3,000 nM vs. Ki = 0.11 nM for the (+)-enantiomer) [14,15,17].

In this study, we estimated non-displaceable binding of [^{18}F]FP-(+)-DTBZ directly within the pancreas using PET imaging of baboons with [^{18}F]FP-(+)-DTBZ and its enantiomer, [^{18}F]P-(−)-DTBZ and compared this pancreatic non-displaceable binding with that of the renal cortex and spleen estimated with [^{18}F]FP-(+)-DTBZ, the spleen being another potential reference tissue. In addition, PET imaging of the same baboon's brains was also performed so that a direct comparison of the magnitude of specific pancreatic VMAT2 binding with the specific striatal VMAT2 binding could be made. We found that the specific binding signals were of similar magnitude suggesting that accurate quantification of VMAT2 binding in the pancreas can be made as in the striatum.

2. Materials and methods

2.1. Animals

A total of five adult male baboons (*Papio ursinus*, 22 ± 6 kg) had multiple PET scans. Four baboons had both [^{18}F]FP-(+)-DTBZ and [^{18}F]FP-(−)-DTBZ abdominal scans; three had an additional [^{18}F]FP-(+)-DTBZ brain scan and one had only a [^{18}F]FP-(+)-DTBZ brain scan. Multiple scans in the same animal were separated by at least 2 weeks between scans. Study procedures were approved by the Institutional Animal Care and Use Committees of the Columbia University.

2.2. Radiochemistry

Both [^{18}F]FP-(+)-DTBZ and its inactive enantiomer, [^{18}F]FP-(−)-DTBZ were prepared from their respective enantio-pure desmethyl precursors obtained from Monomerchem, Inc (Research Triangle Park, NC, USA) as per previously described methods [14]. The radiochemical purity of either radiotracer was greater than 98%, and the specific radioactivity was 37–74 TBq/mmol (1000–2200 Ci/mmol) at synthesis completion.

2.3. Positron Emission Tomography (PET) Procedure

Animals were scanned on an HR + scanner (Siemens, Knoxville, TN, USA) in a two-(abdomen) or three (brain)-dimensional mode under 2% isoflurane anesthesia for 120 min with 22 frames of increasing frame length (3 × 15 s, 2 × 60 s, 4 × 120 s, 2 × 300 s and 10 × 600 s) starting with an intravenous injection (over 30 s) of either [^{18}F]FP-(+)-DTBZ or [^{18}F]FP-(−)-DTBZ (115 ± 49 MBq). Vital signs were monitored (DataScope Corp, Paramus, NJ, USA) and body temperature was maintained at 37 ºC. A 10 min transmission scan was acquired before the tracer injection for attenuation correction. PET data were corrected for attenuation, scatter, and randoms and reconstructed using filtered back-projection with a Shepp filter (cutoff frequency = 0.5 cycles). For the input function measurement, multiple arterial samples were concurrently collected via a femoral artery using an automated sampling system during the first 4 min (11 samples) and manually thereafter at longer intervals at 5, 6, 8, 12, 16, 20, 30, 50, 70, 90, and 120 min (a total of 22 samples). Six samples collected at 2, 4, 12, 10, 50 and 70 min were analyzed by radio-high-performance liquid chromatography (HPLC) to determine the fraction of un-metabolized parent radioactivity. The data points were fitted with a sum of two exponential functions. Additionally, plasma fractions (f_P) of radiotracer unbound to plasma protein were determined using the ultrafiltration method previously described [18].

2.3. PET Data Analysis

For [^{18}F]FP-(+)-DTBZ abdominal scans, regions of interest (ROIs) were manually defined by visual inspection over the pancreas (≈6 cm^3) and spleen (≈10 cm^3) on transverse slices and the renal cortex (right + left ≈ 12 cm^3) on coronal slices of summed images over 120 min by MI who is a radiologist and nuclear medicine physician experienced in anatomical and functional images of the abdomen. For [^{18}F]FP-(−)-DTBZ abdominal scans, the summed images were co-registered to corresponding [^{18}F]FP-(+)-DTBZ summed images by using the iterative co-registration module in biomedical image data analysis software, PMOD (PMOD Technologies Ltd., Zurich, Switzerland); the same ROIs defined on the latter were used with minor modifications when needed as assessed by visual inspection. For [^{18}F]-FP-(+)-DTBZ brain scans, T1 weighted 1.5 T magnetic resonance (MR) scans obtained previously were co-registered to the summed images in PMOD. These images were manually reoriented along the anterior–posterior commissural line. ROIs were manually drawn over the striatum (right + left ≈ 1.8 cm^3) and cerebellum (≈6 cm^3) on the fused PET/MR images. ROI time activity curve (TAC) data were decay corrected and expressed as SUV, which is the tissue radioactivity normalized by injected tracer dose and body weight. TAC data were fitted with standard compartment models for reversibly binding radiotracers to receptors (transporters) using metabolite-corrected plasma TAC data as input functions to estimate the total distribution volume, V_T (mL/cm^3) using kinetic analysis modules implemented in PMOD .

For [^{18}F]FP-(+)-DTBZ, target tissue V_T containing VMAT2 (pancreas or striatum) was defined by the sum of the non-displaceable (free + nonspecific binding) (V_{ND}) and specific binding (V_S) compartment distribution volumes. V_S is proportional to VMAT2 density in tissue (B_{max}) and is defined by $V_s = \frac{f_P B_{max}}{K_D}$ where K_D is the dissociation constant and f_P is the free radiotracer fraction in plasma. V_T in the reference tissue (renal cortex, spleen or cerebellum), containing no appreciable amounts of VMAT2 was equal to V_{ND} in that organ. For the