Nuclear Medicine and Biology 43 (2016) 131–139

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

Kinetics modeling and occupancy studies of a novel C-11 PET tracer for VAChT in nonhuman primates

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ARTICLE INFO

Article history:
Received 12 July 2015
Received in revised form 29 October 2015
Accepted 5 November 2015

Keywords:
Tracer kinetics
Vesicular acetylcholine transporter
(−)-[^{11}C]**TZ659**
Binding potential
Occupancy

ABSTRACT

Introduction: Deficits in cholinergic function have been found in the aged brain and in neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD). The vesicular acetylcholine transporter (VAChT) is a reliable biomarker for the cholinergic system. We previously reported the initial *in vitro* and *ex vivo* characterization of (−)-[^{11}C]**TZ659** as a VAChT specific ligand. Here, we report the *in vivo* specificity, tracer kinetics, and dose-occupancy studies in the nonhuman primate brain.

Methods: MicroPET brain imaging of (−)-[^{11}C]**TZ659** was performed under baseline conditions in two male macaques. Tracer kinetic modeling was carried out using a two-tissue compartment model (2TCM) and Logan plot with arterial blood input function and using a simplified reference tissue model (SRTM) and Logan plot (LoganREF) without blood input. Specificity for VAChT was demonstrated by pretreatment with (+)-pentazocine, (−)-vesamicol, or *S*-(−)-eticlopride. Target occupancy (Occ) was calculated following pretreatment with escalating doses of (−)-vesamicol.

Results: Baseline PET imaging revealed selective retention in the striatum with rapid clearance from the cerebellar hemispheres as a reference region. Total volume of distribution (V_T) values derived from both 2TCM and Logan analysis with blood input revealed ~3-fold higher levels of (−)-[^{11}C]**TZ659** in the striatum than the cerebellar hemispheres. Injection of (−)-vesamicol either as a blocking or displacing agent significantly reduced striatal uptake of (−)-[^{11}C]**TZ659**. In contrast, pretreatment with the sigma-1 ligand (+)-pentazocine had no impact. Pretreatment with the *S*-(−)-eticlopride, a dopamine D_2–like receptor antagonist, increased striatal uptake of (−)-[^{11}C]**TZ659**. Striatal binding potential (BP_{ND}, range of 0.33–1.6 with cerebellar hemispheres as the reference region) showed good correlation ($r^2 = 0.97$) between SRTM and LoganREF. Occupancy studies found that ~0.0057 mg/kg of (−)-vesamicol produced 50% VAChT occupancy in the striatum.

Conclusion: (−)-[^{11}C]**TZ659** demonstrated specific and reversible VAChT binding and favorable pharmacokinetic properties for assessing the density of VAChT in the living brain.

1. Introduction

The cholinergic hypothesis states that functional disturbances in cholinergic activity in the brain of aged subjects and patients with dementia play an important role in memory loss and related cognitive problems. Thus, restoration of cholinergic function may reduce the severity of the cognitive loss [1,2]. Although pathological and pharmacological studies have provided considerable supporting evidence [3], the cholinergic hypothesis has been challenged by post-mortem findings that choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) are not reduced in neocortical tissues of patients with mild AD [4,5]. These findings may not accurately reflect *in vivo* cholinergic function considering the stability of these enzymes. Noninvasive *in vivo* imaging techniques are urgently needed to study changes in cholinergic function in patients with cognitive dysfunction [6,7]. PET imaging with a suitable radiotracer could provide a highly sensitive non-invasive

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; AIR, automated image registration; BP_{ND}, binding potential (non-displaceable); ChAT, choline acetyltransferase; CNS, central nervous system; C_R, radiotracer concentration in the reference region; C_T, radiotracer concentration in the target region; EOB, end of bombardment; DVR, distribution volume ratio; [^{18}F]FEOBV, (−)-5-^{18}F-fluoroethoxybenzovesamicol; HD, Huntington's disease; HPLC, high-performance liquid chromatography; i.v., intravenous injection; K_1, transfer constant from plasma to specific target tissue (mL g^{-1} min^{-1}); k_2, transfer constant from tissue to plasma (min^{-1}); k_3, association constant for ligand-binding site (min^{-1}); k_4, dissociation constant for ligand-binding site (min^{-1}); K_d, dissociation constant; LoganREF, Logan Reference; MP-RAGE, magnetization-prepared rapid gradient-echo; nAChR, nicotinic acetylcholine receptor; NHP, nonhuman primate; Occ, target occupancy; PD, Parkinson's disease; PET, positron emission tomography; p.i., post injection; ROI, region of interest; SPECT, single-photon emission computed tomography; SRTM, simplified reference tissue model; SD, standard deviation; SUV, standardized uptake value; TAC, time activity curve; 2TCM, two-tissue compartment model; VAChT, vesicular acetylcholine transporter; V_T, volume of distribution.

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http://dx.doi.org/10.1016/j.nucmedbio.2015.11.003
0969-8051/

imaging modality that is able to directly quantify cholinergic deficits in living subjects. As a primary signaling molecule for cholinergic neurons, newly synthesized ACh is transported into synaptic vesicles by the vesicular acetylcholine transporter (VAChT) which is a very slow transporter [8–12]. VAChT acts as a limiting factor for release of the neurotransmitter (ACh) and plays a critical role in the cholinergic system [2]. VAChT is considered to be a reliable marker for cholinergic function and a suitable target for molecular imaging with PET [13,14]. (−)-Vesamicol binds in a stereoselective, non-competitive manner to VAChT [15,16]. It acts as an allosteric antagonist of ACh uptake by presynaptic vesicles, thus inhibiting storage and release of ACh in the cholinergic nerve terminal. Although the pharmacology of (−)-vesamicol and the localization of its binding site to cholinergic terminals were described in the 1980s, the high affinity of (−)-vesamicol for the sigma-1 receptor in the central nervous system (CNS) limited its utility for VAChT imaging. Numerous modified (−)-vesamicol analogs have been reported as potent and selective VAChT inhibitors [17–23]. Although many promising ligands proceeded to subsequent radiolabeling for preliminary evaluation in rodents, only a limited number of them have been evaluated in nonhuman primate (NHP) or human subjects [18,19,21,24–26]. Recently, the results of radiation dosimetry and the first PET imaging studies of (−)-5-^{18}F-fluoroethoxybenzovesamicol ([^{18}F]FEOBV) were reported for ten human subjects [24]. Although equilibrium kinetics in the brain of both NHP and human subjects show delayed equilibrium of >360 min post injection (p.i.), [^{18}F]FEOBV offers advantages over SPECT ligands for cholinergic terminal brain imaging [24].

Our group reported a number of VAChT inhibitors containing a carbonyl group attached to the 4-position of the piperidine ring and reported the structure–activity relationships of these new compounds [18,19,26,27]. The most promising ligands were radiolabeled, and we had performed preliminary evaluation in rodents and NHPs. Among these, (−)-[^{11}C]**TZ659** demonstrated favorable initial results during *in vivo* evaluation in rats and preliminary CNS imaging studies in a male macaque [27,28]. Here we further demonstrate *in vivo* binding specificity of (−)-[^{11}C]**TZ659** for VAChT in healthy adult male NHPs under physiological conditions (baseline) and different pharmacological challenge conditions. Our results revealed that (−)-[^{11}C]**TZ659** binds specifically to the VAChT-enriched striatum. The uptake of (−)-[^{11}C]**TZ659** was both blocked and displaced using the known VAChT ligand, (−)-vesamicol. Pretreatment with a sigma-1 receptor ligand did not impact striatal uptake of (−)-[^{11}C]**TZ659**, while pretreatment with the dopamine D_2–like receptor antagonist (−)-eticlopride increased striatal uptake of (−)-[^{11}C]**TZ659**. To estimate the dose for 50% occupancy of VAChT in the striatum, a series of PET studies of (−)-[^{11}C]**TZ659** after pre-treatment of the same subject using different doses of (−)-vesamicol were performed; these studies demonstrated that (−)-[^{11}C]**TZ659** PET imaging can be used to calculate VAChT occupancy.

2. Materials and methods

2.1. Radiosynthesis

The synthesis of (−)-**TZ659** and the radiolabeling of (−)-[^{11}C]**TZ659** were accomplished as previously described [27]. The radiochemical yield was 40–50% (decay corrected to end of bombardment (EOB)) with a radiochemical purity >99%, the chemical purity of >95%, and the specific activity was >74 GBq/μmol (decay corrected to EOB).

2.2. Nonhuman primate microPET studies

2.2.1. Subjects

All animal experiments were conducted in compliance with the Guide for the Care and Use of Research Animals under protocols approved by the Washington University School of Medicine Animal Studies Committee. PET scans of the NHP brain were carried out on two adult male cynomolgus macaques weighing 5.5–7.5 kg following established procedures [26,29–31]. Subject A underwent four baseline scans (including two scans with two arterial blood sampling) and seven scans following pharmacological intervention. Subject B underwent two baseline scans (including one scan with arterial blood sampling). Known compounds were purchased from Sigma Aldrich; (−)-vesamicol was resolved in-house from racemic vesamicol; all doses were freshly prepared for each study and aseptically filtered for intravenous (i.v.) injection. As described below, binding specificity was evaluated in NHP subject A following pretreatment with the sigma-1 receptor compound (+)-pentazocine, the dopamine D_2–like receptor antagonist *S*-(−)-eticlopride hydrochloride, and displacement with (−)-vesamicol. Subject A also underwent a series of scans following pretreatment using escalating doses of (−)-vesamicol prior to administration of (−)-[^{11}C]**TZ659**.

2.2.2. PET data acquisition

Imaging studies of (−)-[^{11}C]**TZ659** were carried out on a microPET Focus 220 scanner (Concorde/CTI/Siemens Microsystems, Knoxville, TN). Animals were fasted for 12 h before the PET scan, each animal was initially anesthetized with ketamine (10 mg/kg) and glycopyrrolate (0.013 mg/kg) intramuscularly for transportation to the PET suite. Upon arrival at the scanner, the subject was intubated and anesthetized with ~2% isoflurane in oxygen. A percutaneous catheter was placed in the femoral vein for injection of tracer or pharmacological agents; an additional percutaneous catheter was placed in the contralateral femoral artery to permit arterial blood sampling as needed. The monkey's head was positioned supine in the head holder with the brain in the center of the field of view. Anesthesia was maintained at 0.75–2.0% during the scan and core temperature was maintained at ~37 °C; vital signs were monitored every 5 min. After a 10 min transmission scan to confirm positioning of the brain within the scanner; a 45 min transmission scan was performed for attenuation correction. After transmission scans, a 120 min dynamic (3 × 1-min, 4 × 2-min, 3 × 3-min, and 20 × 5-min frames) emission scan was acquired after i.v. injection of 299.7–418.1 MBq of (−)-[^{11}C]**TZ659** in 10% ethanol saline solution.

Occupancy of (−)-vesamicol for VAChT was determined by pretreatment of the animal using escalating doses (0.01, 0.05, 0.125, and 0.25 mg/kg) of (−)-vesamicol, a potent allosteric antagonist of VAChT, administered i.v. to NHP Subject A ~6 min prior to injection of (−)-[^{11}C]**TZ659**. Pretreatment using the sigma-1 receptor ligand (+)-pentazocine (1.0 mg/kg, i.v., 12 min prior to tracer injection) was similarly performed to determine specificity for radiotracer binding towards VAChT *versus* the sigma-1 receptor. The dopamine D_2–like receptor antagonist *S*-(−)-eticlopride [32,33] was used to demonstrate the change in striatal cholinergic activity following treatment with *S*-(−)-eticlopride (0.025 mg/kg) administered i.v. 5 min prior to tracer injection. Displacement studies were conducted in NHP subject A by treatment with 0.3 mg/kg (−)-vesamicol, administered i.v. 20 min post radiotracer injection.

2.2.3. PET image processing

PET scans from all studies were corrected by using individual attenuation and model-based scatter correction, and reconstructed using filtered back projection as described previously [34]. The reconstructed resolution in the PET image was <2.0 mm full width half maximum for all 3 dimensions at the center of the field of view. The subject's magnetization-prepared rapid gradient-echo (MP-RAGE) MR image (collected from 3 T Trio MRI Scanner), and the summed 30 frames PET images were co-registered using AIR method [35,36]. For quality control of the co-registration process, the co-registered PET and MRI were superimposed using Vidi or Analyzer program (AnalyzeDirect Inc., Overland Park, KS) and any misalignments could be detected by a misposition of brain edges on both images. For quantitative analyses, three-dimensional regions of interest (ROIs) for striatum, frontal cortex,