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Preclinical evaluation of a promising C-11 labeled PET tracer for imaging phosphodiesterase 10A in the brain of living subject

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ABSTRACT

Phosphodiesterase 10A (PDE10A) plays a key role in the regulation of brain striatal signaling. A PET tracer for PDE10A may serve as a tool to evaluate PDE10A expression *in vivo* in central nervous system disorders with striatal pathology. Here, we further characterized the binding properties of a previously reported radioligand we developed for PDE10A, [^{11}C]TZ1964B, in rodents and nonhuman primates (NHPs). The tritiated counterpart [^{3}H]TZ1964B was used for *in vitro* binding characterizations in rat striatum homogenates and *in vitro* autoradiographic studies in rat brain slices. The carbon-11 labeled [^{11}C]TZ1964B was utilized in the *ex vivo* autoradiography studies for the brain of rats and microPET imaging studies for the brain of NHPs. MicroPET scans of [^{11}C]TZ1964B in NHPs were conducted at baseline, as well as with using a selective PDE10A inhibitor MP-10 for either pretreatment or displacement. The *in vivo* regional target occupancy (Occ) was obtained by pretreating with different doses of MP-10 (0.05–2.00 mg/kg). Both *in vitro* binding assays and *in vitro* autoradiographic studies revealed a nanomolar binding affinity of [^{3}H]TZ1964B to the rat striatum. The striatal binding of [^{3}H]TZ1964B and [^{11}C]TZ1964B was either displaced or blocked by MP-10 in rats and NHPs. Autoradiography and microPET imaging confirmed that the specific binding of the radioligand was found in the striatum but not in the cerebellum. Blocking studies also confirmed the suitability of the cerebellum as an appropriate reference region. The binding potentials (BP_{ND}) of [^{11}C]TZ1964B in the NHP striatum that were calculated using either the Logan reference model (LoganREF, 3.96 ± 0.17) or the simplified reference tissue model (SRTM, 4.64 ± 0.47), with the cerebellum as the reference region, was high and had good reproducibility. The occupancy studies indicated a MP-10 dose of 0.31 ± 0.09 mg/kg (LoganREF)/0.45 ± 0.17 mg/kg (SRTM) occupies 50% striatal PDE10A binding sites. Studies in rats and NHPs demonstrated radiolabeled TZ1964B has a high binding affinity and good specificity for PDE10A, as well as favorable *in vivo* pharmacokinetic properties and binding profiles. Our data suggests that [^{11}C]TZ1964B is a promising radioligand for *in vivo* imaging PDE10A in the brain of living subject.

Introduction

Phosphodiesterase 10A (PDE10A) is expressed primarily in the medium spiny neurons (MSNs) of the striatum, which is a major input site into basal ganglia of the mammalian brain (Soderling et al., 1999; Strick and Menniti, 2010). Nigrostriatal terminals release dopamine that engages dopaminergic receptors on MSNs. These receptor interactions lead to changes in cyclic nucleotides mediated by PDE10A. Dysfunction of nigrostriatal transmission and MSN function contributes to multiple neurologic and psychiatric illnesses and may provide novel targets for therapeutic interventions (Nishi et al., 2011). Thus, PDE10A may serve as a reliable biomarker for disease progression or a potential target for therapeutic interventions in various central nervous system (CNS) diseases, such as Parkinson disease, schizophrenia, Huntington disease, and addiction (Celen et al., 2013). Moreover, molecular imaging of PDE10A by PET provides a potential *in vivo* biomarker of striatal PDE10A function.

Over the past 10 years, tremendous efforts have been made to develop specific PET tracers for imaging PDE10A in the brain. Our group firstly reported the synthesis of a PDE10A PET radiotracer, carbon-11 radiolabeled papaverine (PDE10A inhibition IC_{50} = 36 nM) in 2010 (Tu

Abbreviations: AIR, Automated Image Registration; BP_{ND}, binding potential (non-displaceable); CNS, central nervous system; C_R, reference region tracer radioactivity concentration; C_T, target region tracer radioactivity concentration; DMF, *N,N*-dimethylformamide; DVR, volume of distribution ratio; LoganREF, Logan Reference; MP-RAGE, magnetization-prepared rapid gradient echo; MSN, medium spiny neuron; NHPs, nonhuman primates; Occ, target occupancy; PDE10A, phosphodiesterase 10A; PSL, photo-stimulated luminescence; ROI, region of interest; SD, Sprague–Dawley; SRTM, simplified reference tissue model; SUV, standardized uptake value; TRV, percentage test–retest variability; VAChT, vesicular acetylcholine transporter; VMAT2, vesicular monoamine transporter 2.

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et al., 2010). Due to the low retention of the tracer ([^{11}C]papaverine) in rat and monkey striatum, the more potent and specific PDE10A inhibitor MP-10 (IC_{50} = 0.18 nM) was selected for radiolabeling. However, a radiolabeled metabolite capable of penetrating the blood–brain-barrier limits the clinical utility of [^{11}C]MP-10 for PET quantification of PDE10A (Plisson et al., 2011; Tu et al., 2011). Recently our group reported a series of pyrazole group-containing analogues with a methoxy group on the quinoline fragment, which displayed high potency and selectivity for PDE10A (Li et al., 2013). Among these compounds, TZ1964B has high PDE10A binding potency with an IC_{50} value of 0.40 ± 0.02 nM and high selectivity for PDE10A versus PDE3A/3B and PDE4A/4B with IC_{50} > 1500 nM (Li et al., 2013). Biodistribution in normal rats and MP-10 pretreatment in rats revealed that [^{11}C]TZ1964B, named as [^{11}C]1 previously, had high striatal accumulation and good binding specificity for PDE10A (Fan et al., 2014). MicroPET studies in nonhuman primates (NHP) consistently showed good tracer retention in the striatum with rapid clearance from non-target brain regions, and a stable metabolism profile that only one hydrophilic radiometabolite was detected (Fan et al., 2014).

In the present study, we further evaluated the *in vitro* binding properties of the ligand using its tritiated counterpart [^{3}H]TZ1964B, and characterized the *in vivo* tracer kinetics and binding characteristics of [^{11}C]TZ1964B in the brains of NHPs, to determine whether this radioligand could permit quantification of PDE10A availability.

Materials and methods

Radioligand preparation

The tritiated compound was custom synthesized by American Radiolabeled Chemicals, Inc. (St. Louis, MO). The carbon-11 labeled compound [^{11}C]TZ1964B was successfully synthesized through *O*-methylation of the corresponding precursor, following our previous report (Fan et al., 2014).

Compound preparation

Reagents and standard compounds for *in vitro* binding assays were purchased from Sigma (St. Louis, MO) and Tocris Biosciences (R&D Systems, Minneapolis, MN). Novel compounds were synthesized in-house. Test compounds (Table 1) were dissolved in *N,N*-dimethylformamide (DMF) or ethanol to create a stock solution; the desired concentration for *in vitro* assays was subsequently obtained by further dilution in the Tris–HCl buffer (50 mM Tris/HCl, 8.30 mM $MgCl_2$, 1 mM EDTA, pH 7.5). In the blocking and displacement studies of microPET scans, the solution of MP-10 (0.50 mg/mL) was achieved with 10% PEG300/30% cyclodextrin solution/60% water.

Table 1
Summary of the PDE10A binding affinity of a series compounds obtained from competitive binding study with [^{3}H]TZ1964B using P20 fractions of rat striatum homogenates.

Drugs	Selectivity	K_i, nM
TZ19106B	PDE10A	0.015 ± 0.002
MP-10	PDE10A	0.20 ± 0.06
TZ1982T	PDE10A	0.42 ± 0.10
TZ1914B	PDE10A	36.89 ± 4.51
9c	PDE10A	251.40 ± 44.67
9a	PDE10A	>1000
Papaverine	PDE10A	>1000
Vinpocetine	PDE1	>1000
Pentazocine	Sigma 1	>1000
ISO-1	Sigma2	>1000
Ditolylguanidine (DTG)	Sigma 1/2	>1000
WAY-100135	5-HT1A	>1000
Haloperidol	D2	>1000
Eticlopride	D2	>1000
Vesamicol	VAChT	>1000

Experimental animals

All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals under protocols approved by Washington University's Animal Studies Committee. Mature, male Sprague–Dawley (SD) rats (Charles River Laboratories, Inc., Wilmington, MA) were used for *in vitro* binding assays and *in vitro* autoradiography. Tail vein injections were performed under light inhalation anesthesia (1–2% isoflurane/oxygen) and euthanasia was done under surgical plane anesthesia. Two male adult cynomolgus monkeys, weighing on average 4–6 kg, served as subjects for microPET studies.

In vitro binding assay in rat brain homogenates

Brain homogenate preparation

Subcellular fraction was carried out as described by Xie et al. (2006). Rats were euthanized, and bilateral striatum were dissected and homogenized. Tissue homogenization was carried out in cold (4 °C) homogenization buffer (0.32 M sucrose, 4 mM HEPES-NaOH, 1 mM EDTA, pH 7.4) by vigorous vortexing. The homogenate was then centrifuged for 10 min at 800 × *g* to yield the pellet (P0) and the supernatant (S0). P0 was washed in five volumes of lysis buffer, centrifuged again to yield P1 and S1. S0 and S1 were pooled and centrifuged for 15 min at 9000 × *g* yielding S2 and P2. The supernatant S2 was further spun at 100,000 × *g* for 60 min using the Beckman Ti45 rotor to yield the high speed membrane fraction P20. P20 was re-suspended into the Tris–HCl buffer. Aliquots were stored at −80 °C until use. The protein concentration of the suspension was determined using the DC protein assay (Bio-Rad, Hercules, CA).

Radioligand binding assay

The kinetic analysis of [^{3}H]TZ1964B (~2 nM) binding to rat striatum homogenates P20 (~80 μg/mL) was carried out to measure the association (K_{on}) and dissociation (K_{off}) rates. Association and dissociation curves were obtained by recording the amount of the radioligand bound specifically as a function of time at a constant concentration of both the radioligand and the enzyme. Dissociation experiments were carried out by adding 10 μM of MP-10 to the reaction mixture after equilibrium was achieved and the specific binding of the radioligand as a function of time was determined. The values of K_{on} and K_{off} were determined by nonlinear regression analysis using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

For the saturation binding assay, rat striatum homogenates P20 were diluted and incubated for 60 min with [^{3}H]TZ1964B in a total volume of 150 μL at 25 °C in 96-well polypropylene plates (Fisher Scientific, Pittsburgh, PA). Each well contained 20 μg protein while the concentrations of the radioligand ranged from 0.20 nM to 10.00 nM. Reactions were terminated by the addition of 100 μL of Tris–HCl buffer at 4 °C, then samples were harvested and filtered rapidly using a 96-well glass fiber filtration plate (Millipore, Billerica, MA) presoaked with 100 μL Tris–HCl buffer for 1 h. Each filter was washed with 5 × 200 μL Tris–HCl buffer then transferred to a scintillation vial with 2 mL of scintillation fluid and counted on a Wallac 1450 MicroBeta TriLux liquid scintillation counter (Perkin Elmer, Boston, MA). The equilibrium dissociation constant (K_d) and maximum number of binding sites (B_{max}) were determined by nonlinear regression analysis of one-site saturation binding model using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

For competitive binding assays, rat striatum homogenates P20 (4 μg protein) were incubated with [^{3}H]TZ1964B and each test compound in a total volume of 150 μL in 96 well plates for 60 min at 25 °C. The final concentration of the radioligand in each assay was 2 nM. Concentrations of test compounds ranging from 0.03 nM to 10 μM were added to provide inhibition curves. After incubation, samples were washed 5 times, bound radioactivity counted and analyzed by nonlinear