Published in final edited form as:

Nucl Med Biol. 2010 July ; 37(5): 587–591. doi:10.1016/j.nucmedbio.2010.03.007.

SPECT Imaging with the Serotonin Transporter Radiotracer [¹²³I] *p* ZIENT in Nonhuman Primate Brain

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Abstract

Introduction—Serotonin dysfunction has been linked to a variety of psychiatric diseases; however, an adequate SPECT radioligand to probe the serotonin transporter system has not been successfully developed. The purpose of this study was to characterize and determine the in vivo selectivity of iodine-123 labeled 2β-carbomethoxy-3β-(4'-(Z)-2-iodoethenyl)phenyl)nortropine, [¹²³I]*p* ZIENT, in nonhuman primate brain.

Methods—Two ovariectomized female baboons participated in 9 studies (1 bolus and 8 bolus to constant infusion at a ratio of 9.0 h) to evaluate [¹²³I]*p* ZIENT. To evaluate the selectivity of [¹²³I]*p* ZIENT the serotonin transporter blockers fenfluramine (1.5, 2.5 mg/kg) and citalopram (5 mg/kg), the dopamine transporter blocker methylphenidate (0.5 mg/kg) and the norepinephrine transporter blocker nioxetine (1 mg/kg) were given at 8 h post-radiotracer injection.

Results—In the bolus to constant infusion studies equilibrium was established by 4–8 h. [¹²³I]*p* ZIENT was 93% and 90% protein bound in the 2 baboons and there was no detection of lipophilic radiolabeled metabolites entering the brain. In the high density serotonin transporter regions (diencephalon and brainstem) fenfluramine and citalopram resulted in 35–71% and 129–151% displacement, respectively, whereas methylphenidate and nioxetine did not produce significant changes (<10%).

Conclusion—These findings suggest that [¹²³I]*p* ZIENT is a favorable compound for in vivo SPECT imaging of serotonin transporters with negligible binding to norepinephrine and dopamine transporters.

Keywords

SPECT; Brain imaging; Serotonin Transporter; Nonhuman Primate; [¹²³I]*p* ZIENT

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1. Introduction

Disturbances in serotonin neurotransmission are involved in a variety of psychiatric disorders including depression [1], anxiety [2], psychoses [3] and alcoholism [4]. Specifically, the serotonin transporter is responsible for removal of serotonin from the synapse, which ideally results in a homeostatic serotonergic tone, but in some individuals this is disturbed. For example, individuals with major depression have reduced serotonin transporter availability compared to healthy subjects [5], which may predict treatment response to selective serotonin reuptake inhibitors (SSRIs) that act at the serotonin transporter [6]. Additionally, patients with generalized social anxiety disorder have higher serotonin transporter availability compared to healthy subjects [7]. The importance of understanding the biological underpinnings of these disorders has led to a search for development of a selective high-affinity serotonin transporter radioligand.

Several radiotracers with serotonin transporter binding have been developed and widely used, such as the tropane derivatives [^{123}I]beta-CIT [8,9] and [^{123}I]nor-beta-CIT [10,11]; however, these radiotracers are limited by poor selectivity for the serotonin transporter versus the dopamine transporter. The most successful radiotracers have been labeled with carbon-11 for PET imaging, most notably [^{11}C]DASB [12,13], and related structures such as [^{11}C]MADAM [14, 15], which bind with high affinity and specificity. More limited success has been observed with SPECT agents, such as [^{123}I]ADAM [16,17]. In general, these radiotracers are limited in their ability to measure serotonin transporters throughout the cortex, which is critical to understanding the role of executive function including goal-oriented behavior in psychiatric disorders. [^{123}I]p ZIENT (2 β -carbomethoxy-3 β -(4-((Z)-iodoethenyl)phenyl)nortropane is a tropane analog previously developed and synthesized [18] that demonstrates high affinity ($K_D = 0.03$ nM) and high specificity for binding to the 5-HT transporter compared to the dopamine (100-fold) and norepinephrine (500-fold) transporters. The purpose of the present study was to evaluate and determine the selectivity of [^{123}I]p ZIENT as a single photon emission computed tomography (SPECT) radiotracer for the serotonin transporter in nonhuman primate brain.

2. Materials and Methods

2.1 General

No-carrier-added sodium [^{123}I]iodide in 0.1 N NaOH (radionuclidic purity >99.8%) was obtained from MDS Nordion (Vancouver, BC, Canada). Other reagents were of analytical grade and were obtained from conventional chemical suppliers. Preparative HPLC was carried out on 5- μm Novapak C18 (Waters Corp., Milford, MA, USA) columns (300 $\times$ 3.9-mm stainless steel) with a mobile phase of $\text{CH}_3\text{OH}/\text{H}_2\text{O}/\text{Et}_3\text{N}$ (80/20/0.2, vol/vol/vol) and a flow rate of 1.0 mL/min. Blood samples were collected at the midpoint of each scan to evaluate differences in metabolism and protein binding of [^{123}I]p ZIENT between animals and studies. After centrifugation of whole blood, the plasma was separated and treated with an equal volume of acetonitrile and the denatured protein was removed by centrifugation. The supernatant was analyzed on a high-sensitivity HPLC system, consisting of a Spectra-Physics SP8810 pump, a Waters 484 ultraviolet (UV) detector and home-built in-line flow-through NaI (T1) scintillation detector and rate meter, using a mobile phase of $\text{CH}_3\text{OH}/\text{H}_2\text{O}/\text{Et}_3\text{N}$ (85/15/0.2, vol/vol/vol) on a radial compression module RCM-100 (Waters) and a flow rate of 1.0 mL/min. Total plasma radioactivity was measured in an automatic well-type gamma-counter (LKB 1282 Compugamma CS with 2-in. crystal; Wallac Oy, Turku, Finland), with a window spanning 159 keV. The counting efficiency of the counter (efficiency=0.687) was determined using sources of ^{123}I calibrated in a gas ionization chamber (Capintec CRC-727 Dose Calibrator) with geometry similar to that of the blood samples. All radioactivity measurements were decay-corrected to the time of radiopharmaceutical administration using $T_{1/2}=13.2$ h.

2.2 Chemistry

[¹²³I]*p* ZIENT was prepared by iododestannylation of the trimethylstannyl precursor with sodium [¹²³I]iodide and peracetic acid as oxidizing agent at room temperature, as described previously [18]. Purification by reverse phase HPLC with concomitant UV and radioactivity detection, allowed identity confirmation of [¹²³I]*p* ZIENT by comparison to its corresponding unlabelled analog. The final dosage form was in the form of sterile isotonic saline containing 5% (vol/vol) ethanol and 0.2 mM L-ascorbic acid (final pH of 5–6). Based on the elution profile, [¹²³I]*p* ZIENT was obtained with a no-carrier-added radiochemical yield of 32.9±13.5% (n=9) and radiochemical purity of 95.1±3.4% (n=9).

2.3 In Vivo SPECT Imaging

Two ovariectomized female baboons (*Papio anubis*, 15 and 20 kg) participated in 9 studies separated by 2 to 4 weeks. These studies were carried out under institutional animal care protocols in compliance with federal regulations. Animals were fasted for 18–24 hours prior to the study. Animals were initially anesthetized with ketamine (10 mg/kg, IM), and then maintained on isoflurane (2–3%) anesthesia via an endotracheal tube for the duration of the study. Glycopyrrolate (10 µg/kg, IM), a long-acting peripheral anticholinergic drug that does not cross the blood brain barrier, was administered with the initial ketamine injection to decrease respiratory and digestive secretions. Vital signs including heart rate, respiration rate, oxygen saturation and body temperature were continuously monitored and recorded every 30 minutes. Body temperature was kept constant at 37 °C with a heated water blanket. An IV line was placed in one leg for bolus injection and infusion of the radiotracer and for injection of nonradioactive drugs. A second IV line was placed in the other leg in order to obtain blood samples and for infusion of Lactated Ringers solution, which was maintained at a rate of 1.8 mL/kg/h for the duration of the study. In one study (the bolus only) an arterial line was placed to collect blood samples for kinetic modeling. The baboon's head was immobilized in the gantry with a vacuumed beanbag (Olympic Medical, Seattle, WA, USA). The animals participated in bolus only and equilibrium (bolus plus constant infusion) studies. To assess the pharmacological specificity of [¹²³I]*p* ZIENT, the serotonin transporter blockers fluoxetine (1.5, 2.5 mg/kg, IV) and citalopram (5 mg/kg, IV), the dopamine transporter blocker methylphenidate (0.5 mg/kg, IV), and the norepinephrine transporter blocker nisoxetine (1 mg/kg, IV), were administered at 8 h post-radiotracer injection in the equilibrium studies. All drugs were obtained from Sigma (St. Louis, MO), mixed with saline, and administered at the described doses.

2.3.1 SPECT data acquisition—SPECT data were acquired with the nonhuman primate brain-dedicated multislice CERASPECT camera (Digital Scintigraphics, Waltham, MA, USA). The camera has a resolution in all three axes of approximately 12 mm full width at half-maximum measured with an ¹²³I line source in a 20 cm water-filled cylindrical phantom. The distribution of [¹²³I]*p* ZIENT was assessed after one bolus only (277.5 MBq) and in eight bolus (163.5 ± 22.3 MBq) plus constant infusion (17.6 ± 1.9 MBq/h) studies at a ratio of 9.0 h for up to 10 h. Brain images (128 × 128 × 64 matrix; pixel size = 1.67 × 1.67 mm; slice thickness = 1.67 mm; voxel volume = 4.66 mm³) were acquired into a 159 keV (± 10%) energy window, in step and shoot mode at 15 min each for up to 600 min total/study and up to 37 acquisitions/study. For the equilibrium scan with citalopram, 40 acquisitions were obtained during an 11 h study. Images were reconstructed using a ramp and Butterworth filter (cutoff = 0.65 cm, power factor = 10). Magnetic resonance imaging (MRI) scans were obtained for each animal with a 1.5 Tesla GE Signa device (General Electric, Milwaukee, WI, USA). Axial images were acquired using a spoiled GRASS (gradient recall acquisition in the steady state) sequence with TR = 25 ms, TE = 5 ms, NEX = 2, matrix = 256 × 192, field of view = 16 cm.

2.3.2 Image Analysis—SPECT images were reconstructed, attenuation corrected, and analyzed as previously described [19]. Briefly, reconstructed images were coregistered to each animal's MRI and region-of-interest (ROI) analyses were performed. Regions chosen were those known to contain serotonin transporters including diencephalon, brainstem, basal ganglia and right and left hippocampus and cortical regions including the right and left temporal and occipital. The cerebellum (average of left and right hemispheres) was measured as a reference region, e.g., a region devoid of serotonin transporters. The activity in the whole brain was also measured in the bolus only study using a contour formed around the edge of the brain on the MRI.

Average regional activities were decay-corrected to the time of injection and were expressed as kBq/cc and as percent injected dose (%ID) for whole brain in the bolus only study. For equilibrium studies, the primary outcome measure is the binding potential (BP_{ND}), which is the ratio of specific to nonspecific binding with the cerebellum as the reference region. Equilibrium was obtained between 4-8 h, operationally defined as <5% change/hr. To determine the displacement of [^{123}I]p ZIENT by the displacement agents, the percent change in BP_{ND} pre (average of 2 scans prior to the displacement) and post (average of 2 final scans) displacement was calculated and expressed as a percent.

3. Results

3.1 Pharmacokinetics

By the ultrafiltration method, plasma [^{123}I]p ZIENT was $93.1 \pm 0.3\%$ and $89.8 \pm 0.7\%$ protein bound in the two non-human primates tested. Arterial plasma samples following injection of [^{123}I]p ZIENT were analyzed for nonpolar, potentially brain permeable metabolites. Reversed phase HPLC, after plasma protein precipitation with acetonitrile, allowed an operational definition of the components of plasma radioactivity (Table 1) as two metabolites with polarity greater than that of [^{123}I]p ZIENT: (1) lipophilic fraction which decreased over time; (2) a transient polar fraction and (3) a polar component that increased over time. The lipophilic component was identified as unmetabolized [^{123}I]p ZIENT, which rapidly decreased from 69%, at the time of peak level of radioactivity in arterial plasma (4-7 min), to 9% at 60 min and 4% at 180 min. The abundance of the combined two polar metabolite fractions as a percent of total plasma activity increased from 31% at 3 min to 96% at 180 min post-injection. The transient fraction reached its peak at 3-5 min (26%), then decreased to 1% at 180 min. The characterization of the polar metabolites was not deemed necessary because of their improbable brain permeability.

3.2 SPECT data

After administration of [^{123}I]p ZIENT by bolus IV injection, whole brain uptake occurred at 74 min and corresponded to 2.3% of the original injected dose. The regional uptake of [^{123}I]p ZIENT was assessed with bolus and equilibrium studies. The regional rank order of potency [brainstem > diencephalon > basal ganglia > hippocampus > cingulate cortex > occipital cortex > cerebellum] was consistent between bolus and equilibrium studies and is consistent with the known anatomical distribution of 5-HT transporters (Figure 1). Using the bolus to constant infusion paradigm, equilibrium was established in all studies between 4-8 h with < 5% change per hour (Figure 1).

The in vivo pharmacological specificity of [^{123}I]p ZIENT was assessed by displacement studies with the selective 5-HT transporter blockers fluoxetine and citalopram, the DA transporter blocker methylphenidate, and the norepinephrine transporter blocker, nisoxetine. Fluoxetine and citalopram produced a robust, rapid displacement of [^{123}I]p ZIENT in all regions studied but no significant changes were seen after administration of methylphenidate or nisoxetine

(Table 2). In addition, with the arterial blood data collected in the bolus only study, MRTM2 analysis was conducted using cerebellum as the reference region. BP_{ND} values using MRTM2 (diencephalon: 1.48, brainstem: 1.34, basal ganglia: 0.97, hippocampus: 0.49, temporal cortex: 0.39) were consistent with BP_{ND} values calculated as an average from the equilibrium scans prior to displacement in the same animal (diencephalon: 1.04, brainstem: 1.34, basal ganglia: 0.81, hippocampus: 0.82, temporal cortex: 0.22). These BP_{ND} values were notably lower than those in the second animal (diencephalon: 3.83, brainstem: 4.32, basal ganglia: 2.9, hippocampus: 2.07, temporal cortex: 1.28) illustrating the individual variability in serotonin transporter availability.

4. Discussion

In this study, we examined the time course, distribution and specificity of [^{123}I]p ZIENT in baboon brain. Importantly, we detected no radiometabolites that are capable of entering the brain. Since we could not detect the formation of lipophilic radiolabeled metabolites capable of entering the brain, the calculation of the [^{123}I]p ZIENT input function and subsequent quantification of binding sites by tracer kinetic modeling could be performed in the future without correcting for lipophilic metabolites. Consistent with a previous study [18], after bolus injection, [^{123}I]p ZIENT entered the brain rapidly and reached a peak uptake in the highest density regions (diencephalon, brainstem, and basal ganglia) between 130 and 277 min post-injection, and in lower density regions (temporal and occipital cortex) peaked as early as 50 to 74 minutes. Whole brain uptake peaked at 74 min and was 2.3% of the injected dose. Although bolus injection of [^{123}I]p ZIENT appears to approach an equilibrium, bolus to constant infusion at a ratio of 9.0 h resulted in a sustained equilibrium between 4-8 hr with <5% change/hr. Additionally, there is a consistency in BP_{ND} values obtained from MRTM2 and at equilibrium further supporting the use of equilibrium studies with this tracer.

In a series of constant infusion studies, the specificity of [^{123}I]p ZIENT was evaluated with displacement by the serotonin transporter blockers fluoxetine and citalopram, the dopamine transporter blocker methylphenidate, and the norepinephrine transporter blocker nisoxetine. Generally, these results confirm that [^{123}I]p ZIENT uptake in brain primarily reflects binding to 5-HT transporters. Specifically, moderate doses of fluoxetine (1.5-2.5 mg/kg) resulted in displacement of the radiotracer by 46-53% in the diencephalon and brainstem. A high dose of citalopram (5 mg/kg) resulted in robust displacement of 69-72% in diencephalon and brainstem. These findings suggest that [^{123}I]p ZIENT is a favorable compound for in vivo SPECT imaging of serotonin transporters with negligible binding to norepinephrine and dopamine transporters.

[^{123}I]p ZIENT was the first compound prepared and studied of the ZIENT-like compounds. [^{123}I]p ZIENT was targeted for SPECT because of the ease of incorporating an I-123 into the structure. In general, this study suggests [^{123}I]p ZIENT is a good SPECT compound because it has slow kinetics resulting in high binding in SERT-rich regions compared to the cerebellum, a SERT-devoid region. [^{123}I]p ZIENT has similar slow kinetics to [^{123}I]ADAM but higher signal to background. The slow kinetics of [^{123}I]p ZIENT can be attributed to its very high SERT binding affinity (~0.05 nM). This is the highest reported SERT binding affinity of a SPECT radioligand with attractive physiochemical properties. Analogs of pZIENT have also been prepared as PET compounds when labeled with F-18. Specifically, $\beta\text{FepZIENT}$ is a PET SERT radioligand that was designed based upon pZIENT. The iodovinyl pharmacophore was retained for its high SERT binding affinity and selectivity over DAT and NET. The F-18 was introduced in the ester as a fluoroethyl group. This PET radiotracer had similar pharmacologic and imaging properties to pZIENT. Thus, radiolabeling $\beta\text{FepZIENT}$ with I-123 would show no advantage over pZIENT. $\beta\text{FemZIENT}$, which has a SERT affinity of approximately 0.5 nM is a PET SERT radioligand designed based upon mZIENT and results in a radioligand with

faster kinetics and better compatibility for PET. The slow kinetics of pZIENT make it less desirable as a C-11 labeled PET compound than as a F-18 labeled β FEpZIENT. In general, the results from this study and comparisons to similar tracers suggest [^{123}I]p ZIENT is a suitable compound for in vivo SPECT imaging of serotonin transporters.

Acknowledgments

The authors gratefully acknowledge the technical assistance of Louis Amici, Nina Sheung, Gary Wisniewski, and Eileen O. Smith. This research was sponsored by US Department of Veterans Affairs (Schizophrenia Research Center, Research Enhancement Award Program (REAP) Center on “Neural Mechanisms and Treatment Response in Depression”), the Office of Health and Environmental Research, U.S. Department of Energy under Grant No. DE-FG02-97ER62637, NARSAD and National Institutes of Health grants KO1 DA020651 and KO1 AA00288.

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Figure 1.

Time-activity curves for $[^{123}\text{I}]p$ ZIENT for the total and free parent in the blood (kBq/mL) (upper panels) and in the brain (kBq/mL) (lower panels) after a bolus injection (left) of 278 MBq and a bolus to constant infusion study (right) of a bolus of 161 MBq plus constant infusion of 17.8 MBq/h at a ratio of 9.0 h for up to 10 h. Brain regions are diencephalon (Dien), brainstem (Bstem), hippocampus (HA), basal ganglia (BG), temporal insular cortex (TIC), occipital cortex (OC) and cerebellum (CB).

Table 1
Components of plasma radioactivity

Time (min)	Relative proportion (%)		
	Parent Compound $t_R=15.5$ min	“Transient Fraction” $t_R=7.5$ min	“Polar Fraction” $t_R=4.5$ min
1	69	19	12
3	69	26	5
5	65	25	10
10	43	16	41
30	16	6	78
60	10	3	87
120	5	1	94
180	4	1	95
360	3	1	96

Table 2

Percent Displacement in [¹²³I]p ZIENT (BP_{ND})

	Diencephalon	Brainstem	Basal Ganglia	Hippocampus	Temporal Cortex
NET					
Nisoxetine (1 mg/kg, n=2)	5%	2%	4%	9%	8%
DAT					
Methylphenidate (0.5 mg/kg, n=2)	-5%	-11%	-4%	-8%	-1%
SERT					
Fluoxetine (1.5 mg/kg, n=1)	47%	52%	54%	35%	100%
(2.5 mg/kg, n=1)	53%	46%	11%	18%	20%
Citalopram (5 mg/kg, n=1)	69%	72%	41%	60%	38%

% Displacement is the percent change from pre- to post-displacement calculated as ((pre-post)/pre)*100. Pre refers to the 2 scans immediately preceding the displacement. Post refers to the 2 final scans of the study.