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Characterization of [^{11}C]RO5013853, a novel PET tracer for the glycine transporter type 1 (GlyT1) in humans

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ABSTRACT

We characterize a novel radioligand for the glycine transporter type 1 (GlyT1), [^{11}C]RO5013853, in humans. Ten healthy male volunteers, 23–60 years of age, were enrolled in this PET study; seven subjects participated in the evaluation of test-retest reliability and three subjects in whole body dosimetry. Subjects were administered intravenous bolus injections of approximately 1100 MBq (30 mCi) [^{11}C]RO5013853 with a high specific activity of about 481 GBq (13 Ci)/μmol. Standard compartmental model analysis with arterial plasma input function, and an alternative noninvasive analysis method which was evaluated and validated by occupancy studies in both baboons and humans, were performed. Mean parameter estimates of the volumes of distribution (V_T) obtained by a 2-tissue 5-parameter model were higher in the cerebellum, pons, and thalamus (1.99 to 2.59 mL/mL), and lower in the putamen, caudate, and cortical areas (0.86 to 1.13 mL/mL), with estimates showing less than 10% difference between test and retest scans. Tracer retention was effectively blocked by the specific glycine reuptake inhibitor (GRI), bitopertin (RG1678). [^{11}C]RO5013853 was safe and well tolerated. Human dosimetry studies showed that the effective dose was approximately 0.0033 mSv/MBq, with the liver receiving the highest absorbed dose.
In conclusion, quantitative dynamic PET of the human brain after intravenous injection of [^{11}C]RO5013853 attains reliable measurements of GlyT1 binding in accordance with the expected transporter distribution in the human brain. [^{11}C]RO5013853 is a radioligand suitable for further clinical PET studies. Full characterization of a novel radiotracer for GlyT1 in humans is provided. The tracer has subsequently been used to assess receptor occupancy in healthy volunteers and to estimate occupancy at doses associated with best efficacy in a clinical trial with schizophrenic patients with predominantly negative symptoms.

Introduction

Glycine transporter type 1 (GlyT1) is a target of interest in the development of novel drugs for schizophrenia. GlyT1 inhibition is one of the key strategies to address *N*-methyl-D-aspartate receptor (NMDA-R) hypofunction which is postulated to play an important role in the pathophysiology of schizophrenia (Harrison and Weinberger, 2005; Hashimoto, 2011; Krystal et al., 1994; Millan, 2005; Sanger, 2004; Stone, 2009). Promising clinical findings were recently reported in schizophrenic patients with predominantly negative symptoms who were treated with the glycine reuptake inhibitor (GRI) bitopertin (RG1678) (Umbricht et al., 2010). Positron emission tomography (PET) data indicate that low to medium target occupancy was associated with the best efficacy (Umbricht et al., 2011).

A radioligand specific for GlyT1 with appropriate molecular properties for a PET imaging probe is a key tool for the clinical development of a GRI. Such a radioligand allows assessment of brain penetration of candidate drugs and enables the quantification and characterization of transporter occupancy. RO5013853 ([5-methanesulfonyl-2-((*S*)-2,2,2-trifluoro-1-methyl-ethoxy)-phenyl]-[5-(tetrahydro-pyran-4-yl)-1,3-dihydro-isoindol-2-yl]-methanone) was identified as a high affinity ligand for

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GlyT1 suitable for PET radiochemistry and was successfully radiolabeled yielding [^{11}C]RO5013853 (Pinard et al., 2011). Furthermore, it was selected as the better of two PET ligands evaluated in a study in non-human primates presented in this issue (Borroni et al., 2013–this issue).

Here we report the full characterization of [^{11}C]RO5013853 as a novel PET radioligand for GlyT1 in humans. Objectives were as follows: (1) to examine and analyze the specific uptake and kinetics of [^{11}C]RO5013853 in the human brain; (2) to assess the test–retest reliability of quantitative PET measurements between two sessions; (3) to determine human radiation dosimetry following injection of a single microdose of [^{11}C]RO5013853; (4) to report rodent radiation dosimetry for suitability of the microdose of the radiotracer for human use; and (5) to report the acute safety and tolerability of [^{11}C]RO5013853 administered intravenously at microdose levels in healthy humans.

Materials and methods

PET study

Subjects and design

This single-center, open label, non-randomized study was conducted in accordance with the Declaration of Helsinki Principles. The study received ethical approval from the Johns Hopkins School of Medicine Institutional Review Board (Baltimore, MD) and Chesapeake Research Review, Inc. (Columbia, MD) and was conducted under an exploratory U.S. IND following appropriate animal toxicology studies of the unlabeled ligand and radiation dosimetry. All participants provided written, informed consent after receiving oral and written descriptions of study procedures and aims.

The study was conducted in 10 healthy male volunteers, 23 to 60 years of age, with a body mass index ranging from 21.6 to 29.7 kg/m^2. Eligibility criteria included: no significant medical or surgical history; no history of head trauma with prolonged loss of consciousness; no neurologic conditions; no history of alcohol and/or drug abuse or addiction within the last 2 years; heart rate between 40 and 100 beats per minute; no family history of congenital long QT syndrome or sudden death; and a clinically unremarkable brain magnetic resonance imaging (MRI) scan at screening. Subjects were to be medication-free for 14 days or 5 times the elimination half-life (whichever was longer) prior to the first PET scan and throughout the study. Subjects entered the clinic the day before the PET scan and remained in-house until the day after the PET scan. Seven subjects were to be scanned twice, in order to study test-retest reliability of outcome parameters, and 3 subjects underwent whole body PET scans for evaluation of human radiation dosimetry.

Safety assessments

Safety assessments included: continuous medical monitoring during and following the radioligand injection for approximately 24 hours, adverse event reporting, blood chemistry profile, including liver and renal function tests, complete blood count, electrocardiogram, urinalysis, and a physical examination.

Radioligand

[^{11}C]RO5013853 is a highly selective ligand for GlyT1 and was synthesized as previously described (Pinard et al., 2011). Briefly, an appropriate desmethyl precursor was radiolabeled with [^{11}C]methyl iodide and purified by reverse-phase high-performance liquid chromatography (HPLC). A typical synthesis produced 4132.9 ± 1465.2 MBq (111.7 ± 39.6 mCi) (mean ± SD, hereafter) of [^{11}C]RO5013853. The end of synthesis specific radioactivity was 494 ± 296 GBq (13 ± 8 Ci)/μmol. Analytical HPLC showed a radiochemical purity of 100%. [^{11}C]RO5013853 was provided as a sterile, pyrogen-free injectable solution (14:1 normal saline:ethanol).

Table 1 lists the radioactivity dose (MBq [mCi]), mass (μg), mass per kg (ng/kg), and specific activity (GBq [Ci]/μmol) for individual subjects and scans. For the first PET session, subjects (n = 7) received an average dose of 1111.11 ± 52.17 MBq (30.03 ± 1.41 mCi) (mean ± SD), with mass of 1.62 ± 0.91 μg, mass per kg of 19.57 ± 11.70 ng/kg, and specific activity of 496 ± 388 GBq (13 ± 10 Ci)/μmol. The administered dose for the second PET session averaged 1082.99 ± 51.8 MBq (29.27 ± 1.40 mCi), with mass of 1.31 ± 0.45 μg, mass per kg of 15.19 ± 4.98 ng/kg, and specific activity of 459 ± 178 GBq (12 ± 5 Ci)/μmol. The administered doses, masses, and specific activities of [^{11}C]RO5013853 were not significantly different across the two sessions. The injected radioactivity was consistent with ALARA (as low absorbed radiation as possible) principles for the scientific question asked and based on rodent and verified by human radiation dosimetry.

The maximal allowed mass dose per injection was 20 μg RO5013853 which fulfilled the definition of a microdose (Food and Drug Administration, 2006).

Blood sampling and metabolite analysis

For derivation of the plasma input function, arterial blood was initially sampled frequently with increasingly prolonged intervals throughout the scan for 90 minute post injection. Total radioactivity was measured in more than 30 samples using a gamma counter that was cross-calibrated against the PET activity measurements. Selected blood samples collected at 0, 5, 15, 30, 45, 60, and 90 minutes were analyzed by HPLC for the presence of [^{11}C]RO5013853 and its radiolabeled metabolites using a general method developed previously for PET radiotracers (Hilton et al., 2000).

PET scanning

Following placement of a radial arterial catheter (for the input function) and a venous catheter (radioligand injection), seven

Table 1
Injected radioactivity dose, mass, mass per kg, and specific activity per subject per scan [n = 7 subjects].

Data subject	ID	Scan number	Injected dose (MBq) [mCi]	Mass (μg)	Mass per kg (ng/kg)	Specific activity (GBq/μmol) [Ci/μmol]
1	101	1	1095.94 [29.62]	2.83	30.43	191 [5]
		2	1084.47 [29.31]	1.65	17.74	324 [9]
2	102	1	1079.29 [29.17]	2.35	30.92	227 [6]
		2	1175.86 [31.78]	1.73	22.76	335 [9]
3	106	1	1135.53 [30.69]	2.47	33.38	227 [6]
		2	1091.5 [29.50]	0.72	9.73	746 [20]
4	107	1	1013.80 [27.40]	1.16	13.18	430 [12]
		2	1072.26 [28.98]	1.47	16.70	359 [10]
5	109	1	1143.67 [30.91]	0.43	4.67	1301 [35]
		2	1025.64 [27.72]	1.08	11.74	468 [13]
6	108	1	1161.80 [31.40]	0.93	9.69	614 [17]
		2	1107.04 [29.92]	1.74	18.12	313 [8]
7	110	1	1147.37 [31.01]	1.18	14.75	480 [13]
		2	1025.27 [27.71]	0.76	9.50	667 [18]