ITI-007 demonstrates brain occupancy at serotonin 5-HT_{2A} and dopamine D₂ receptors and serotonin transporters using positron emission tomography in healthy volunteers

Robert E. Davis^{1,2}, Kimberly E. Vanover¹, Yun Zhou³, James R. Brašić³, Maria Guevara³, Blanca Bisuna³, Weiguo Ye³, Vanessa Raymond³, William Willis³, Anil Kumar³, Lorena Gapasin^{4,5}, D. Ronald Goldwater⁴, Sharon Mates¹ and Dean F. Wong³

(1) Intra-Cellular Therapies, Inc. (ITI), 3960 Broadway, 6th floor, New York, NY 10032, USA

(2) 3-D Pharmaceutical Consultants, San Diego, CA, USA

(3) Johns Hopkins University School of Medicine, Baltimore, MD, USA

(4) PAREXEL International, Baltimore, MD, USA

(5) Present address: Johns Hopkins University School of Medicine, Baltimore, MD, USA

Abstract

Rationale

Central modulation of serotonin and dopamine underlies efficacy for a variety of psychiatric therapeutics. ITI-007 is an investigational new drug in development for treatment of schizophrenia, mood disorders, and other neuropsychiatric disorders.

Objectives

The purpose of this study was to determine brain occupancy of ITI-007 at serotonin 5-HT_{2A} receptors, dopamine D₂ receptors, and serotonin transporters using positron emission tomography (PET) in 16 healthy volunteers.

Methods

Carbon-11-MDL100907, carbon-11-raclopride, and carbon-11-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile (carbon-11-DASB) were used as the radiotracers for imaging 5-HT_{2A} receptors, D₂ receptors, and serotonin transporters, respectively. Brain regions of interest were outlined using magnetic resonance tomography (MRT) with cerebellum as the reference region. Binding potentials were estimated by fitting a simplified reference tissue model to the measured tissue-time activity curves. Target occupancy was expressed as percent change in the binding potentials before and after ITI-007 administration.

Results

Oral ITI-007 (10–40 mg) was safe and well tolerated. ITI-007 rapidly entered the brain with long-lasting and dose-related occupancy. ITI-007 (10 mg) demonstrated high occupancy (>80 %) of cortical 5-HT_{2A} receptors and low occupancy of striatal D₂ receptors (~12 %). D₂ receptor occupancy increased with dose and significantly correlated with plasma concentrations ($r^2 = 0.68$, $p = 0.002$). ITI-007 (40 mg) resulted in peak occupancy up to 39 % of striatal D₂ receptors and 33 % of striatal serotonin transporters.

Conclusions

The results provide evidence for a central mechanism of action via dopaminergic and serotonergic pathways for ITI-007 in living human brain and valuable information to aid dose selection for future clinical trials.

Keywords

PET Antidepressant Antipsychotic Brain imaging Neuroimaging Dopamine receptor
Serotonin receptor Human

Introduction

Modulation of serotonergic and dopaminergic neurotransmission in the central nervous system has proven to be an effective means of treating a variety of psychiatric and neurological disorders. Dopamine D₂ receptor blockade, for example, is thought to mediate the ability of antipsychotic drugs to control positive symptoms (e.g., hallucinations and delusions) in patients with schizophrenia (Snyder 1976) whereas serotonin reuptake inhibition is thought to mediate antidepressant efficacy (Benfield et al. 1986; Benfield and Ward 1986). The possibility that antagonism of 5-HT_{2A} receptors may act synergistically with antagonism of dopamine receptors to control psychotic behavior emerged with the introduction of clozapine. At therapeutic doses in patients with schizophrenia, clozapine exhibits mean dopamine D₂ receptor occupancy (RO) of approximately 45 %, whereas the 5-HT_{2A} RO is approximately 90 % (Nordstrom et al. 1995). Many other atypical antipsychotic drugs have less selectivity for 5-HT_{2A} receptors relative to dopamine receptors and only achieve high 5-HT_{2A} RO at doses that also demonstrate high (>50 %) striatal D₂ RO. For example, risperidone shows no or little separation between 5-HT_{2A} and D₂ RO, depending on the dose, with ≥78 % occupancy of 5-HT_{2A} receptors and ≥75 % occupancy of D₂ receptors at a 6 mg/day dose and approximately 83 % 5-HT_{2A} RO and 72 % D₂ RO at a dose of 3 mg/day (Farde et al. 1995; Nyberg et al. 1999). Olanzapine also shows little separation between 5-HT_{2A} RO (68–78 %) and striatal D₂ RO (85–93 %; Kessler et al. 2005). These atypical antipsychotic drugs with relatively high striatal D₂ RO elevate prolactin levels (Bushe et al. 2008) and can induce extrapyramidal motor side effects (extrapyramidal symptoms (EPS)) at therapeutic doses (Rummel-Kluge et al. 2012). Importantly, selective 5-HT_{2A} receptor antagonism has been shown to enhance D₂-receptor-antagonist-mediated antipsychotic efficacy while reducing motor side effects and hyperprolactinemia in animal models (Gardell et al. 2007; Wadenberg et al. 1996). Moreover, antidepressant efficacy corresponding to occupancy of serotonin transporters (Meyer et al. 2004b; Meyer 2007) can be enhanced by 5-HT_{2A} receptor antagonism with reduced sexual side effects (Aizenberg et al. 1997; Marek et al. 2005; Pullar et al. 2000). A medication that combines potent 5-HT_{2A} receptor antagonism with optimal dopamine D₂ receptor modulation and optimal serotonin reuptake inhibition, never before achieved to our knowledge, may present an ideal balance of serotonergic and dopaminergic neurotransmission for the treatment of psychosis and mood disorders. By definition, this ideal balance would provide for robust antipsychotic and antidepressant efficacy mediated by D₂ receptors and serotonin transporters, respectively, with minimal side effects associated with engagement of these targets.

ITI-007 [4-((6bR,10aS)-3-methyl-2,3,6b,9,10,10a-hexahydro-1H,7H-pyrido[3',4':4,5]pyrrolo[1,2,3-de]quinoxalin-8-yl)-1-(4-fluoro-phenyl)-butan-1-one 4-methylbenzenesulfonate] represents a novel small-molecule therapeutic agent designed to act synergistically through serotonergic, dopaminergic, and glutamatergic pathways (Li et al. 2014; Snyder et al. 2015). Specifically, ITI-007 combines potent 5-HT_{2A} receptor antagonism with regionally specific modulation of dopaminergic intra-cellular phosphoprotein pathways and with serotonin reuptake inhibition. ITI-007 exhibits K_i s of 0.54 nM at 5-HT_{2A} receptors, 32 nM at D₂ receptors, and 61 nM at serotonin transporters (Li et al. 2014). ITI-007 has dual actions at dopamine D₂ receptors in vivo, acting as a post-synaptic D₂ receptor antagonist and as a pre-synaptic partial agonist at D₂ receptors with mesolimbic/mesocortical selectivity (Snyder et al. 2015). ITI-007 also exhibits affinity for D₁ receptors (K_i = 52 nM; Li et al. 2014) and indirectly modulates glutamatergic activity in dopamine-rich mesolimbic brain regions by increasing the phosphorylation of the GluN2B subunit of N-methyl-d-aspartate (NMDA) channels (Snyder et al. 2015). In total, ITI-007 has a unique pharmacological

profile useful for the treatment of schizophrenia and related psychoses as well as mood disorders.

The in vitro profile of ITI-007 suggests that 5-HT_{2A} receptors could be fully saturated before significant dopamine D₂ RO is achieved. This is not possible with other antipsychotics wherein significant dopamine D₂ RO and/or other potent receptor interactions are seen even at modest levels of 5-HT_{2A} RO. With ITI-007, full occupancy of 5-HT_{2A} RO is achieved at low doses, and then, the dose increased to bring in just enough dopamine receptor modulation and/or serotonin reuptake inhibition. This would allow optimal efficacy to be achieved without incurring unwanted side effects. Such a profile is not achieved even by the dopamine D₂ receptor partial agonist which requires very high levels of D₂ RO for antipsychotic efficacy (Yokoi et al. 2002); aripiprazole is a partial agonist both pre- and post-synaptically and has a higher affinity for D₂ over 5-HT_{2A} receptors, in contrast to the pre-synaptic partial agonism and post-synaptic antagonism at D₂ receptors by ITI-007 and potent 5-HT_{2A} receptor antagonism. Moreover, ITI-007 has minimal interaction with histamine, serotonin 5-HT_{2C}, and muscarinic acetylcholine receptors that translate to a lack of sedation, metabolic disturbance, and cognitive dysfunction.

The present study sought to determine if the unique in vitro receptor interaction profile of ITI-007 translated to a unique RO profile in the brain of healthy volunteers using positron emission tomography (PET) with appropriate ligands for labeling 5-HT_{2A} receptors, D₂ receptors, and serotonin transporters. The relationship between ITI-007 dose and receptor occupancies as well as the relationship between ITI-007 plasma concentrations and receptor occupancies was also investigated. Safety and tolerability of ITI-007 after a single administration as an oral dose solution were evaluated.

Subjects and methods

Participants and ethical approval

Sixteen healthy male subjects between the ages of 18 and 45 years old were recruited for the study. Subjects had to have a body mass index (BMI) of 19–30 kg/m² with a minimum weight of 60 kg at screening. Subjects were excluded if they had any current or history of significant medical issue (e.g., organ abnormality or disease; renal, hepatic, gastrointestinal, cardiovascular, or hematological disease; neurological condition; psychiatric illness; alcohol and/or drug abuse).

This study was conducted jointly at the Johns Hopkins University (JHU) School of Medicine/Johns Hopkins Hospital (JHH) and PAREXEL Early Phase Clinical Unit (EPCU; Baltimore, MD) and was approved by Institutional Review Boards for each institution (JHU School of Medicine: Office of Human Subjects Research, Baltimore, MD, USA and Chesapeake Research Review, Inc., Columbia, MD, USA) in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Study design

This study was conducted as an open-label study. After providing written informed consent, subjects underwent a screening visit to determine eligibility within 28 days of the baseline PET scan. Subjects were admitted to the PAREXEL EPCU for the collection of baseline

safety assessments and overnight housing. The following day, subjects were transported to JHH/JHU PET center. Test drug administration took place on study day 1 at JHU followed by PET scan(s), safety assessments, and blood collections for pharmacokinetic determinations for approximately 8 h post-dose. Subjects were discharged after the last assessment on study day 1 and returned to PAREXEL EPCU for a safety follow-up visit on study days 5 to 7.

ITI-007 was administered at JHH/JHU as a single oral dose using an oral solution reconstituted from drug powder in degassed Sprite Zero® within 24 h before administration. Subjects received one baseline PET scan and up to three post-dose scans, as illustrated in Fig. 1. PET scans to image D₂ receptors were conducted at various time points in groups receiving 10 mg (*N* = 2), 20 mg (*N* = 4), 30 mg (*N* = 4), or 40 mg (*N* = 2) ITI-007. Occupancy of serotonin 5-HT_{2A} receptors at 10 mg ITI-007 (*N* = 2) and serotonin transporters at 40 mg ITI-007 (*N* = 2) was each determined in separate groups only at the time of peak brain occupancy (~30–120 min post-dose).

Fig. 1 Timeline of scans relative to dose administration

Radiotracers and positron emission tomography

Carbon-11 radiolabeled compounds were manufactured at Johns Hopkins Hospital Nuclear Medicine Radiochemistry Facility according to the center's standard operating procedures and in compliance with US Pharmacopoeia (USP) <823>, "Radiopharmaceuticals for positron emission tomography: compounding." Each subject received a new batch of radiotracer manufactured and administered just before each PET scan.

Carbon-11-raclopride ([¹¹C]-RAC) was used for imaging D₂ dopamine receptors (Ribeiro et al. 2005; Schlaepfer et al. 1997; Slifstein et al. 2006). Carbon-11-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile ([¹¹C]-DASB) (Mathis et al. 2003; Wilson et al. 2002) was used to measure serotonin transporter binding (Frankle et al. 2005; Guttman et al. 2007; McCann et al. 2008; Meyer et al. 2004a; Reimold et al. 2008). Carbon-11-MDL100907 ([¹¹C]-MDL) was used for imaging 5-HT_{2A} receptors (Bhagwagar et al. 2006; de Paulis 2001; Hilton et al. 2000; Hinz et al. 2007; Hirani et al. 2003). The injected activity of radioisotopes administered in this study was approximately 20 mCi, and the radiation exposure was approximately 0.6- to 0.7-rem effective dose equivalent for each PET scan. A maximum of four PET scans was performed on each participant; thus, the total cumulative exposure during this study was less than 3.0-rem effective dose equivalent. The radiotracer was administered, followed by a 90-min dynamic PET scan.

PET images were acquired on a high-resolution research tomograph (HRRT). The reconstructed dynamic PET images were attenuation- and decay-corrected and expressed in units of concentration ($\mu\text{Ci}/\text{mmol}$). In addition to the PET scan, each participant had a volumetric magnetic resonance imaging (MRI) scan for co-registration with the PET data to enhance anatomical definitions. The MRI data were acquired using a spoiled gradient recalled acquisition in the steady state (GRASS) (SPGR) sequence on a GE 1.5 T Signa Camera. To minimize movement during the MRI and PET scans, each participant was fitted with a thermoplastic facemask that was secured to the scanner bed during imaging.

Subjects receiving [^{11}C]-MDL or [^{11}C]-DASB had blood samples drawn through an arterial line for determination of total and metabolite-associated radioactivity in plasma. Before start of a [^{11}C]-MDL or [^{11}C]-DASB PET scan, subject had a venous catheter inserted into one of his arms to administer the radiotracer, and a second venous catheter or radial arterial line was inserted for blood sampling. Radioactivity and radiotracer metabolite concentrations in plasma were measured within the JHU facility using a validated high-performance liquid chromatography (HPLC) method/appropriate radioactive analytical method (Hilton et al. 2000). These data were used for construction of a metabolite-corrected plasma input function to calculate specific uptake of the radiotracer in different regions of the brain.

Pharmacokinetics of ITI-007

Blood samples (approximately 7 mL) for ITI-007 (and two metabolites) used for drug concentration determination were collected into polypropylene tubes containing sodium heparin immediately pre-dose (0 h) and at approximately 1, 3.5, and 8 h post-dose. Immediately after blood collection, the tube was inverted gently and placed on ice. Samples were centrifuged at 4 °C for 10 min at $2500 \times g$ within 15 min of collection and then stored frozen at approximately -80 °C until analysis. Plasma samples were analyzed for ITI-007 and metabolite concentrations using validated HPLC/tandem mass spectrometry (HPLC/MS/MS) bioanalytical assays. The assay was validated at a level of detection of 0.05 to 50 ng/mL for the parent and 0.2 to 100 ng/mL for the metabolites.

Safety

Safety was monitored during the study by the performance of the following procedures and assessments at regular intervals: vital signs (three-positional blood pressure and heart rate, oral body temperature, and respiratory rate), 12-lead electrocardiograms (ECGs), clinical laboratory testing (hematology, clinical chemistry, and urinalysis), adverse event (AE) assessments, and physical examinations.

Data analysis

Regions of interest (ROIs) were drawn for PET data: caudate and putamen for D₂ RO; midbrain, thalamus, caudate, putamen, and pons for serotonin transporters; frontal cortex for 5-HT_{2A} RO; and cerebellum used as a reference region. Target occupancies of ITI-007 were calculated as the percent change of non-displaceable binding potential (BP_{ND}) from baseline: $\text{Occupancy (\%)} = 100 \times (\text{BP}_{\text{ND}}(\text{baseline}) - \text{BP}_{\text{ND}}(\text{blocking})) / \text{BP}_{\text{ND}}(\text{baseline})$. Specific binding (i.e., BP_{ND}) in all ROIs was obtained by using simplified reference tissue modeling (SRTM) for [^{11}C]-RAC only. Parametric maps of the BP_{ND} were created from the PET ROI data as follows: For [^{11}C]-RAC, ROIs were defined on co-registered MRI images and then applied to dynamic PET images to obtain time-activity curves for use in kinetic modeling, in

this case, using SRTM. Then, using the linear regression with spatial constraint method (Zhou et al. 2003), parametric maps of binding potential were generated. ROIs were then applied to the parametric maps to obtain mean regional BP_{ND}s. For [¹¹C]-DASB and [¹¹C]-MDL, distribution volume (DV) maps were generated using multigraphical analysis using plasma input (Zhou et al. 2010). BP_{ND} images were created from the DV maps using $DV/DV_{\text{cerebellum}} - 1$ [, where cerebellum was the reference region.

RO was calculated as the percent change in BP_{ND} in the blocking vs. baseline scans. Plasma concentrations of the blocking drug and RO were fitted to a concentration-occupancy model, to estimate a half maximal effective concentration (EC₅₀). Individual subject ITI-007 and metabolite plasma concentration data were correlated with the individual levels of D2 RO.

Results

Enrollment

Sixteen (16) subjects were enrolled; all completed the study and were included in the analyses. Participants had a mean age of 30.7 years (± 6.4 standard deviation) with an average BMI of 24.6 (± 3.0). Ten subjects (62.5 %) were Black/African American, and six (37.5 %) were Caucasian. Of the 16 subjects enrolled, two (12.5 %) were of Hispanic/Latino ethnicity.

Positron emission tomography

The main outcome variables from the PET data were non-displaceable binding potential (BP_{ND}), and RO. Baseline BP_{ND} ranged from 1.5 to 3.6 for [¹¹C]-RAC, from 1.7 to 5.8 for [¹¹C]-DASB, and from 1.6 to 2.9 for [¹¹C]-MDL. Estimates of BP_{ND} derived from ROIs directly by SRTM correlated closely with estimates of BP_{ND} derived from the parametric maps, with less than a 5 % difference in BP_{ND} estimates between the two methods for all subjects.

Table 1 summarizes the mean striatal D₂ RO, average of the putamen and caudate occupancies for each subject, and averaged across subjects by dose and time. Taken together, these data illustrate that ITI-007 rapidly enters the brain and occupies striatal D₂ receptors, with peak RO within 30 min to 1 h after oral administration. Striatal D₂ RO increased with higher doses of ITI-007. Striatal D₂ RO after ITI-007 administration was measureable in scans starting as late as 7.5 h after administration of 40 mg ITI-007.

Table 1 Striatal D₂ receptor occupancy of ITI-007 by dose and time

	Mean (individual values) striatal D₂ % receptor occupancy		
ITI-007 dose (mg)	30 min–1 h post-dose	2–3.5 h post-dose	5–7.5 h post-dose
10	11.56 % (6.36, 16.75)		
20	19.03 % (18.46, 19.60)	8.19 % (8.03, 8.35)	
30	26.53 % (17.82, 30.18, 31.58)	18.17 % (13.01, 18.33, 23.17)	12.05 % (6.16, 7.27, 22.27)
40	28.76 % (18.70, 38.81)	23.25 % (19.30, 27.19)	13.83 % (10.17, 17.48)

At a dose of 10 mg administered approximately 30 min before the PET scan, ITI-007 demonstrated high occupancy (>80 %) of cortical 5-HT_{2A} receptors (Table 2). At a dose of 40 mg administered approximately 30 min before the PET scan, ITI-007 demonstrated occupancy of striatal serotonin transporters (8–33 %; Table 3) in the same range as its D₂ RO (19–39 %).

Table 2 Cortical 5-HT_{2A} receptor occupancy by 10 mg ITI-007

Mean (Individual Values) 5-HT_{2A} % Receptor Occupancy						
Pre-frontal cortex	Orbital cortex	Temporal cortex	Occipital cortex	Superfrontal cortex	Parietal cortex	Cingulate cortex
87.7 % (83.8, 91.6)	87.3 % (85.5, 89.2)	83.9 % (81.1, 86.7)	83.3 % (78.4, 88.3)	87.4 % (83.4, 91.4)	84.7 % (78.9, 90.5)	81.0 % (78.9, 83.0)

Table 3 Serotonin transporter occupancy by 40 mg ITI-007

Mean (individual values) serotonin transporter % occupancy				
Caudate	Putamen	Thalamus	Pons	Midbrain
20.7 % (8.3, 33.1)	24.1 % (19.0, 29.2)	21.1 % (9.9, 32.4)	19.3 % (10.8, 27.7)	27.5 % (11.5, 43.6)

Pharmacokinetic-pharmacodynamic relationships

Plasma concentrations of ITI-007 (parent and two metabolites) were measured. Striatal D₂ RO was significantly correlated with ITI-007 parent plasma concentrations ($r^2 = 0.68$, $p = 0.002$; Fig. 2a) and with ITI-007 total exposure ($r^2 = 0.75$, $p = 0.0005$; Fig. 2b).

Fig. 2 Percent striatal D₂ receptor occupancy as a function of **a** ITI-007 parent plasma level (ng/mL) or **b** total exposure (parent+metabolites (ng/mL))

When the D₂ RO data were plotted against plasma concentration of ITI-007 (parent) and fitted to a concentration-occupancy model (% Occupancy = $100 \times [\text{Conc} / (\text{EC}_{50} + \text{Conc})]$), the predicted EC₅₀ was estimated to be 24 ng/mL of parent if B_{max} was assumed to be 100 and, as a sensitivity analysis, was determined to be 26 ng/mL of parent if B_{max} was assumed to be 90 (Fig. 3).

Fig. 3 Percent striatal D₂ receptor occupancy projections

There was little variability with regard to 5-HT_{2A} RO between the two subjects tested, and these two subjects showed similar ITI-007 plasma levels. Average occupancy for the two subjects evaluated was 88 % of 5-HT_{2A} receptors in the pre-frontal cortex with a range of 84 % minimum (corresponding to 0.72 ng/mL of parent and 2.55 ng/mL of parent plus

metabolites) and 92 % maximum or peak (corresponding to 2.16 ng/mL of parent and 5.33 ng/mL of parent plus metabolites). Moreover, these plasma levels were similar to those achieved at the 10-mg ITI-007 dose level in the subjects from the D₂ RO portion of the experiment.

With regard to serotonin transporter occupancy, one subject with 15.6 ng/mL ITI-007 (parent) and 35.1 ng/mL total (parent + metabolites) concentrations showed approximately 31 % striatal serotonin transporter occupancy, whereas the other subject with 18.3 ng/mL ITI-007 (parent) and 61.7 ng/mL total (parent + metabolites) concentrations showed only 14 % serotonin reuptake inhibition. However, this range of plasma levels observed at 40 mg ITI-007 in the serotonin transporter occupancy subjects was similar to the range measured at the same dose in the D₂ RO subjects.

Safety

Oral administration of 10–40 mg ITI-007 was safe and well tolerated in healthy volunteers. There were no dose-related or serious AEs. There were no clinically significant findings in safety data, including physical exams and clinical laboratory tests. There were no clinically relevant differences identified in mean observed vital sign data within each ITI-007 dose group over time or between doses, and there were no clinically relevant mean changes from baseline. There were no findings on ECG measures considered to be clinically significant. Mean values of the corrected QT interval using the formulae of Bazett (QTcB) and Fridericia (QTcF) were <430 ms, and QT values were <500 ms at all time points throughout the study for all dose groups.

Discussion

ITI-007 rapidly penetrated into the brain with extended, dose-related, and plasma-concentration-related striatal D₂ RO in healthy volunteers. A low dose of 10 mg ITI-007 essentially fully saturated cortical 5-HT_{2A} receptors with lesser striatal D₂ RO, consistent with the 5-HT_{2A} receptor selectivity of ITI-007 in vitro and in animal models (Snyder et al. 2015). A higher dose of ITI-007 (40 mg) produced similar striatal D₂ RO and striatal serotonin transporter occupancy. Taken together, these data demonstrate that low doses of ITI-007 are predominantly serotonin 5-HT_{2A} receptor selective, while higher doses also engage dopamine D₂ receptors and serotonin transporters.

Serotonin 5-HT_{2A} receptor occupancy

ITI-007 demonstrated relatively high selectivity for serotonin 5-HT_{2A} receptors at low doses. The pattern of high RO of 5-HT_{2A} receptors at low doses and large separation between other pharmacological activities were predicted based on in vivo animal studies. At low doses (0.2 mg/kg, p.o.) in rodents, ITI-007 selectively inhibits brain 5-HT_{2A} receptors and blocks quipazine-induced head twitches (Snyder et al. 2015). Serotonin 5-HT_{2A} receptor antagonism has been strongly linked with sleep maintenance (Vanover and Davis 2010). 5-HT_{2A} receptor binding may also influence impulsive aggression (Meyer et al. 2008; Rosell et al. 2010). Moreover, selective 5-HT_{2A} receptor antagonists have been shown to enhance D₂-receptor-mediated antipsychotic-like efficacy in animal models (Gardell et al. 2007; Wadenberg et al. 2001; Wadenberg et al. 2000; Wadenberg et al. 1996) without altering or even decreasing D₂-receptor-mediated extrapyramidal motor side effects and hyperprolactinemia (Gardell et al.

2007; Wadenberg et al. 1996), likely due to a complex and regionally selective interaction between 5-HT_{2A} and dopamine receptors in the brain (see review by Alex and Pehek 2007). Serotonin 5-HT_{2A} receptor antagonism can also enhance serotonin-transporter-inhibition-mediated antidepressant-like efficacy in animal models (Marek et al. 2005). Taken together, the data suggest a role for 5-HT_{2A} RO in the treatment of a wide range of psychiatric disorders.

Dopamine D₂ receptor occupancy

Early data suggested that greater than 60 % striatal D₂ RO was required for antipsychotic clinical response and greater than 80 % striatal D₂ RO was associated with extrapyramidal side effects (Farde et al. 1992; Nordstrom et al. 1993), and this D₂ RO relationship appeared to hold true for classical antipsychotics (Kapur et al. 2000). However, clozapine, with relatively more potent 5-HT_{2A} receptor antagonism than earlier antipsychotic drugs, was noted to exhibit antipsychotic efficacy at lower levels of D₂ RO (Farde et al. 1992). Subsequent studies confirmed that both clozapine and quetiapine are exceptions to the “rule” and exhibit antipsychotic efficacy with less than 50 % striatal D₂ RO (Kessler et al. 2006). Similarly, ITI-007’s high 5-HT_{2A} RO and relative selectivity for 5-HT_{2A} over D₂ receptors suggests that ITI-007 will demonstrate antipsychotic efficacy at relatively low levels of striatal D₂ RO. In contrast, not all atypical antipsychotics achieve efficacy at low levels D₂ RO. Lurasidone’s antipsychotic efficacy is associated with >60 % striatal D₂ RO (Wong et al. 2013), and aripiprazole’s antipsychotic efficacy is associated with greater than 90 % D₂ RO (Yokoi et al. 2002). Aripiprazole may achieve antipsychotic efficacy at only relatively higher D₂ RO compared to most other antipsychotics due to its partial agonism at D₂ receptors. Partial agonism at D₂ receptors may be associated with lower levels of some forms of extrapyramidal side effects, such as parkinsonism, despite high levels of D₂ occupancy (Grunder et al. 2003), but may be associated with relatively high rates of akathisia (Mamo et al. 2007; Poyurovsky et al. 2008). Interestingly, 5-HT_{2A} receptor antagonism has been suggested to attenuate D₂-receptor-mediated akathisia and a high 5-HT_{2A}/D₂ occupancy ratio, which aripiprazole lacks, has been suggested as “essential” to prevent antipsychotic-induced akathisia (Poyurovsky et al. 2008).

Clozapine and quetiapine, two other antipsychotic drugs with higher affinity for 5-HT_{2A} receptors than for D₂ receptors, exhibit higher levels of extrastriatal D₂ RO than striatal D₂ RO using [18 F]-fallypride as the radiotracer (Grunder et al. 2006; Kessler et al. 2006; Vernaleken et al. 2010). This profile is thought to contribute to the lower incidence of extrapyramidal side effects with these drugs compared to other antipsychotics. The potent cortical 5-HT_{2A} RO demonstrated in the present study suggests low liability of extrapyramidal side effects with ITI-007. Future investigation of extrastriatal D₂ RO with ITI-007 would be of interest to see if the pattern of higher extrastriatal than striatal D₂ RO holds true for ITI-007. In contrast to quetiapine and clozapine and other antipsychotics, ITI-007 exhibits the additional occupancy of serotonin transporters in the same dose range as its striatal D₂ RO.

Serotonin transporter occupancy

Serotonin transporter occupancy of about 80–90 % by selective serotonin reuptake inhibitors is associated with antidepressant efficacy (Meyer 2007; Meyer et al. 2004b). As mentioned above, serotonin 5-HT_{2A} receptor antagonism can enhance serotonin-reuptake-inhibition-mediated antidepressant-like efficacy in animal models (Marek et al. 2005). This suggests

that in combination with 5-HT_{2A} receptor antagonism, antidepressant efficacy can be achieved at lower levels of serotonin reuptake inhibition and/or for greater therapeutic efficacy (Marek et al. 2003). Moreover, combining atypical antipsychotic activity (i.e., 5-HT_{2A} and D₂ receptor antagonism) with serotonin reuptake inhibition provides for synergistic activity than either mechanism alone (Reus et al. 2012; Tohen et al. 2010). Unique among antipsychotics, measurable serotonin transporter occupancy was found at doses of ITI-007 that similarly occupy D₂ receptors. These results suggest that ITI-007 combines antipsychotic and antidepressant mechanisms in a single compound.

Limitations

The estimated striatal D₂ RO EC₅₀ for ITI-007 of 24–26 ng/mL (parent) was based on the ascending portion of the curve without reaching full saturation of D₂ receptors. Therefore, D₂ receptor occupancies above doses of 40 mg ITI-007 with associated parent plasma concentrations of 18 ng/mL had to be extrapolated. The raclopride radiotracer binds to D₂ and D₃ dopamine receptor subtypes, so displacement of raclopride by ITI-007 may be partially due to displacement at D₃ receptors. However, this is unlikely because ITI-007 showed little or no affinity for dopamine D₃ receptors in vitro as tested in a broad panel of receptors (Snyder et al. 2015). Additionally, the sample size of the study was small with two to four subjects per dose per radioligand which may have affected the accuracy of the projection of an EC₅₀ value. However, the low dose range of 10–40 mg ITI-007 showed a predictable dose relationship with plasma concentrations highly correlated with striatal D₂ RO and with good correspondence to in vitro receptor affinity (Li et al. 2014).

Conclusions

These data provide the first demonstration for a central mechanism of action via serotonergic and dopaminergic pathways for ITI-007 in living human brain. Moreover, these data in healthy volunteers correspond well to the preclinical data and provide further evidence for a separation between ITI-007's 5-HT_{2A} receptor antagonism and its other dopaminergic and serotonergic modulation (Table 4). This separation of pharmacological effects provides an opportunity to treat different symptoms and/or different neuropsychiatric disorders at different doses of ITI-007. The present data provide valuable information to aid dose selection for future efficacy clinical trials with ITI-007.

Table 4 In vitro binding affinity and human receptor occupancy profiles of ITI-007 and antipsychotic drugs

In vitro binding affinity K_i (nM) for 5-HT _{2A} versus D ₂ receptors ^a					
	ITI-007	Clozapine	Risperidone	Olanzapine	Aripiprazole
5-HT _{2A}	0.5	9.6	0.5	2.5	9
D ₂	32	190	5.9	31	1.6
Serotonin Transporters	62	>1000	>1000	>1000	240–405
Selectivity for 5-HT _{2A} versus D ₂ receptors in vitro					
	ITI-007	Clozapine	Risperidone	Olanzapine	Aripiprazole
Ratio D ₂ /5-HT _{2A}	60	20	12	12	0.18
5-HT _{2A} versus D ₂ receptor occupancy at efficacious antipsychotic dose ^b					

	ITI-007	Clozapine	Risperidone	Olanzapine	Aripiprazole
5-HT _{2A}	Projected ≥90 %	90 %	≥78 %	68–78 %	54–60 %
D ₂	Projected ≤50 %	45 %	≥72 %	85–93 %	>90 %

^aIn vitro receptor binding affinities from Snyder et al. (2015) and derived from the NIMH Psychoactive Drug Screening Program (PDSP) Database (Roth et al. 2004)

^bReceptor occupancy data using PET: ITI-007 data projected to the efficacious antipsychotic dose of 60 mg ITI-007 (Vanover et al. 2014) based on the present data determined at lower doses; clozapine data from Farde et al. (1992) and Kessler et al. (2006); risperidone data from Farde et al. (1995) and Nyberg et al. (1999); olanzapine data from Kessler et al. (2005); and aripiprazole data from Mamo et al. (2007) and Yokoi et al. (2002)

Acknowledgments

The authors thank the study team at PAREXEL Early Phase Clinical Unit in Baltimore, MD, especially Syed Faridi and the clinical team at The Johns Hopkins University School of Medicine, including Babar Hussain and Stephen Condouris. The authors also gratefully acknowledge the efforts of Robert F Dannals and his team in the Department of Radiology and Radiological Science at The Johns Hopkins University School of Medicine. The authors also thank Lawrence P Wennogle for his helpful comments on the manuscript. Importantly, the authors thank the volunteers for their participation in the study.

Funding

The clinical study described in this manuscript was sponsored and funded by Intra-Cellular Therapies, Inc. (ITI).

Authors' contribution

The authors from ITI (RE Davis, KE Vanover, and S Mates) were involved in all aspects of the study, including the original concept and study design, the analysis and interpretation of the data, and the writing and approval of the manuscript.

Conflict of interest

Intra-Cellular Therapies, Inc. (ITI) sponsored this study. K Vanover and S Mates are employees of ITI. R Davis is a paid consultant to ITI. DR Goldwater is an employee of PAREXEL International, and L Gapasin was an employee of PAREXEL International at the time that this study was conducted. DF Wong discloses financial relationships with Intra-Cellular Therapies, Inc., Amgen, Avid, Bristol Myers Squibb, Lilly, Merck, Otsuka, Roche, Sanofi Aventis, and Wyeth. Y Zhou, JR Brašić, MR Guevara, B Bisuna, W Ye, V Rayment, A Kumar, and W Willis have no disclosures. ITI owns or controls the intellectual property that covers ITI-007, the investigational new drug evaluated in this study and described in this manuscript.

References

1. Aizenberg D, Gur S, Zemishlany Z, Granek M, Jeczmien P, Weizman A (1997) Mianserin, a 5-HT_{2a/2c} and alpha 2 antagonist, in the treatment of sexual dysfunction induced by serotonin reuptake inhibitors. *Clin Neuropharmacol* 20(3):210–214
2. Alex KD, Pehek EA (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol Ther* 113(2):296–320
3. Benfield P, Ward A (1986) Fluvoxamine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. *Drugs* 32(4):313–334
4. Benfield P, Heel RC, Lewis SP (1986) Fluoxetine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. *Drugs* 32(6):481–508
5. Bhagwagar Z, Hinz R, Taylor M, Fancy S, Cowen P, Grasby P (2006) Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL 100,907. *Am J Psychiatry* 163(9):1580–1587
6. Bushe C, Shaw M, Peveler RC (2008) A review of the association between antipsychotic use and hyperprolactinaemia. *J Psychopharmacol* 22(2 Suppl):46–55
7. de Paulis T (2001) M-100907 (Aventis). *Curr Opin Investig Drugs* 2(1):123–132
8. Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G (1992) Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. *Arch Gen Psychiatry* 49(7):538–544
9. Farde L, Nyberg S, Oxenstierna G, Nakashima Y, Halldin C, Ericsson B (1995) Positron emission tomography studies on D2 and 5-HT₂ receptor binding in risperidone-treated schizophrenic patients. *J Clin Psychopharmacol* 15(1 Suppl 1):19S–23S
10. Frankle WG, Narendran R, Huang Y, Hwang DR, Lombardo I, Cangianno C et al (2005) Serotonin transporter availability in patients with schizophrenia: a positron emission tomography imaging study with [11C]DASB. *Biol Psychiatry* 57(12):1510–1516
11. Gardell LR, Vanover KE, Pounds L, Johnson RW, Barido R, Anderson GT et al (2007) ACP-103, a 5-hydroxytryptamine 2A receptor inverse agonist, improves the antipsychotic efficacy and side-effect profile of haloperidol and risperidone in experimental models. *J Pharmacol Exp Ther* 322(2):862–870
12. Grunder G, Carlsson A, Wong DF (2003) Mechanism of new antipsychotic medications: occupancy is not just antagonism. *Arch Gen Psychiatry* 60(10):974–977
13. Grunder G, Landvogt C, Vernaleken I, Buchholz HG, Ondracek J, Siessmeier T et al (2006) The striatal and extrastriatal D2/D3 receptor-binding profile of clozapine in patients with schizophrenia. *Neuropsychopharmacology* 31(5):1027–1035
14. Guttman M, Boileau I, Warsh J, Saint-Cyr JA, Ginovart N, McCluskey T et al (2007) Brain serotonin transporter binding in non-depressed patients with Parkinson's disease. *Eur J Neurol* 14(5):523–528
15. Hilton J, Yokoi F, Dannals RF, Ravert HT, Szabo Z, Wong DF (2000) Column-switching HPLC for the analysis of plasma in PET imaging studies. *Nucl Med Biol* 27(6):627–630

16. Hinz R, Bhagwagar Z, Cowen PJ, Cunningham VJ, Grasby PM (2007) Validation of a tracer kinetic model for the quantification of 5-HT_{2A} receptors in human brain with [(11)C]MDL 100,907. *J Cereb Blood Flow Metab* 27(1):161–172
17. Hirani E, Sharp T, Sprakes M, Grasby P, Hume S (2003) Fenfluramine evokes 5-HT_{2A} receptor-mediated responses but does not displace [(11)C]MDL 100907: small animal PET and gene expression studies. *Synapse* 50(3):251–260
18. Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000) Relationship between dopamine D₂ occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. *Am J Psychiatry* 157(4):514–520
19. Kessler RM, Ansari MS, Riccardi P, Li R, Jayathilake K, Dawant B, Meltzer HY (2005) Occupancy of striatal and extrastriatal dopamine D₂/D₃ receptors by olanzapine and haloperidol. *Neuropsychopharmacology* 30(12):2283–2289
20. Kessler RM, Ansari MS, Riccardi P, Li R, Jayathilake K, Dawant B et al (2006) Occupancy of striatal and extrastriatal dopamine D₂ receptors by clozapine and quetiapine. *Neuropsychopharmacology* 31(9):1991–2001
21. Li P, Zhang Q, Robichaud AJ, Lee T, Tomesch J, Yao W, Beard JD, Snyder GL, Zhu H, Peng Y, Hendrick JP, Vanover KE, Davis RE, Mates S, Wennogle LP (2014) Discovery of a tetracyclic quinoxaline derivative as a potent and orally active multifunctional drug candidate for the treatment of neuropsychiatric and neurological disorders. *J Med Chem* 57(6):2670–2682
22. Mamo D, Graff A, Mizrahi R, Shammi CM, Romeyer F, Kapur S (2007) Differential effects of aripiprazole on D₂, 5-HT₂, and 5-HT_{1A} receptor occupancy in patients with schizophrenia: a triple tracer PET study. *Am J Psychiatry* 164:1411–1417
23. Marek GJ, Carpenter LL, McDougale CJ, Price LH (2003) Synergistic action of 5-HT_{2A} antagonists and selective serotonin reuptake inhibitors in neuropsychiatric disorders. *Neuropsychopharmacology* 28(2):402–412
24. Marek GJ, Martin-Ruiz R, Abo A, Artigas F (2005) The selective 5-HT_{2A} receptor antagonist M100907 enhances antidepressant-like behavioral effects of the SSRI fluoxetine. *Neuropsychopharmacology* 30(12):2205–2215
25. Mathis CA, Wang Y, Holt DP, Huang GF, Debnath ML, Klunk WE (2003) Synthesis and evaluation of [(11)C]-labeled 6-substituted 2-arylbenzothiazoles as amyloid imaging agents. *J Med Chem* 46(13):2740–2754
26. McCann UD, Szabo Z, Vranesic M, Palermo M, Mathews WB, Ravert HT et al (2008) Positron emission tomographic studies of brain dopamine and serotonin transporters in abstinent (+/-)3,4-methylenedioxymethamphetamine (“ecstasy”) users: relationship to cognitive performance. *Psychopharmacology* 200(3):439–450
27. Meyer JH (2007) Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. *J Psychiatry Neurosci* 32(2):86–102
28. Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N et al (2004a) Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Arch Gen Psychiatry* 61(12):1271–1279
29. Meyer JH, Wilson AA, Sagrati S, Hussey D, Carella A, Potter WZ et al (2004b) Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [(11)C]DASB positron emission tomography study. *Am J Psychiatry* 161(5):826–835
30. Meyer JH, Wilson AA, Rusjan P, Clark M, Houle S, Woodside S et al (2008) Serotonin_{2A} receptor binding potential in people with aggressive and violent behaviour. *J Psychiatry Neurosci* 33(6):499–508

31. Nordstrom AL, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C et al (1993) Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. *Biol Psychiatry* 33(4):227–235
32. Nordstrom AL, Farde L, Nyberg S, Karlsson P, Halldin C, Sedvall G (1995) D1, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients. *Am J Psychiatry* 152(10):1444–1449
33. Nyberg S, Eriksson B, Oxenstierna G, Halldin C, Farde L (1999) Suggested minimal effective dose of risperidone based on PET-measured D2 and 5-HT2A receptor occupancy in schizophrenic patients. *Am J Psychiatry* 156(6):869–875
34. Poyurovsky M, Weizman R, Weizman A (2008) Aripiprazole's receptor pharmacology and extrapyramidal side effects. *Am J Psychiatry* 165:398
35. Pullar IA, Carney SL, Colvin EM, Lucaites VL, Nelson DL, Wedley S (2000) LY367265, an inhibitor of the 5-hydroxytryptamine transporter and 5-hydroxytryptamine(2A) receptor antagonist: a comparison with the antidepressant, nefazodone. *Eur J Pharmacol* 407(1–2):39–46
36. Reimold M, Batra A, Knobel A, Smolka MN, Zimmer A, Mann K et al (2008) Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: a [¹¹C]DASB PET study. *Mol Psychiatry* 13(6):606–613
37. Reus GZ, Abelaira HM, Agostinho FR, Ribeiro KF, Vitto MF, Luciano TF et al (2012) The administration of olanzapine and fluoxetine has synergistic effects on intracellular survival pathways in the rat brain. *J Psychiatr Res* 46(8):1029–1035
38. Ribeiro MJ, Ricard M, Bourgeois S, Lievre MA, Bottlaender M, Gervais P et al (2005) Biodistribution and radiation dosimetry of [¹¹C]raclopride in healthy volunteers. *Eur J Nucl Med Mol Imaging* 32(8):952–958
39. Rosell DR, Thompson JL, Slifstein M, Xu X, Frankle WG, New AS et al (2010) Increased serotonin 2A receptor availability in the orbitofrontal cortex of physically aggressive personality disordered patients. *Biol Psychiatry* 67(12):1154–1162
40. Roth BL, Sheffler DJ, Kroeze WK (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev* 3:353–359
41. Rummel-Kluge C, Komossa K, Schwarz S, Hunger H, Schmid F, Kissling W et al (2012) Second-generation antipsychotic drugs and extrapyramidal side effects: a systematic review and meta-analysis of head-to-head comparisons. *Schizophr Bull* 38(1):167–177
42. Schlaepfer TE, Pearlson GD, Wong DF, Marengo S, Dannals RF (1997) PET study of competition between intravenous cocaine and [¹¹C]raclopride at dopamine receptors in human subjects. *Am J Psychiatry* 154(9):1209–1213
43. Slifstein M, Hwang DR, Martinez D, Ekelund J, Huang Y, Hackett E et al (2006) Biodistribution and radiation dosimetry of the dopamine D2 ligand ¹¹C-raclopride determined from human whole-body PET. *J Nucl Med* 47(2):313–319
44. Snyder SH (1976) The dopamine hypothesis of schizophrenia: focus on the dopamine receptor. *Am J Psychiatry* 133(2):197–202
45. Snyder GL, Vanover KE, Zhu H, Miller DB, O'Callaghan JP, Tomesch J, Li P, et al (2015) Functional profile of a novel modulator of serotonin, dopamine, and glutamate neurotransmission. *Psychopharmacology (Berl)* 232(3):605–621
46. Tohen M, Case M, Trivedi MH, Thase ME, Burke SJ, Durell TM (2010) Olanzapine/fluoxetine combination in patients with treatment-resistant depression: rapid onset of therapeutic response and its predictive value for subsequent overall response in a pooled analysis of 5 studies. *J Clin Psychiatry* 71(4):451–462

47. Vanover KE, Davis RE (2010) Role of 5-HT_{2A} receptor antagonists in the treatment of insomnia. *Nat Sci Sleep* 2:139–150
48. Vanover KE, Davis RE, Ereshefsky L, Mates S (2014) Positive results for the treatment of schizophrenia: a randomized double-blind placebo- and active-controlled Phase 2 study. *Schizophr Res* 153:S278–S279
49. Vernaleken I, Janouschek H, Raptis M, Hellmann S, Veselinovic T, Brocheler A et al (2010) Dopamine D_{2/3} receptor occupancy by quetiapine in striatal and extrastriatal areas. *Int J Neuropsychopharmacol* 13(7):951–960
50. Wadenberg ML, Salmi P, Jimenez P, Svensson T, Ahlenius S (1996) Enhancement of antipsychotic-like properties of the dopamine D₂ receptor antagonist, raclopride, by the additional treatment with the 5-HT₂ receptor blocking agent, ritanserin, in the rat. *Eur Neuropsychopharmacol* 6(4):305–310
51. Wadenberg ML, Kapur S, Soliman A, Jones C, Vaccarino F (2000) Dopamine D₂ receptor occupancy predicts catalepsy and the suppression of conditioned avoidance response behavior in rats. *Psychopharmacology* 150(4):422–429
52. Wadenberg MG, Browning JL, Young KA, Hicks PB (2001) Antagonism at 5-HT_{2A} receptors potentiates the effect of haloperidol in a conditioned avoidance response task in rats. *Pharmacol Biochem Behav* 68(3):363–370
53. Wilson AA, Ginovart N, Hussey D, Meyer J, Houle S (2002) In vitro and in vivo characterisation of [¹¹C]-DASB: a probe for in vivo measurements of the serotonin transporter by positron emission tomography. *Nucl Med Biol* 29(5):509–515
54. Wong DF, Kuwabara H, Brašić JR, Stock T, Maini A, Gean EG et al (2013) Determination of dopamine D₂ receptor occupancy by lurasidone using positron emission tomography in healthy male subjects. *Psychopharmacology* 229:245–252
55. Yokoi F, Grunder G, Biziere K, Stephane M, Dogan AS, Dannals RF et al (2002) Dopamine D₂ and D₃ receptor occupancy in normal humans treated with the antipsychotic drug aripiprazole (OPC 14597): a study using positron emission tomography and [¹¹C]raclopride. *Neuropsychopharmacology* 27(2):248–259
56. Zhou Y, Endres CJ, Brašić JR, Huang SC, Wong DF (2003) Linear regression with spatial constraint to generate parametric images of ligand-receptor dynamic PET studies with a simplified reference tissue model. *NeuroImage* 18(4):975–989
57. Zhou Y, Ye W, Brašić JR, Wong DF (2010) Multi-graphical analysis of dynamic PET. *NeuroImage* 49(4):2947–2957