Original Paper

Neurotransmitter transporter occupancy following administration of centanafadine sustained-release tablets: A phase 1 study in healthy male adults

Journal of Psychopharmacology
2023, Vol. 37(2) 164–171

DOI: 10.1177/02698811221140008
journals.sagepub.com/home/jop

David Matuskey[1,2,3], Jean-Dominique Gallezot[1], Nabeel Nabulsi[1], Shannan Henry[1], Kristen Torres[1], Mark Dias[1], Gustavo A Angarita[2], Yiyun Huang[1], Susan E Shoaf[4], Richard E Carson[1] and Shailly Mehrotra[4]

Abstract

Background: Centanafadine is an inhibitor of reuptake transporters for norepinephrine (NET), dopamine (DAT) and serotonin (SERT).

Aims: This phase 1, adaptive-design positron emission tomography study investigated the occupancy time course of NET, DAT, and SERT and the relationship to centanafadine plasma concentrations.

Methods: Healthy adult males received centanafadine sustained-release 400 mg/day for 4 days ($N=6$) or 800 mg in a single day ($N=4$). Assessments included safety monitoring; time course of occupancy of NET, DAT, and SERT; and centanafadine plasma concentrations.

Results: Transporter occupancy was numerically higher for NET versus DAT or SERT. For NET, estimated (mean ± standard error [SE]) maximal observable target occupancy (TO_{max}) and concentration at half maximal occupancy (IC_{50}) were 64 ± 7% and 132 ± 65 ng/mL, respectively, for all regions and 82 ± 13% and 135 ± 97 ng/mL after excluding the thalamus, which showed high nonspecific binding. For DAT and SERT, TO_{max} could not be established and was assumed to be 100%; estimated IC_{50} (mean ± SE) values were 1580 ± 186 ng/mL and 1,760 ± 309 ng/mL, respectively. For centanafadine, the estimated in vivo affinity ratio was 11.9 ± 6.0 (mean ± SE) for NET/DAT, 13.3 ± 7.0 for NET/SERT, and 1.1 ± 0.2 for DAT/SERT. DAT and SERT occupancies at a plasma concentration of 1400 ng/mL were estimated to be 47 and 44%, respectively.

Conclusions: High occupancy at NET and moderate occupancy at DAT and SERT was observed at peak concentrations achieved following 400 mg total daily doses of centanafadine.

Keywords

Positron emission tomography imaging, centanafadine, norepinephrine transporter, dopamine transporter, serotonin transporter

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a neurobehavioral condition characterized by hyperactivity, inattentiveness, and impulsivity that is likely to have a lasting impact as children become adults (Caye et al., 2016; Barbaresi et al., 2013; Sharma and Couture, 2014). ADHD affects an estimated 4.4% of adults in the United States (Kessler et al., 2006).

Two classes of pharmacotherapy are available for the treatment of ADHD. The first class includes stimulants such as methylphenidate, which are believed to act through reuptake inhibition of dopamine and norepinephrine as well as inverse agonism of dopamine transporters, and amphetamines, which act primarily through release of dopamine and norepinephrine (Heal et al., 2013, 2014). The second class includes nonstimulants, such as the norepinephrine reuptake inhibitor atomoxetine and the α-adrenergic agonists guanfacine and clonidine, which are typically less effective than stimulants (Cortese et al., 2018). The usefulness of available pharmacotherapies is limited in some patients by adverse reactions, lack of efficacy, and abuse liability (Kolar et al., 2008).

Centanafadine is an inhibitor of norepinephrine, dopamine, and serotonin transporters (Bymaster et al., 2012). In vitro studies have determined that centanafadine has inhibitory activity at the norepinephrine transporter (NET; half-maximal inhibitory concentration [IC_{50}] 6 nM), the dopamine transporter (DAT; IC_{50} 38 nM), and the serotonin transporter (SERT; IC_{50} 83 nM) (Bymaster et al., 2012).

[1]Department of Radiology and Biomedical Imaging, Yale University School of Medicine, New Haven, CT, USA
[2]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA
[3]Department of Neurology, Yale University School of Medicine, New Haven, CT, USA
[4]Otsuka Pharmaceutical Development & Commercialization, Inc., Princeton, NJ, USA

Corresponding author:
Susan E Shoaf, Otsuka Pharmaceutical Development & Commercialization, Inc., 508 Carnegie Center, Princeton, NJ 08540, USA.
Email: Susan.Shoaf@otsuka-us.com

Centanafadine has a half-life of 4.5 h (Wigal et al., 2020). The efficacy and safety of centanafadine administered twice daily, every 5 h, as sustained-release (SR) tablets have been demonstrated in phase 2 and 3 clinical studies in adults with ADHD (Wigal et al., 2020). Across clinical studies, centanafadine was generally well tolerated with a low incidence of treatment-emergent adverse events (AEs) common to ADHD therapies, including decreased appetite, headache, nausea, dry mouth, upper respiratory tract infection, and diarrhea (Wigal et al., 2020).

This was a phase 1, adaptive positron emission tomography (PET) imaging study using tracers and methodology to investigate the time course of occupancy at NET, DAT, and SERT by centanafadine and to explore the relationship between centanafadine plasma concentrations and transporter occupancy.

Materials and methods

Participants

All subjects provided written informed consent after complete explanation of study procedures. The study protocol was approved by the Yale University Human Investigation Committee and the Yale University Radiation Safety Committee. The study was conducted in accordance with the protocol, Food and Drug Administration regulations, International Conference on Harmonisation for Good Clinical Practice Guideline (E6), and the ethical principles derived from the Declaration of Helsinki and Council for International Organizations of Medical Science guidelines. The study included adult (18–45 years of age) males with a body mass index between 19 and 32 kg/m^2 who were in good health without psychiatric or medical diagnoses as determined by medical history, physical examination, electrocardiogram (ECG), serum/urine biochemistry, hematology, and serology tests. Subjects who were current smokers or smoked within the past 6 months were excluded. Study procedures took place while subjects were residing in an inpatient clinical research unit.

End points

The primary end points of the study were determination of NET, DAT, and SERT occupancy by centanafadine in the human brain at different time points and plasma concentrations of centanafadine. Additionally, safety end points were also examined and included AEs, vital signs, ECGs, and clinical laboratory tests and scores on the Columbia-Suicide Severity Rating Scale. A full description of safety assessments can be found in eMethods Section 1.

Study design

Using a flexible trial design, NET, DAT, and SERT occupancy results obtained from the first six subjects (cohort 1) were used to inform changes to centanafadine doses, radioligands, and timing of the post-dose scans for an additional four subjects (cohort 2) (eTable 1). Cohort 1 received a total daily dose (TDD) of centanafadine 400 mg for 4 days and cohort 2 received a TDD of centanafadine 800 mg for 1 day. Each TDD consisted of equal doses of centanafadine-SR tablets administered orally twice daily approximately 5 ($\pm$1) h apart. There were no changes to the protocol or the planned analysis during the study.

Data acquisition

PET scans were performed on the Siemens Biograph mCT PET scanner (Siemens Medical Solutions USA, Inc., Malvern, PA, USA), which uses a low-dose computerized tomography scan to correct for attenuation. PET images were reconstructed into 27 or 33 frames, depending on the radiotracer used, containing 111 axial slices of 200 $\times$ 200 voxels each (2.04 $\times$ 2.04 $\times$ 2.0 mm^3). Corrections for attenuation, normalization, scatter, randoms, and dead time used were performed and images reconstructed with the ordered subset-expectation maximization algorithm (2 iterations, 21 subsets). Head motion was tracked using the Polaris Vicra® optical tracking system (NDI Systems, Waterloo, Canada). A full description of how MR and PET scans were performed is provided in eMethods Sections 2 and 3, respectively.

Reductions in specific binding of established ligands, defined as binding potential with respect to non-displaceable radiotracer concentration in brain tissue (BP_{ND}), were used to evaluate transporter occupancy. Radiotracers were injected over 1 min using an infusion pump and tracer uptake was determined by PET. Regions of interest (ROIs) for data analysis were selected for each tracer based on the established literature. The NET ligand was (S,S)-[^{11}C]methylreboxetine ([^{11}C]MRB) and PET scans were 120 min long (33 frames) (Ding et al., 2010). For each tracer, regions of interest (ROIs) were selected based on specific binding (i.e. only ROIs known to have higher and reliable specific binding in vivo) (Comley et al., 2013; DeLorenzo et al., 2011; Ding et al., 2010; Hannestad et al., 2010; Logan et al., 2007; Sasaki et al., 2012). For NET, the selected ROIs for data analysis were the hypothalamus, thalamus, locus coeruleus, and paracentral lobule cortex, with the occipital cortex as the reference region. The DAT ligand was (*E*)-*N*-(3-iodopro-2-enyl)-2β-carbo[^{18}F]fluroethoxy-3β-(4′-methylphenyl)nortropane ([^{18}F]FE-PE2I) and PET scans were 90 min long (27 frames) (Sasaki et al., 2012). For DAT, the selected ROIs for data analysis were the caudate, putamen, pallidum, substantia nigra, and ventral striatum, with the cerebellum as the reference region. The SERT ligand was 3-amino-4-(2-[^{11}C]methylaminomethylphenylsulfanyl)benzonitrile ([^{11}C]DASB) and PET scans were 90 min long (27 frames) (Comley et al., 2013; DeLorenzo et al., 2011). For SERT, the selected ROIs for data analysis were the amygdala, caudate, putamen, thalamus, hippocampus, and cingulate, frontal, occipital, parietal, and temporal cortices, with the cerebellum as the reference region.

Baseline scans for subjects included magnetic resonance imaging for anatomical brain mapping and $\leq$2 PET scans with different ligands. After receiving the last dose of centanafadine, subjects underwent up to three PET scans, using up to two different ligands at 2–3 h post-dose, 4–6 h post-dose, and 25–29 h post-dose. Each subject underwent no more than five PET scans, receiving a maximum potential of 2775 MBq of radioactivity, approximately 555 MBq per injection of [^{11}C]MRB and [^{11}C]DASB, or 222 MBq per injection of [^{18}F]FE-PE2I. Actual injection parameters are shown in Table 1.

Serial pharmacokinetic (PK) blood samples (4 mL each) were collected before, during, and after each post-dose PET scan for the determination of centanafadine concentrations in the plasma. The

Table 1. Administered radioactivity (MBq) and mass dose (μg/kg) of radiotracers.

	[11C]MRB		[11C]FE-PE2I		[11C]DASB	
	MBq	μg/kg	MBq	μg/kg	MBq	μg/kg
Baseline	417 (152)	0.015 (0.009)	133 (62)	0.004 (0.002)	374 (203)	0.011 (0.007)
Post-dose 1	430 (156)	0.021 (0.001)	147 (60)	0.007 (0.003)	249 (232)	0.009 (0.009)
Post-dose 2	462 (129)	0.016 (0.008)	173 (6)	0.008 (0.003)	388 (227)	0.019 (0.007)

Values in parenthesis are ± SD. [11C]MRB: (S,S)-[11C]methylreboxetine; [11C]DASB: 3-amino-4-(2-[11C]methylaminomethylphenylsulfanyl) benzonitrile; [18F]FE-PE2I: (*E*)-*N*-(3-iodopro-2-enyl)-2β-carbo[18F]fluroethoxy-3β-(4′-methylphenyl)nortropane; SD: standard deviation.

average of the three concentrations was used for analysis. Concentrations were quantified by a validated assay using high performance liquid chromatography with tandem mass spectrometry. The lower limit of quantification (LLOQ) was 5.00 ng/mL.

Statistical analysis

No formal sample-size calculations were performed for this study. The imaging analysis dataset included subjects with a baseline PET scan and at least one evaluable post-dose scan ($n=10$).

The quantification of BP_{ND} and drug occupancy relies on hypotheses about the properties of the tracer in the various regions of interest. In particular, it is assumed that the level of nonspecific (i.e. non-displaceable) binding is the same in the reference region and in all the transporter-rich regions. If this hypothesis is invalid, the occupancies estimated using BP_{ND} values are biased. This bias tends to be larger for tracers with low specific binding (resulting in a small BP_{ND}), like [11C]MRB used for NET studies. It has been observed in past studies that [11C]MRB nonspecific binding is higher in the thalamus (the only larger ROI with specific binding) than in the occipital cortex (used as the reference region) (Ding et al., 2010; Gallezot et al., 2014). Because of this limitation of the tracer, we used the term apparent target occupancy (TO_{app}). The TO_{app} values were computed for each PET scan after centanafadine administration using an occupancy plot constructed with the difference of baseline and post-drug binding potential. Time-activity curves (TACs) were generated for PET images using specific ROIs for each tracer as established in the literature (Ding et al., 2010; Gallezot et al., 2014). MR images for each subject were transformed to a template MR image. A full description of MR image co-registration and PET image motion correction processing, as well as computation of the tracers' binding potentials and apparent target occupancies are provided in eMethods Sections 4 and 5, respectively.

The PK/pharmacodynamic (PD) analysis dataset included subjects with TO_{app} measurements and plasma centanafadine concentrations taken before, during, and after the nominal scan time. Individual data from all subjects were pooled for PK/PD analysis. Modeling was conducted to quantify the relationship between TO_{app} and centanafadine concentrations for each transporter. In model 1, maximum apparent occupancy (TO_{max}) was assumed to be 100% and the centanafadine concentration that produces half of the maximum occupancy (IC_{50}) was estimated. The final occupancy estimates were then computed as $TO=TO_{app}$). In model 2, both TO_{max} and IC_{50} were estimated. The final occupancy estimates were then computed as $TO=TO_{app}/TO_{max}$). Model selection was based on either estimated correlation between the model parameters or *F*-test. A full description of IC_{50} computation is provided in eMethods Section 6.

Results

Subject disposition and demographics

A total of 10 subjects (cohort 1, $n=6$; cohort 2, $n=4$) were enrolled, treated, and completed the study (eSupplement, Figure 1). Subject demographics and baseline characteristics are presented in Table 2. Results of safety assessments are provided in eResults, Section 1, and in eTable2.

PK/PD data

Centanafadine concentrations (mean of concentration values before, during and after each post-dose scan) ranged from Figure 1. TACs for the NET, DAT, and SERT ligands are shown in eSupplement Figure 2. Occupancy at the post-dose 1 scan is visible based on the lower tracer uptake compared to the baseline scans due to competition from centanafadine. For NET and DAT, lower brain uptake is clearly visible in the post-dose 1 scans. The SERT TACs are more complex owing to the substantial change in the tracer input function due to SERT blockade, as shown previously for similar SERT tracers (Huang et al., 2004).

Apparent occupancy values for all three binding sites were determined with occupancy plots (Figure 2). Across centanafadine concentrations, TO_{app} tended to be numerically higher for NET than for DAT or SERT. At post-dose scan 1, NET TO_{app} ranged from 40 to 68% across the centanafadine concentration range of 221–2580 ng/mL. Similarly, DAT TO_{app} at post-dose scan 1 ranged from 22 to 58% across the centanafadine concentration range of 587–2580 ng/mL. For post-dose scan 1, SERT TO_{app} ranged from 12 to 59% across the centanafadine concentration range of 358–1390 ng/mL.

To establish relationships between centanafadine concentrations and transporter occupancies, modeling was used. For NET, estimated (mean ± standard error [SE]) TO_{max} and IC_{50} were 64 ± 7% and 132 ± 65 ng/mL, respectively (Figure 3(a)). After correcting apparent occupancy estimates for TO_{max} effect, the final occupancy estimate ranged from 62 to 106% for NET. For DAT and SERT, TO_{max} could not be established and was assumed to be 100%, based on results from previous studies (Kim et al., 2014; Parsey et al., 2006); thus the final occupancy estimates are equal to the apparent occupancy estimates for these two targets; the estimated IC_{50} (mean ± SE) values were 1580 ± 186 and 1760 ± 309 ng/mL, respectively (Figure 3(b) and (c)). For centanafadine, the estimated in vivo affinity ratio was 11.9 ± 6.0

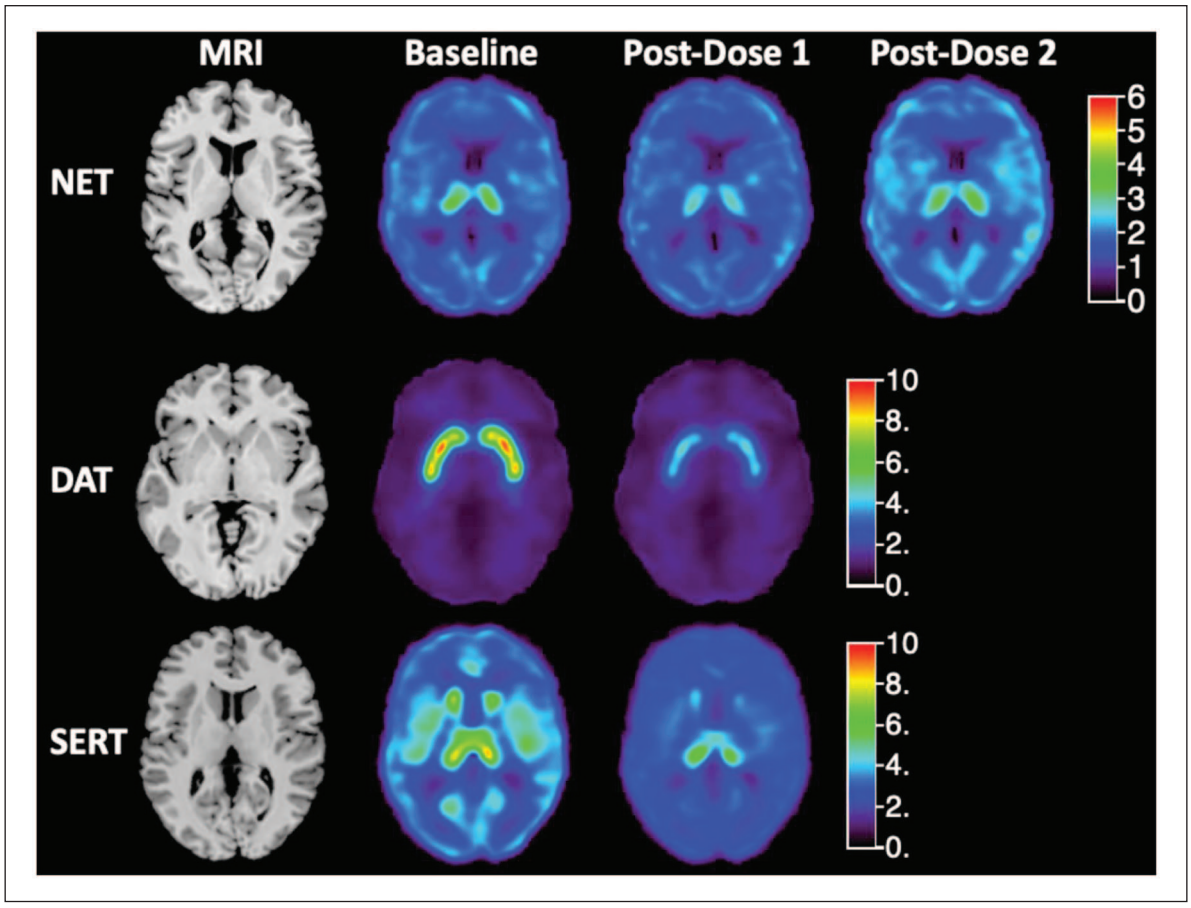

Figure 1. PET imaging results for centanafadine occupancy. PET: positron emission tomography.

Table 2. Study population demographics and baseline characteristics.

Demographic/baseline characteristics	Cohort 1 Centanafadine 400 mg ($n=6$)	Cohort 2 Centanafadine 800 mg ($n=4$)	Total ($N=10$)
Age, mean (SD) (years)	32.5 (7.9)	31.8 (8.5)	32.2 (7.6)
Sex, *n* (%)			
Male	6 (100.0)	4 (100.0)	10 (100.0)
Race, *n* (%)			
Black or African American	3 (50.0)	3 (75.0)	6 (60.0)
Caucasian	3 (50.0)	1 (25.0)	4 (40.0)
Ethnicity, *n* (%)			
Hispanic or Latino	4 (66.7)	0 (0.0)	4 (40.0)
Not Hispanic or Latino	2 (33.3)	4 (100.0)	6 (60.0)
Weight, mean (SD) (kg)	87.9 (8.7)	77.8 (16.3)	83.9 (12.5)
Height, mean (SD) (cm)	178.9 (10.3)	176.8 (10.5)	178.1 (9.8)
BMI, mean (SD) (kg/m^2)	27.5 (1.7)	24.9 (4.8)	26.4 (3.3)

Percentages are based on the number of enrolled subjects.
BMI: body mass index; SD: standard deviation.

(mean ± SE) for NET/DAT, 13.3 ± 7.0 for NET/SERT, and 1.1 ± 0.2 for DAT/SERT.

Additional analyses of occupancy values in each region revealed that the apparent NET occupancy was significantly lower in the thalamus than the other regions (eSupplement, Figure 3). When the original analysis of NET data was conducted without the thalmus region (eSupplement, Figure 4), mean TO_{max} and IC_{50} were 82 ± 13% and 135 ± 97 ng/mL, respectively,

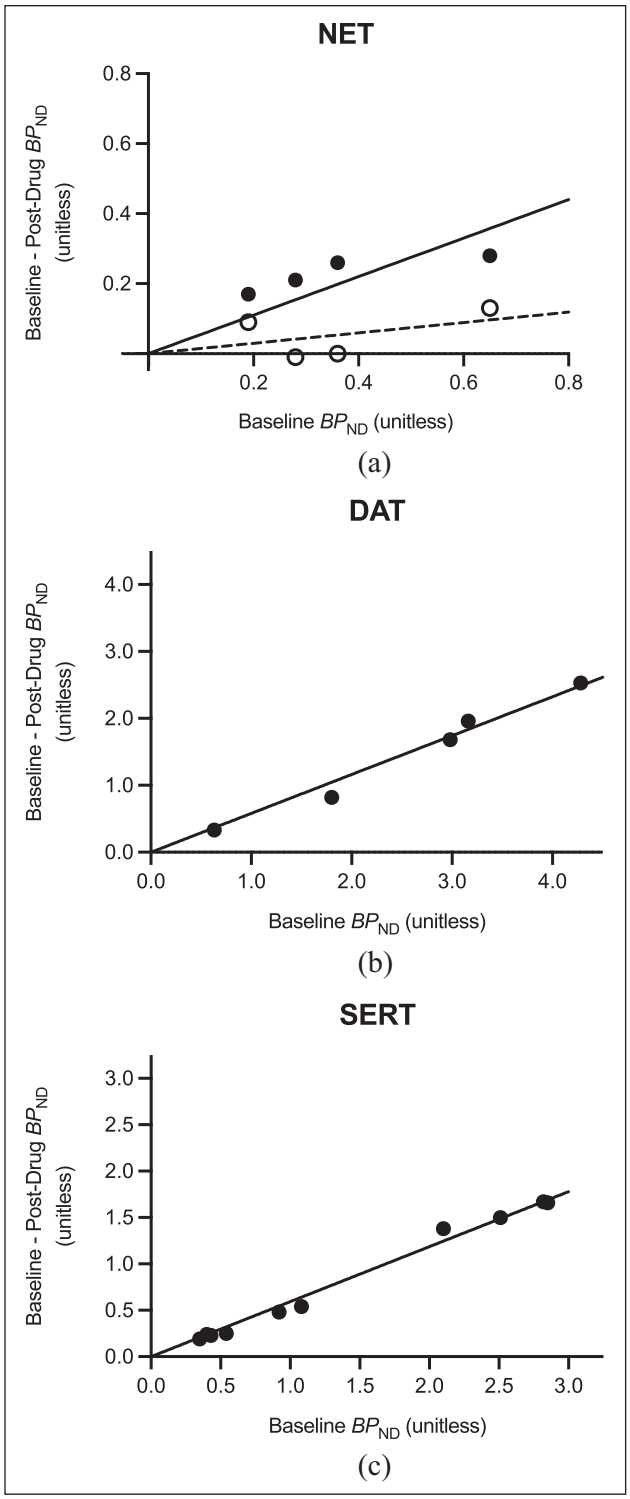

Figure 2. Typical occupancy plots for transporters of interest. (a) Occupancy plots for NET studies with [11C]MRB, (b) DAT studies with [18F]FE-PE2I, and (c) SERT studies with [11C]DASB. Solid circles show "post-dose one" scan data, open circles show "post-dose two" scan data.

indicating that the estimate of IC_{50} was not greatly affected despite that TO_{max} in the thalamus was estimated at 38% whereas TO_{max} in the hypothalamus, locus coeruleus, and paracentral lobule were 79, 88, and 97%, respectively. For DAT and SERT, such significant difference in TO_{max} was not observed between brain regions (eSupplement, Figures 5 and 6).

Discussion

This study demonstrates the feasibility of investigating centanafadine in vivo to: (1) evaluate the degree and time course of NET, DAT, and SERT occupancy in male humans at clinically relevant concentrations; and (2) describe the relationship between plasma concentration and NET, DAT, and SERT occupancy. To achieve these goals, centanafadine was administered using different dosing regimens. The selection of the doses provided a broad range of centanafadine concentrations and took into consideration the design of the phase 3 randomized controlled trials in adults with ADHD and PK information obtained from phase 1 studies. Centanafadine was generally safe and well tolerated. The results showed the highest occupancy at NET followed by DAT and SERT. Centanafadine had an in vivo affinity for NET nearly 12- and 13-fold greater than for DAT and SERT, respectively.

When analyzing the relationship between apparent occupancy TO_{app} and PK values, we tested two models: model 1, assuming that the maximal apparent occupancy was 100%; and model 2, allowing the maximal occupancy to be different from 100%. PET occupancy studies using BP_{ND} estimates, or the occupancy plot, rely on the assumption that tracer nonspecific binding is homogenous throughout the brain, and violation of this hypothesis leads to biased apparent occupancy estimates. If nonspecific binding is higher in a target region than in the reference region, then BP_{ND} values cannot be reduced to zero even in the presence of high drug concentration, and the apparent occupancy will remain lower than 100%. Conversely, if nonspecific binding is lower in a target region than in the reference region, negative BP_{ND} values and apparent occupancies higher than 100% can be observed. In a previous [^{18}F]FE-PE2I study (Kim et al., 2014), model 1 was used. In a previous [^{11}C]DASB study, model 2 was used, but TO_{max} was not found to be significantly different from 100%, thus model 2 effectively reduced to model 1 for this tracer (Parsey et al., 2006). In this study, model 1 was the preferred model for both [^{18}F]FE-PE2I and [^{11}C]DASB. Conversely, model 2 was the preferred model for [^{11}C]MRB, and TO_{max} was estimated to be 64% when using all regions of interest. This is similar to the results obtained in a previous study with methylphenidate (Hannestad et al., 2010): [^{11}C]MRB BP_{ND} values did not to decrease to zero even in the presence of a large dose of methylphenidate, with a TO_{max} value of only 59% in the thalamus (see eMethods 6 for more details of the comparison between the two studies).

TO_{max} estimation by target region revealed that NET occupancy values were lower (38%) in the thalamus than the other regions (79–97%). The lower apparent occupancy in the thalamus was most likely caused by higher nonspecific binding of [^{11}C]MRB in the thalamus as noted above. The lower apparent NET occupancy in the thalamus negatively affected overall apparent NET occupancy estimated based on regression lines of the occupancy plots (example in Figure 2) and thus a secondary analysis was conducted without the thalamus. Thus, the lower TO_{max} value seen in this study for NET is likely due to a property of the tracer [^{11}C]MRB, not a property of centanafadine, and apparent occupancy estimates obtained from the occupancy plots should be corrected for TO_{max} for proper biological interpretation of the study (i.e. true occupancy = apparent occupancy/TO_{max}).

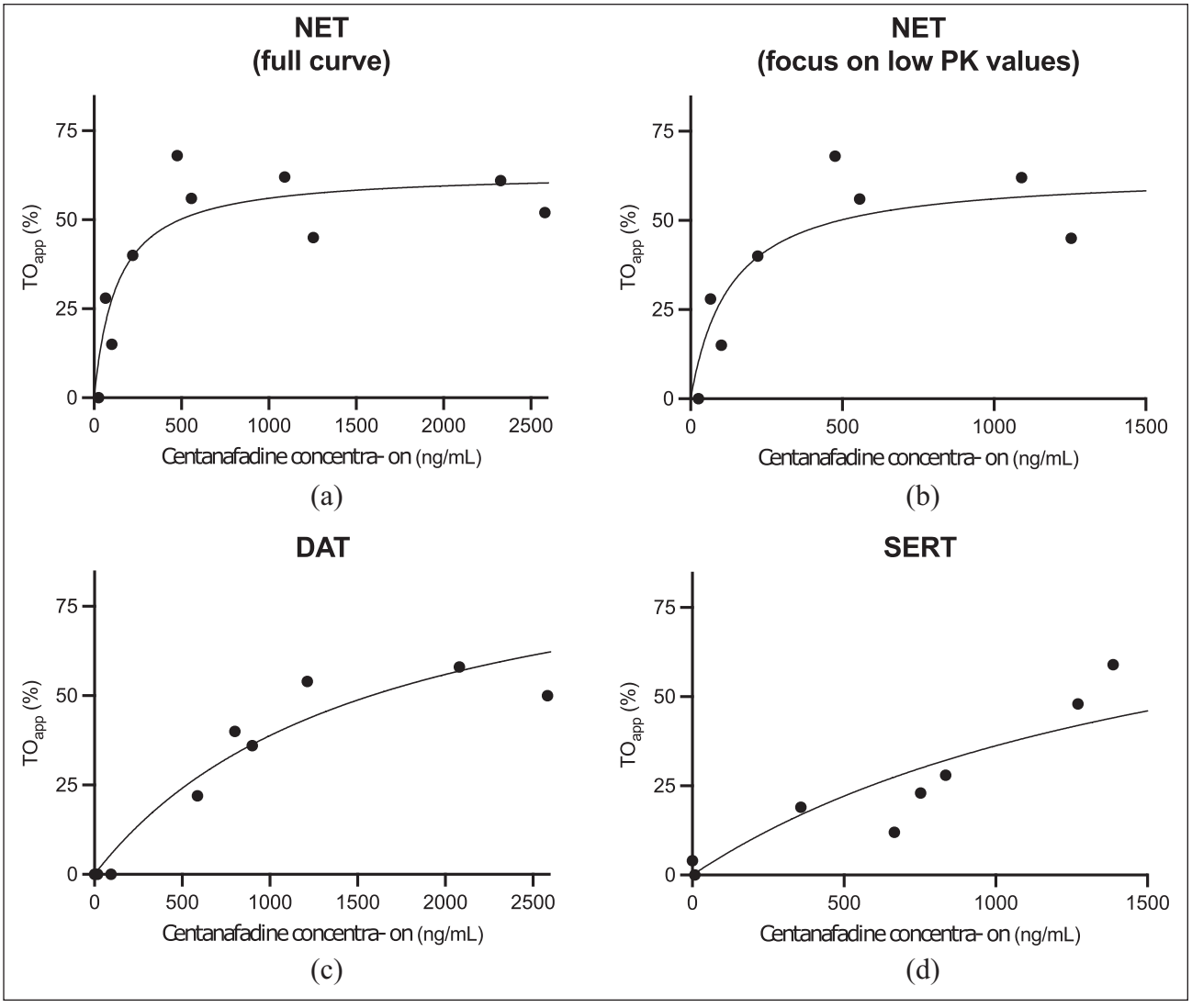

Figure 3. Transporter occupancy–plasma concentration relationship. (a and b) The relationship between plasma concentration (PK) and transporter occupancy (TO) for NET, (c) DAT, and (d) SERT.

Comparing [^{11}C]MRB with [^{18}F]FE-PE2I and [^{11}C]DASB, it should be noted that the binding potentials of [^{11}C]MRB are much lower than those of [^{18}F]FE-PE2I and [^{11}C]DASB. Occupancy studies using tracers with low specific binding are affected more by small variations in nonspecific binding, and thus model 2 is more likely to be needed for tracers with low specific binding such as [^{11}C]MRB. In addition, it is worth noting that in this study it was not fully possible to test if model 2 is needed for [^{18}F]FE-PE2I and [^{11}C]DASB, since the range of plasma concentrations for centanafadine in the [^{18}F]FE-PE2I and [^{11}C]DASB studies was too narrow (maximum concentrations were only about four times minimum concentrations, while there was a factor of 11 between maximum and minimum concentrations in the [^{11}C]MRB studies). Finally, the expected occupancy at the mean peak centanafadine concentration (Cmax) of 1400 ng/mL obtained following 400 mg TDD of SR tablet was computed based on the estimated IC_{50}. High occupancy was determined for NET (91%), while occupancies for SERT and DAT were lower (44.3 and 47.1%, respectively).

The results of this trial differ from those of a previously reported SPECT study with centanafadine (NVI-EB-1020-104) (McKinney et al., 2016). In the SPECT trial, the occupancy at NET was similar to those at DAT and SERT. However, the apparent NET occupancy in the SPECT study was not explicitly corrected for the effect of differences in nonspecific binding between NET-rich regions. In addition, such differences may be even higher for the SPECT tracer [^{123}I]INER than for the PET tracer [^{11}C]MRB, as suggested by the previous work showing that an 80 mg dose of atomoxetine only reduced [^{123}I]INER BP_{ND} in the thalamus by 17%, while a 50 mg dose of atomoxetine reduced [^{11}C]MRB BP_{ND} in the thalamus by 55% (Logan et al., 2007). Therefore, the TO_{max} measured with [^{123}I]INER SPECT may be much lower than the 64% estimated with [^{11}C]MRB PET in this study, and the apparent occupancy measured with [^{123}I]INER likely underestimates the true NET occupancy by a large factor. In addition, the SPECT study predicted occupancy for DAT of 13.8% at a concentration of 1151 ng/mL, while in this study it was 42%. Similarly, the predicted SERT occupancy was 14.4% at

907 ng/mL, while in this study SERT occupancy was 36% (Unpublished data). The higher occupancies observed at DAT and SERT in the current study may be explained by methodological challenges involving the time required for SPECT assessment. In the previous study (McKinney et al., 2016), DAT and SERT BP_{ND} values were estimated using a bolus tracer injection protocol and equilibrium analysis with the radiotracer [^{123}I]β-CIT at 4 h post tracer injection for SERT and 18 h post tracer injection for DAT due to the need to wait up to 25 h to reach equilibrium with [^{123}I]β-CIT in the DAT rich-regions (Unpublished data; Laruelle et al., 1994) The different time scales used for the PET measurements (90 min) as compared to the SPECT measurements (4 and 18 h) and the dynamic changes in PK during the longer SPECT studies contribute to the differences seen between the two studies for DAT and SERT occupancies, and likely were also contributing factors to the differences seen in NET occupancy.

The current results were similar, however, to an in vitro study that showed centanafadine had higher affinity for NET than SERT (Bymaster et al., 2012). In vitro, the IC_{50} values of centanafadine for NET, DAT, and SERT were measured to be 6, 38, and 83 nM, respectively. Therefore, the NET:SERT, NET:DAT, and DAT:SERT affinity ratios were 13.8, 6.3, and 2.2, respectively. The major difference in the current work was the numerically higher estimated NET:DAT ratio, suggesting that centanafadine has relatively lower in vivo affinity for DAT when compared to in vitro data.

At concentrations achieved in ADHD clinical studies, occupancies of SERT and DAT are expected to be lower than or equal to the highest occupancy levels in this study, and about linearly proportional to dose, while the occupancy of NET is expected to be more in the saturated, nonlinear portion of the PK/receptor occupancy relationship. A limitation of this study is that only healthy male subjects were studied due to the unknown effects of the drug on reproduction, with the possibility of population heterogenicity to affect statistical power. While a single gender is a relative strength in a smaller sized cohort, results may be different for females and clinical populations as previous studies have shown that receptors can differ both by gender and in patients with psychiatric disorders (Maron et al., 2011; Matuskey et al., 2019; Nishizawa et al., 1997; Wong et al., 2012). A larger balanced study in the future is needed to answer if possible gender differences exist. Finally, inherent limitations of the tracers, including restricted anatomical distribution and differential binding potentials affecting signal-to-noise ratios, may preclude definitive statements about neurotransmitter relationships in vivo.

Despite these caveats, this study was very innovative in the design of multiple PET scans (i.e. 5) at different times and with separate tracers to understand the occupancy of three neurotransmitter transporters by centanafadine: Based on in vivo imaging of multiple transmitter transporters, centanafadine is found to have the highest affinity for NET, followed by DAT and SERT. These findings can help inform mechanism of action, target engagement and occupancy at clinically relevant concentrations, and thus further clinical work with centanafadine.

Acknowledgments

Editorial support for this manuscript was provided by BioScience Communications, New York, NY, USA, and funded by Otsuka Pharmaceutical Development & Commercialization, Inc., Princeton, NJ, USA.

Author contributions

DM was involved in the design of the study, participant screening and care, interpretation of results, and writing and review of the manuscript; he had full access to all study data. J-DG was involved in data analysis and interpretation; manuscript revision. NN contributed to the acquisition and analysis of data. YH contributed to the acquisition and analysis of data. SH contributed to the acquisition and analysis of data. KT contributed to the acquisition and analysis of data. MD contributed to the acquisition and analysis of data. GAA was involved in clinical coverage for subjects while on the inpatient research unit and PET Center, data collection on adverse events, and manuscript drafting/revision. SES was involved in the design of the study, interpretation of results, and writing and review of the manuscript; she had full access to all study data. REC was involved in the design of the study, performance of the imaging, interpretation of results, and writing and review of the manuscript; he had full access to all study data. SM was involved in the design of the study, analysis of drug concentrations, interpretation of results, and review of the manuscript; she had full access to all study data.

Data sharing

To submit inquiries related to Otsuka Clinical Research, or to request access to individual participant data (IPD) associated with any Otsuka clinical trial, please visit https://clinical-trials.otsuka.com/. For all approved IPD access requests, Otsuka will share anonymized IPD on a remotely accessible data sharing platform.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: DM was the principal investigator of the study and has no personal disclosures to state. J-DG, NN, SH, KT, MD, YH, and REC have no disclosures to report. GAA has no conflicts of interest; his research is funded by the Brain and Behavior Research Foundation. SES and SM are employees of Otsuka Pharmaceutical Development & Commercialization, Inc., Princeton, NJ, USA.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was sponsored and supported by Otsuka Pharmaceutical Development & Commercialization, Inc., Princeton, NJ, USA. The work described in this article was funded in part by the State of Connecticut, Department of Mental Health and Addiction Services, but this publication does not express the views of the Department of Mental Health and Addiction Services or the State of Connecticut. The views and opinions expressed are those of the authors.

ORCID iDs

Jean-Dominique Gallezot https://orcid.org/0000-0003-0399-8374
Nabeel Nabulsi https://orcid.org/0000-0001-8129-0051
Susan E Shoaf https://orcid.org/0000-0001-7725-9385

Supplemental material

Supplemental material for this article is available online.

References

Barbaresi WJ, Colligan RC, Weaver AL, et al. (2013) Mortality, ADHD, and psychosocial adversity in adults with childhood ADHD: A prospective study. *Pediatrics* 131: 637–644.

Bymaster FP, Golembiowska K, Kowalska M, et al. (2012) Pharmacological characterization of the norepinephrine and dopamine

reuptake inhibitor EB-1020: Implications for treatment of attention-deficit hyperactivity disorder. *Synapse* 66: 522–532.

Caye A, Swanson J, Thapar A, et al. (2016) Life span studies of ADHD-conceptual challenges and predictors of persistence and outcome. *Curr Psychiatry Rep* 18: 111.

Comley RA, Salinas CA, Slifstein M, et al. (2013) Monoamine transporter occupancy of a novel triple reuptake inhibitor in baboons and humans using positron emission tomography. *J Pharmacol Exp Ther* 346: 311–317.

Cortese S, Adamo N, Del Giovane C, et al. (2018) Comparative efficacy and tolerability of medications for attention-deficit hyperactivity disorder in children, adolescents, and adults: A systematic review and network meta-analysis. *Lancet Psychiatry* 5: 727–738.

DeLorenzo C, Lichenstein S, Schaefer K, et al. (2011) SEP-225289 serotonin and dopamine transporter occupancy: A PET study. *J Nucl Med* 52: 1150–1155.

Ding YS, Singhal T, Planeta-Wilson B, et al. (2010) PET imaging of the effects of age and cocaine on the norepinephrine transporter in the human brain using (S,S)-[(11)C]O-methylreboxetine and HRRT. *Synapse* 64: 30–38.

Gallezot JD, Zheng MQ, Lim K, et al. (2014) Parametric imaging and test-retest variability of (1)(1)C-(+)-PHNO binding to D(2)/D(3) dopamine receptors in humans on the High-Resolution Research Tomograph PET scanner. *J Nucl Med* 55: 960–966.

Hannestad J, Gallezot JD, Planeta-Wilson B, et al. (2010) Clinically relevant doses of methylphenidate significantly occupy norepinephrine transporters in humans in vivo. *Biol Psychiatry* 68: 854–860.

Heal DJ, Smith SL, Gosden J, et al. (2013) Amphetamine, past and present–A pharmacological and clinical perspective. *J Psychopharmacol* 27: 479–496.

Heal DJ, Smith SL and Henningfield JE (2014) CNS stimulants. *Neuropharmacology* 87: 1–3.

Huang Y, Hwang DR, Bae SA, et al. (2004) A new positron emission tomography imaging agent for the serotonin transporter: Synthesis, pharmacological characterization, and kinetic analysis of [11C]2-[2-(dimethylaminomethyl)phenylthio]-5-fluoromethylphenylamine ([11C]AFM). *Nucl Med Biol* 31: 543–556.

Kessler RC, Adler L, Barkley R, et al. (2006) The prevalence and correlates of adult ADHD in the United States: Results from the National Comorbidity Survey Replication. *Am J Psychiatry* 163: 716–723.

Kim W, Tateno A, Arakawa R, et al. (2014) In vivo activity of modafinil on dopamine transporter measured with positron emission tomography and [(1)(8)F]FE-PE2I. *Int J Neuropsychopharmacol* 17: 697–703.

Kolar D, Keller A, Golfinopoulos M, et al. (2008) Treatment of adults with attention-deficit/hyperactivity disorder. *Neuropsychiatr Dis Treat* 4: 107–121.

Laruelle M, Wallace E, Seibyl JP, et al. (1994) Graphical, kinetic, and equilibrium analyses of in vivo [123I] beta-CIT binding to dopamine transporters in healthy human subjects. *J Cereb Blood Flow Metab* 14: 982–994.

Logan J, Wang GJ, Telang F, et al. (2007) Imaging the norepinephrine transporter in humans with (S,S)-[11C]O-methyl reboxetine and PET: Problems and progress. *Nucl Med Biol* 34: 667–679.

Maron E, Toru I, Hirvonen J, et al. (2011) Gender differences in brain serotonin transporter availability in panic disorder. *J Psychopharmacol* 25: 952–959.

Matuskey D, Dias M, Naganawa M, et al. (2019) Social status and demographic effects of the kappa opioid receptor: A PET imaging study with a novel agonist radiotracer in healthy volunteers. *Neuropsychopharmacology* 44: 1714–1719.

McKinney A, Jennings D, Barret O, et al. (2016) Centanafadine SR (CTN-SR) demonstrates brain occupancy at norepinephrine transporter (NET), serotonin transporter (SERT), and dopamine transporter (DAT) using a single photon emission tomography (SPECT) in healthy volunteers (HVS). Abstract presented at the *American Society of Clinical Psychopharmacology*, Scottsdale, Arizona, USA, 30 May 2016 to 3 June 2016.

Nishizawa S, Benkelfat C, Young SN, et al. (1997) Differences between males and females in rates of serotonin synthesis in human brain. *Proc Natl Acad Sci U S A* 94: 5308–5313.

Parsey RV, Kent JM, Oquendo MA, et al. (2006) Acute occupancy of brain serotonin transporter by sertraline as measured by [11C]DASB and positron emission tomography. *Biol Psychiatry* 59: 821–828.

Sasaki T, Ito H, Kimura Y, et al. (2012) Quantification of dopamine transporter in human brain using PET with 18F-FE-PE2I. *J Nucl Med* 53: 1065–1073.

Sharma A and Couture J (2014) A review of the pathophysiology, etiology, and treatment of attention-deficit hyperactivity disorder (ADHD). *Ann Pharmacother* 48: 209–225.

Wigal SB, Wigal T, Hobart M, et al. (2020) Safety and efficacy of centanafadine sustained-release in adults with attention-deficit hyperactivity disorder: Results of Phase 2 studies. *Neuropsychiatr Dis Treat* 16: 1411–1426.

Wong KK, Muller ML, Kuwabara H, et al. (2012) Gender differences in nigrostriatal dopaminergic innervation are present at young-to-middle but not at older age in normal adults. *J Clin Neurosci* 19: 183–184.