SEP-225289 Serotonin and Dopamine Transporter Occupancy: A PET Study

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SEP-225289 is a novel compound that, based on in vitro potencies for transporter function, potentially inhibits reuptake at dopamine, norepinephrine, and serotonin transporters. An open-label PET study was conducted during the development of SEP-225289 to investigate its dopamine and serotonin transporter occupancy. **Methods:** Different single doses of SEP-225289 were administered to healthy volunteers in 3 cohorts: 8 mg ($n = 7$), 12 mg ($n = 5$), and 16 mg ($n = 7$). PET was performed before and approximately 24 h after oral administration of SEP-225289, to assess occupancy at trough levels. Dopamine and serotonin transporter occupancies were estimated from PET using ^{11}C-*N*-(3-iodoprop-2*E*-enyl)-2β-carbomethoxy-3β-(4-methylphenyl) nortropane (^{11}C-PE2I) and ^{11}C-*N,N*-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine (^{11}C-DASB), respectively. Plasma concentration of SEP-225289 was assessed before ligand injection, and subjects were monitored for adverse events. **Results:** Average dopamine and serotonin transporter occupancies increased with increasing doses of SEP-225289. Mean dopamine and serotonin transporter occupancies were 33% ± 11% and 2% ± 13%, respectively, for 8 mg; 44% ± 4% and 9% ± 10%, respectively, for 12 mg; and 49% ± 7% and 14% ± 15%, respectively, for 16 mg. On the basis of the relationship between occupancy and plasma concentration, dopamine transporter IC_{50} (the plasma concentration of drug at 50% occupancy) was determined (4.5 ng/mL) and maximum dopamine transporter occupancy was extrapolated (85%); however, low serotonin transporter occupancy prevented similar serotonin transporter calculations. No serious adverse events were reported. **Conclusion:** At the doses evaluated, occupancy of the dopamine transporter was significantly higher than that of the serotonin transporter, despite similar in vitro potencies, confirming that, in addition to in vitro assays, PET occupancy studies can be instrumental to the drug development process by informing early decisions about indication, dose, and therapeutic potential.

Key Words: dopamine; serotonin; occupancy; positron emission tomography; SEP-225289

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Dysfunction in monoamine transmission has been implicated in the pathophysiology of several major neuropsychiatric disorders, including mood disorders, alcohol and drug abuse, pain disorders, eating disorders, and obesity. Although treatments for these conditions often involve blocking dopamine, norepinephrine, or serotonin reuptake, mounting evidence suggests that targeting multiple neurotransmitter systems simultaneously may increase or broaden efficacy.

For example, recent studies suggest that targeting dopamine or norepinephrine transmission, in addition to targeting transmission of serotonin, may improve treatment outcomes in major depression (*1–3*). Also, on the basis of the potential additional or differential benefits of dopamine reuptake inhibition in combination with serotonin or norepinephrine reuptake inhibition, triple-reuptake inhibitors have been studied for several years (*4*). These drugs have promise in the treatment of substance abuse (alcohol, nicotine, and cocaine) (*5–7*), chronic pain syndromes (*8,9*), and obesity and eating disorders (*10,11*).

On the basis of the numerous clinical applications of therapeutics targeting multiple monoamine systems, one such drug, SEP-225289, was developed by Sunovion Pharmaceuticals Inc. as a potential triple-reuptake inhibitor. Average in vitro potencies for transporter function inhibition by SEP-225289 have been measured (8 ± 7, 17 ± 3, and 10 ± 8 nM, for the dopamine, norepinephrine, and serotonin transporters, respectively) (*12*). However, a potentially more clinically valid gauge of a drug's transporter binding profile is its in vivo occupancy, because differences in temperature, pH, concentration, or other unknown factors can generate discrepancies between in vitro and in vivo affinities (*13–16*).

In vivo drug occupancy can be assessed using PET. Numerous PET studies have been conducted on existing therapeutics to determine the threshold drug receptor (or transporter) occupancies necessary to achieve the therapeutic effect (*17*). Such studies have suggested that the minimum occupancy needed for selective serotonin reuptake inhibitors

is approximately 80% of serotonin transporters (*14,17*), whereas the occupancy of dopamine transporters potentially associated with various clinical effects is less clear (*18*). PET occupancy studies are also becoming increasingly common in early drug development programs (*16,17*). However, despite this, few have examined drug occupancy at multiple monoamine neurotransmitter systems within the same subject, and those that have (*13,15,19–22*) were mostly restricted to neuroleptic compounds.

In vivo occupancy estimates of reuptake inhibitors with multiple monoamine targets are particularly important because occupancy at each transporter type must happen within a fairly narrow dose range, since adverse effects within one system (e.g., nausea and serotonin) may prevent the dose escalation needed to potentially engage additional systems. Consequently, supplementing in vitro, ex vivo, and preclinical target activity profiles of compounds with human imaging can provide information on potential efficacy, side effects, and therapeutic dose ranges, which may help to inform subsequent phases of development. Therefore, in this study, the multiple monoamine transporter occupancy of a nonneuroleptic compound (SEP-225289) was assessed in the same individual using PET and a reference region methodology.

MATERIALS AND METHODS

Subjects

All subjects were healthy volunteers who met the following inclusion criteria: age 18–50 y; body mass index between 16 and 32 kg/m^2; capacity to provide written informed consent; negative results on urine screening for drugs; willingness to refrain from strenuous activity during the study; daily consumption of less than 180 mg of caffeine; and negative pregnancy test and use of an acceptable birth control method throughout the study for women of child-bearing potential. Exclusion criteria consisted of a significant medical illness; a major psychiatric illness, including mental retardation, drug or alcohol abuse or dependence; an axis I diagnosis according to the *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV* (*23*); use of psychotropic medications within 5 half-lives or 30 d; regular alcohol consumption exceeding 7 (for women) or 14 (for men) drinks per week; history of tobacco dependency or use of tobacco or nicotine within 30 d; a first-degree relative with a history of schizoaffective disorder, depression, bipolar disorder, or suicide attempt; 2 or more first-degree relatives with a history of alcohol or substance dependence; or a first-degree family history of schizophrenia if the subject was less than 33 y of age (mean age of onset for schizophrenia plus 2 SDs). Criteria were assessed by the following: history, the Structured Clinical Interview for DSM Disorders (SCID), physical examination, blood tests, urine toxicology, and electrocardiography.

The protocol was approved by the Institutional Review Board of the New York State Psychiatric Institute, and all subjects gave written informed consent after receiving an explanation of the study.

Study Medication

Two phase I clinical studies have been conducted using SEP-225289. In the first, 128 subjects were exposed to single doses of SEP-225289 ranging from 0.2 to 36 mg. In the second, 27 subjects were exposed to multiple doses of SEP-225289 ranging from 1 to 3 mg for 21 d. At the highest single dose administered (36 mg), 7 of the 9 subjects manifested symptoms consistent with stimulant-related exaggerated pharmacology, which resolved within 48 h of taking the drug without any intervention. This resolution was followed by a brief period of depressed mood in 2 subjects. In both studies, the most commonly observed adverse events (AEs) included headache, insomnia, dizziness, nausea, somnolence, diarrhea, and irritability (Sunovion Pharmaceuticals, Inc., unpublished data, 2011).

Selection of dose range for this PET occupancy study was based on the phase I clinical program. To produce drug plasma levels consistent with those observed in steady-state conditions in the clinical studies (i.e., clinically relevant doses), the single doses of SEP-225289 used in this PET study were higher than those that would be administered chronically.

Study Design

Subjects were assigned to 1 of 3 cohorts (A, B, or C), receiving a single dose of 8, 12, or 16 mg of SEP-225289, respectively. The final subject population had a mean age of 28.0 y (SD, 8.3 y) and consisted of cohorts A (2 men, 5 women), B (3 men, 2 women), and C (3 men, 4 women).

Dopamine and serotonin transporter occupancies were estimated in each subject with PET using the radioligands ^{11}C-*N*-(3-iodoprop-2*E*-enyl)-2β-carbomethoxy-3β-(4-methylphenyl)nortropane (^{11}C-PE2I) (*24*) and ^{11}C-*N,N*-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine (^{11}C-DASB) (*25*), respectively. The subjects received a baseline ^{11}C-PE2I and ^{11}C-DASB PET scan, with approximately a 1-h break between scans. The subjects returned 5–10 d after the baseline scans and were administered a single dose of SEP-225289, followed by occupancy scans with ^{11}C-PE2I and ^{11}C-DASB at approximately 24 and 27 h after dose administration, respectively. Because of scheduling constraints, 1 subject, S034, received the ^{11}C-DASB occupancy scan first, followed by the ^{11}C-PE2I occupancy scan, and 1 subject, S018, did not receive an ^{11}C-DASB occupancy scan. The number of hours between study medication administration and PET was chosen to lie between the peak plasma concentration (median, 6–12 h, depending on dose) and the drug half-life (median, 46–64 h, depending on dose), as determined by previous clinical trials (Sunovion Pharmaceuticals, Inc., unpublished data, 2011). Because of the long half-life of SEP-225289, it is unlikely that the time between scans (or ligand order) had any effect on the measured occupancy.

Plasma concentrations of SEP-225289 were assayed from blood samples collected approximately 1 h before administration of a single dose of SEP-225289, immediately before injection of ^{11}C-PE2I and ^{11}C-DASB (~24 and 27 h after dose administration, respectively) and at the follow-up visit 7–14 d after dosing.

Image Acquisition and Analysis

MR images were acquired on a 3-T Signa Advantage system (GE Healthcare), as previously described (*25*). ^{11}C-PE2I and ^{11}C-DASB were prepared as previously described (*25,26*). PET was performed using an ECAT HR+ scanner (Siemens/CTI) (*24,25*). Injected doses for each cohort are shown in Table 1.

All images were analyzed using MATLAB (The MathWorks). Subject motion during the scan was corrected as described previously (*24*). Automatic regions of interest were obtained using nonlinear registration techniques to warp 18 previously manually outlined MR images to the target image, as described elsewhere (*26*). The mean PET image was then coregistered to the segmented MR image to apply the regions of interest to the mean PET image,

TABLE 1
Injected Doses of ^{11}C-PE2I and ^{11}C-DASB for All Cohorts

Cohort	^{11}C-PE2I injected dose		^{11}C-DASB injected dose	
	Mean ± SD	Range	Mean ± SD	Range
A	543.9 ± 138.01 (14.70 ± 3.73)	230.88–696.71 (6.24–18.83)	600.51 ± 78.44 (16.23 ± 2.12)	399.23–672.29 (10.79–18.17)
B	356.31 ± 144.3 (9.63 ± 3.90)	149.11–617.16 (4.03–16.68)	490.62 ± 193.51 (13.26 ± 5.23)	167.24–671.92 (4.52–18.16)
C	542.79 ± 179.82 (14.67 ± 4.86)	141.71–683.76 (3.83–18.48)	591.63 ± 88.06 (15.99 ± 2.38)	441.04–682.28 (11.92–18.44)

Data are in MBq, with mCi in parentheses.

as well as individual PET frames (*26,27*). Time–activity curves were generated by plotting the average activity within a region over the time course of the PET acquisition.

Appropriate modeling methods for both ^{11}C-PE2I and ^{11}C-DASB have been previously determined (*25,26*). In this study, the outcome measure used was $BP_{ND} = f_{ND}B_{avail}/K_D$, where f_{ND} is the fraction of free ligand in the reference region, B_{avail} is the density of available receptors, and K_D is the radioligand equilibrium dissociation constant (*28*). BP_{ND}, the ratio of the concentration of specifically bound to nondisplaceable ligand at equilibrium, was used in this study because it does not require arterial sampling (*28*). Although BP_{ND} measurements are potentially sensitive to changes in the fraction of free ligand in the reference region, there is no evidence to suggest that SEP-225289 would affect these values.

^{11}C-DASB BP_{ND} was calculated in the dorsal caudate, dorsal putamen, and midbrain using likelihood estimation in graphical analysis with the gray matter of the cerebellum as the reference tissue (*25*). ^{11}C-PE2I BP_{ND} was calculated in the dorsal caudate and dorsal putamen using Logan graphical analysis with the cerebellum as the reference tissue (*24*).

Occupancy Measures

Occupancy, the percentage change in ligand binding due to SEP-225289 administration, was calculated as 100% × (baseline BP_{ND} – follow-up BP_{ND})/baseline BP_{ND}. Because of variability in binding estimates, low or no transporter occupancy (which occurred only using ^{11}C-DASB) occasionally resulted in negative values of estimated occupancy. Although negative occupancy estimates are not meaningful, to prevent bias, these negative values were used in the calculation of average occupancy.

Maximal occupancy was estimated by the equation occupancy = (plasma concentration of drug × OCC_{max})/(plasma concentration of drug + IC_{50}), where OCC_{max} is the maximum occupancy and IC_{50} is the plasma concentration of drug at 50% occupancy (*29*). Values for OCC_{max} and IC_{50} were fit using a nonlinear least-squares technique in MATLAB.

Safety Monitoring

Monitoring for AEs, defined as any reaction, side effect, or other undesirable event that occurred in conjunction with the study drug, whether or not the event was considered drug-related, took place from the time informed consent was signed through the end of the study. Subjects were also monitored for serious AEs occurring between the time of informed consent and 30 d after the final dose of SEP-225289.

RESULTS

Occupancy Results

Dopamine and serotonin transporter occupancies are plotted by oral SEP-225289 dose in Figure 1. In the dorsal putamen, average SEP-225289 occupancy was 9.8 (at 8 mg), 3.7 (at 12 mg), and 3.0 (at 16 mg) times higher at the dopamine transporter than the serotonin transporter. In the dorsal caudate, average SEP-225289 occupancy was 5.6 (at 8 mg), 5.1 (at 12 mg), and 5.7 (at 16 mg) times higher at the dopamine transporter than the serotonin transporter.

The relationship between dopamine and serotonin occupancy is explored in Figure 2.

Effects of SEP-225289 on Dopamine and Serotonin Transporter Binding

Figure 3 indicates tracer binding before and after administration of SEP-225289 in the subject with the highest measured serotonin transporter occupancy. Though this subject achieved high serotonin transporter occupancy, differences in

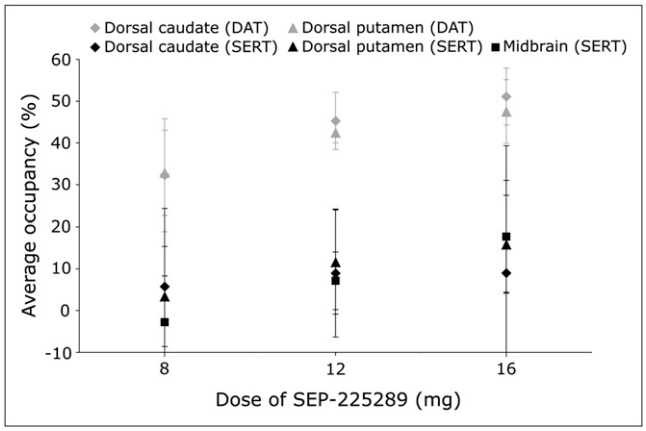

FIGURE 1. Average dopamine and serotonin transporter occupancy vs. oral dose of SEP-225289. Dopamine transporter occupancy was measured in dorsal caudate and dorsal putamen using PET with ^{11}C-PE2I. Serotonin transporter occupancy was measured in dorsal caudate, dorsal putamen, and midbrain using PET with ^{11}C-DASB. Each symbol represents mean value across all subjects in single cohort. Error bars indicate SD across cohort subjects. DAT = dopamine transporter; SERT = serotonin transporter.

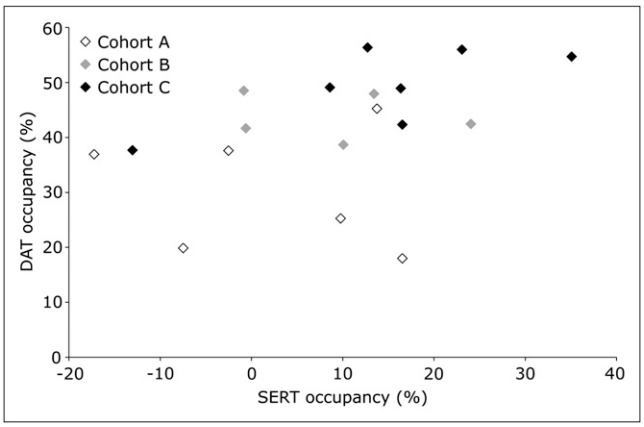

FIGURE 2. Average dopamine transporter occupancy versus average serotonin transporter occupancy. For each subject, mean dopamine transporter occupancy in dorsal caudate and dorsal putamen was calculated and plotted versus mean serotonin transporter occupancy in dorsal caudate, dorsal putamen, and midbrain. DAT = dopamine transporter; SERT = serotonin transporter.

^{11}C-DASB images before and after dosing of SEP-225289 were not as striking as ^{11}C-PE2I binding differences. These results are representative in this study, because every subject achieved higher dopamine transporter occupancy than serotonin transporter occupancy.

As expected, the 24- and 27-h plasma drug levels increased as a function of oral SEP-225289 dose, as shown in Figure 4. The R^2 values for the regression lines for the 24- and 27-h plasma levels were 0.98 and greater than 0.99, respectively.

SEP-225289 plasma levels at 24 h (except for subject S034, for whom the 27-h plasma value was used) versus average occupancy (mean of dorsal caudate and dorsal putamen occupancy), as determined from the ^{11}C-PE2I scans, are shown in Figure 5. The parameters of the best-fit curve to the data were a maximum occupancy of 84.9% and a 4.5 ng/mL drug plasma concentration at 50% occupancy (IC_{50}).

SEP-225289 plasma levels at 27 h (except for subject S034, for whom the 24-h plasma value was used) versus average occupancy (mean of dorsal caudate, dorsal putamen, and midbrain occupancy), as determined from the ^{11}C-DASB scans are shown in Figure 6. The serotonin transporter occupancy values were too low to permit the data to be fit to a curve that allows the calculation of IC_{50} or estimation of maximal occupancy.

Safety Monitoring

Eight AEs occurred in cohort A (8 mg), including nausea and emesis, elevated mood, anxiety, and insomnia. Four AEs occurred in cohort B (12 mg), including 2 subjects who reported having a headache. Twenty-three AEs occurred in the highest dose cohort, cohort C (16 mg), including headache, insomnia, nausea, diarrhea, palpitations, and dizziness. In all cohorts, all AEs believed to be related to the study medication resolved within 32 h, except for insomnia experienced by 1 subject in cohort C that lasted 8 nights. No serious AEs were reported.

DISCUSSION

In this study, PET was performed before and after administration of a single dose of SEP-225289, a potential triple monoamine reuptake inhibitor. Drug occupancy at the dopamine and serotonin transporters was assessed (norepinephrine transporter occupancy was not evaluated) within the same individual, and this occupancy was examined relative to the administered dose and plasma levels of SEP-225289. In addition, subjects were monitored for AEs and serious AEs throughout the study duration. No serious AEs occurred, and most of the AEs reported in this study were of mild or moderate severity and self-limiting.

In vitro data suggested that the functional potencies of SEP-225289 for the dopamine and serotonin transporters were similar. However, in this study, in vivo dopamine transporter occupancy was, on average, at least 3 times greater than that of serotonin transporter, and dopamine transporter

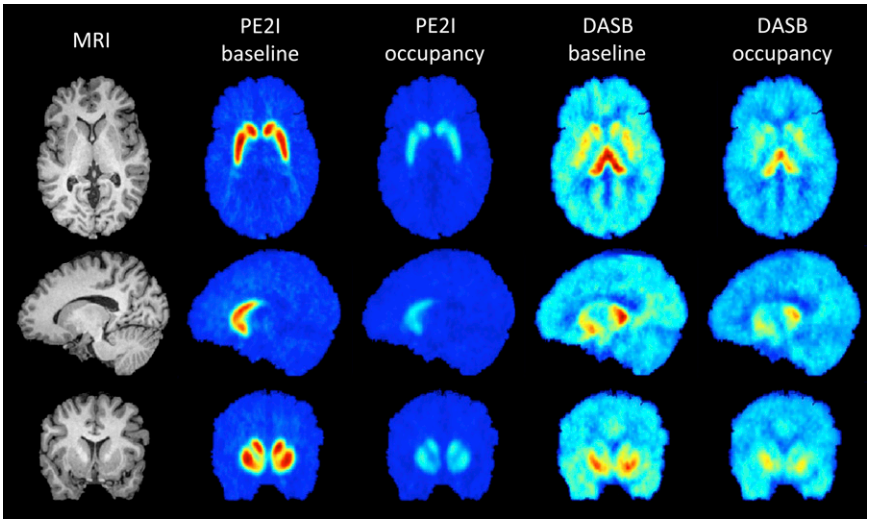

FIGURE 3. MR and mean PET images, before and after administration of SEP-225289 for the subject achieving highest serotonin transporter occupancy. Axial, sagittal, and coronal slices are shown in top, middle, and bottom rows, respectively. Left column shows MR images of subject, for anatomic reference. PET images are means of the last 6 frames of each scan (corresponding to last 60 min of scanning). Baseline scanning occurred 5–10 d before administration of SEP-225289. Difference in binding between baseline and occupancy scans is due to SEP-225289 transporter occupancy. Dopamine transporter occupancy in this subject (55%, as indicated by PE2I PET scans) was markedly higher than serotonin transporter occupancy (35%, as indicated by the DASB PET scans).

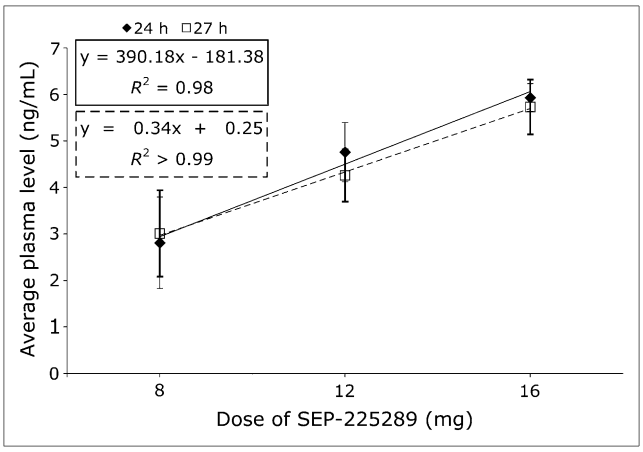

FIGURE 4. SEP-225289 plasma levels vs. dose. Plasma level was averaged over all subjects in each cohort. Error bars indicate SD across cohort subjects.

occupancy exceeded the serotonin transporter occupancy for every subject for whom both were measured. In addition, Figure 2 indicates little correlation between average dopamine and serotonin transporter occupancies. Although in vitro assays are an important first step in determining whether a compound engages its target, because of disparities in temperature, pH, concentration, and other conditions, in vitro affinity may not predict in vivo occupancy. Also, of critical importance is determining the relatively narrow therapeutic dose range for these compounds, which is based on tolerability and therefore not easily predicted from in vitro models. For these reasons, many consider in vivo occupancy studies more useful in predicting a drug's therapeutic potential than in vitro measures.

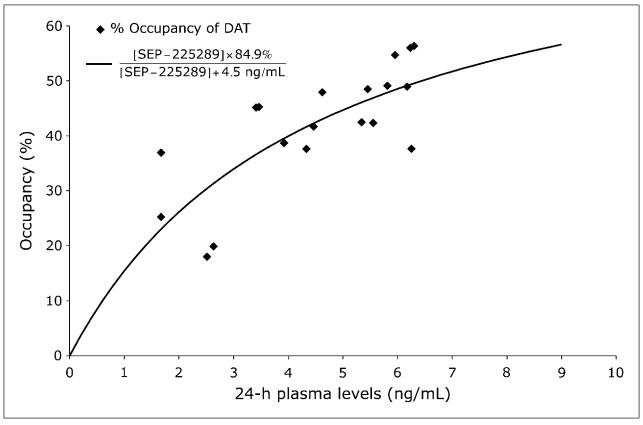

FIGURE 5. Dopamine transporter occupancy vs. plasma level. Percentage occupancy of dopamine transporter as measured with ^{11}C-PE2I PET images is plotted. Curve was fit to data using a nonlinear least-squares method. Parameters for best-fit curve were maximum occupancy of 84.9% and 4.5 ng/mL drug plasma concentration at 50% occupancy. These parameters are indicated in equation of fitted curve shown on top left corner. For 1 subject (S034), 27-h plasma level was used, because this subject received ^{11}C-PE2I scan at approximately 27 h after dose administration. DAT = dopamine transporter.

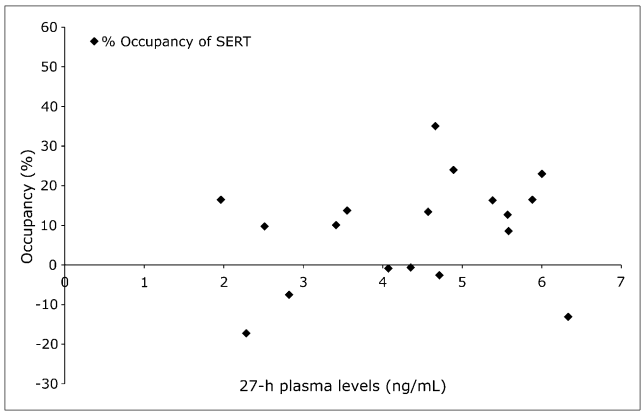

FIGURE 6. Serotonin transporter occupancy vs. plasma level. Percentage occupancy of serotonin transporter as measured with ^{11}C-DASB PET images is plotted. Negative values occur because of measurement noise. For 1 subject (S034), 24-h plasma level was used, because this subject received ^{11}C-DASB scan at approximately 24 h after dose administration. SERT = serotonin transporter.

PET occupancy studies can also provide estimations of drug IC_{50} and extrapolation of maximal occupancy, based on fitted occupancy versus plasma curves (*29*). Mean serotonin transporter occupancy across all cohorts based on ^{11}C-DASB PET scans was 8.8%, and 6 subjects (3 receiving 8 mg, 2 receiving 12 mg, and 1 receiving 16 mg of SEP-225289) attained less than 1% serotonin transporter occupancy, on average. These values were therefore too low for meaningful curve fits. However, the dopamine transporter occupancy as measured by ^{11}C-PE2I was significant. Dopamine transporter occupancies were as high as 56%, allowing the calculation of SEP-225289 plasma concentration at 50% dopamine transporter occupancy (4.5 ng/mL) and an extrapolation of maximal occupancy (85%). Because maximal occupancy is an extrapolation based on current data, this value will need to be verified by in vivo measurements of SEP-225289 occupancy and plasma concentrations at higher doses.

Although SEP-225289 was originally developed as a triple–monoamine reuptake inhibitor based on promising in vitro data, this PET occupancy study suggests that the serotonin transporter occupancy of SEP-225289 is too low to produce an antidepressant effect at these exposures. Even though 3 subjects achieved serotonin transporter occupancies greater than 20%, all serotonin transporter occupancies were well below the 80% threshold established for clinical effectiveness of selective serotonin reuptake inhibitors (*14*). Because of its high dopamine transporter occupancy, however, SEP-225289 is a potentially clinically effective dopamine reuptake inhibitor. Although the therapeutic threshold for dopamine transporter occupancy remains unclear, the dopamine transporter occupancy of SEP-225289 in this study is well above occupancy of bupropion (<22%) (*18*). Additionally, depending on its norepinephrine transporter occupancy, SEP-225289 may be an effective norepinephrine–dopamine reuptake inhibitor.

CONCLUSION

The in vivo target occupancy of novel therapeutics is best assessed early in a development program to provide clinical direction. PET allows the calculation of this target occupancy directly in vivo and augments in vitro, ex vivo, and other preclinical data.

This PET study used a within-subject design to investigate the differential binding of a new drug, SEP-225289, to dopamine and serotonin transporters. Despite similar in vitro potencies, the in vivo occupancies of SEP-225289 at serotonin and dopamine transporters were significantly different, as has been previously observed with other drugs. This study demonstrates the advantages of in vivo occupancy evaluations and the potential contributions of PET to this and similar development programs.

DISCLOSURE STATEMENT

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