Preclinical to Clinical Translation of CNS Transporter Occupancy of TD-9855, a Novel Norepinephrine and Serotonin Reuptake Inhibitor

Jacqueline AM Smith, PhD; DL Patil, PhD; OT Daniels, MD; Y-S Ding, PhD; J-D Gallezot, PhD; S Henry, BS; KHS Kim, MD, PhD; S Kshirsagar, PhD; WJ Martin, PhD; GP Obedencio, BS; E Stangeland, PhD; PR Tsuruda, PhD; W Williams, MD; RE Carson, PhD; ST Patil, MD, PhD

Theravance Biopharma US, Inc., San Francisco, CA (Drs Smith, Bourdet, Daniels, Kim, Kshirsagar, Martin, Obedencio, Stangeland, Tsururda, Williams, and Patil); Yale School of Medicine, New Haven, CT (Drs Ding, Gallezot, Henry, Williams, and Carson).

Correspondence: Jacqueline A Smith, PhD, Theravance Biopharma US, Inc., 901 Gateway Blvd., San Francisco, CA 94080 (jsmith@theravance.com).

Abstract

Background: Monoamine reuptake inhibitors exhibit unique clinical profiles that reflect distinct engagement of the central nervous system (CNS) transporters.

Methods: We used a translational strategy, including rodent pharmacokinetic/pharmacodynamic modeling and positron emission tomography (PET) imaging in humans, to establish the transporter profile of TD-9855, a novel norepinephrine and serotonin reuptake inhibitor.

Results: TD-9855 was a potent inhibitor of norepinephrine (NE) and serotonin 5-HT uptake in vitro with an inhibitory selectivity of 4- to 10-fold for NE at human and rat transporters. TD-9855 engaged norepinephrine transporters (NET) and serotonin transporters (SERT) in rat spinal cord, with a plasma EC_{50} of 11.7 ng/mL and 50.8 ng/mL, respectively, consistent with modest selectivity for NET in vivo. Accounting for species differences in protein binding, the projected human NET and SERT plasma EC_{50} values were 5.5 ng/mL and 23.9 ng/mL, respectively. A single-dose, open-label PET study (4–20 mg TD-9855, oral) was conducted in eight healthy males using the radiotracers [^{11}C]-3-amino-4-[2-[(di(methyl)amino)methyl]phenyl]sulfanylbenzonitrile for SERT and [^{11}C]-([S,S]-methylreboxetine for NET. The long pharmacokinetic half-life (30–40 h) of TD-9855 allowed for sequential assessment of SERT and NET occupancy in the same subject. The plasma EC_{50} for NET was estimated to be 1.21 ng/mL, and at doses of greater than 4 mg the projected steady-state NET occupancy is high (>75%). After a single oral dose of 20 mg, SERT occupancy was 25 (±8)% at a plasma level of 6.35 ng/mL.

Conclusions: These data establish the CNS penetration and transporter profile of TD-9855 and inform the selection of potential doses for future clinical evaluation.

Keywords: norepinephrine and serotonin transporter, pain, PET, TD-9855
Introduction

Dose selection for central nervous system (CNS)-based therapeutics is guided by many factors, including target engagement and the underlying pathophysiology of the disease of interest. For monoamine reuptake inhibitors, where unique clinical profiles emerge from distinct CNS monoamine transporter engagement, the challenge of optimal dose selection is amplified. TD-9855 is an investigational dual norepinephrine (NE) and serotonin (5-HT) reuptake inhibitor with selectivity for NE over 5-HT (NSRI). Dual monoamine reuptake inhibitors offer an opportunity to relieve core symptoms and associated comorbidities across a spectrum of CNS disorders by differential regulation of NE and 5-HT. Here, we used a translational strategy that included rodent pharmacokinetic/pharmacodynamic (PK/PD) modeling and positron emission tomography (PET) imaging in humans to establish the profile of TD-9855. The systematic integration of in vitro and in vivo preclinical data with clinical PET imaging data can provide a quantitative assessment of the CNS transporter profile for monoamine reuptake inhibitors. Since differential transporter engagement may confer therapeutic benefit in different patient populations, our objectives were to confirm central penetration and to inform selection of potential doses of TD-9855 for clinical evaluation.

Central NE levels influence a wide range of neurobiological functions, including mood, attention, and pain (Millan, 2002; Berridge and Waterhouse, 2003; Robbins and Arnsten, 2009; Del et al., 2011; Hamon and Blier, 2013). The multiple actions of NE underscore the diverse therapeutic benefits of NE reuptake inhibitors, which are approved to manage conditions such as pain, like anxiety and depression. Duloxetine has significant serotonergic activity, exhibiting at least 10-fold selectivity for inhibition of serotonin (SERT) over norepinephrine (NET). At the 60 mg dose, duloxetine exhibits >80% occupancy of NET and serotonin (5-HT) reuptake inhibitor with selectivity for NE over 5-HT (NSRI). Dual monoamine reuptake inhibitors offer an opportunity to relieve core symptoms and associated comorbidities across a spectrum of CNS disorders by differential regulation of NE and 5-HT. Here, we used a translational strategy that included rodent pharmacokinetic/pharmacodynamic (PK/PD) modeling and positron emission tomography (PET) imaging in humans to establish the profile of TD-9855. The systematic integration of in vitro and in vivo preclinical data with clinical PET imaging data can provide a quantitative assessment of the CNS transporter profile for monoamine reuptake inhibitors. Since differential transporter engagement may confer therapeutic benefit in different patient populations, our objectives were to confirm central penetration and to inform selection of potential doses of TD-9855 for clinical evaluation.

By its very nature, pain represents a multidimensional experience in which emotional and cognitive dimensions converge with sensory elements to signal actual or potential tissue damage (Neugebauer et al., 2009). Norepinephrine and 5-HT are major components of the descending pain inhibitory control system that runs from the brain stem to the spinal cord (Basbaum and Fields, 1984). In the setting of chronic pain, central modulatory pathways may become dysregulated, yielding altered or reduced levels of NE and 5-HT at supraspinal and/or spinal levels. Dual 5-HT and NE reuptake inhibitors, such as duloxetine (Cymbalta®), are approved for the management of chronic pain conditions as well as for conditions that can be co-morbid with pain, like anxiety and depression. Duloxetine has significant serotonergic activity, exhibiting at least 10-fold selectivity in vitro for inhibition of serotonin (SERT) over norepinephrine transporters (NET). At the 60 mg dose, duloxetine exhibits >80% occupancy of SERT (Takano et al., 2006). Milnacipran (Savella®) is a dual reuptake inhibitor with more apparent noradrenergic activity than duloxetine, but likely insufficient selectivity between NET and SERT to enable differential engagement of each transporter (Tsursuda et al., 2010; Nogami et al., 2013). NE reuptake inhibitors exhibit efficacy in chronic pain but, in contrast, selective 5-HT reuptake inhibitors (SSRIs) do not produce a primary analgesic effect (Max et al., 1992; Fishbain et al., 2000; Finnerup et al., 2005; Atkinson et al., 2007; Verdu et al., 2008; Arnold et al., 2012). SSRIs can augment the activity of a selective NE reuptake inhibitor (Fishbain et al., 2000; Iyengar et al., 2004; Finnerup et al., 2005; Hall et al., 2011), and we hypothesize that an inhibitor with modest selectivity for NET instead would offer the potential for robust pain relief while minimizing any putative serotonergic side effects such as nausea, somnolence, fatigue, and sexual dysfunction (Papakostas, 2008).

Methods

Animals

Adult male Sprague Dawley rats (Charles River) were housed under controlled laboratory conditions (temperature at 21 ± 1°C) on a 12:12 hour light-dark cycle. Animals were given free access to food and water upon arrival to the facility and animals were acclimatized to their holding room for at least 48 hours. Animals were fasted but allowed free access to water for 15–18 hours prior to dosing. All animal experiments were approved by the Institutional Animal Care and Use Committee at Theravance Biopharma US, Inc.

Equilibrium Radioligand Binding and [3H]-Neurotransmitter Uptake

The in vitro pharmacology of TD-9855 (4-[2-(2,4,6-trifluorophenoxy)methyl]phenyl)piperidine; Figure 1) at human recombinant and rat native monoamine transporters was characterized as described previously (Tsursuda et al., 2010; Shen et al., 2013). Radioligands were sourced commercially (Perkin Elmer Life Sciences or GE Healthcare Life Sciences). Briefly, membranes prepared from HEK293 (Human Embryonic Kidney 293) cells stably-transfected with human recombinant SERT (HEK293-hSERT), NET...
(HEK293-hNET), or DAT (CHO-K1-hDAT) were incubated for 1 hr at 22°C in the absence, or presence, of TD-9855 and [3H]-citalopram (1.0 nM) for SERT, [3H]-nisoxetine (2.0 nM) for NET, and [3H]-WIN35428 (3.0 nM) for DAT in 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 0.025% BSA, 100 μM ascorbic acid, pH 7.4. Rat cortical membrane preparations were incubated with [3H]-citalopram (2.0 nM) for SERT or [3H]-nisoxetine (4.0 nM) for NET for 1 hr at 22°C. In neurotransmitter uptake assays HEK293-hSERT, hNET, or hDAT cells, respectively, were pre-incubated for 30 min at 37°C in the absence, or presence, of TD-9855 in 7.5 mM HEPES, 12.5 mM Tris–HCl, 2.2 mM Na-phosphate, 120 mM NaCl, 5 mM KCl, 0.4 mM MgCl2, 7.5 mM glucose, 1.7 mM CaCl2, 250 μM ascorbic acid, 150 μM pargyline, 0.025% BSA, pH 7.4 prior to incubation with [3H]-5-HT (20 nM), [3H]-NE (40 nM), or [3H]-DA (100 nM) for 10 min. Rat cortical synaptosomes were incubated with [3H]-5-HT or [3H]-NE for 6 min and striatal synaptosomes with [3H]-DA for 6 min. Binding and uptake assays were terminated by rapid filtration and radioactivity, determined by liquid scintillation spectroscopy.

Final [3H]-neurotransmitter concentrations were significantly below the respective Km such that pIC50 approximated the absence, or presence, of TD-9855 using the dual-tracer evaluation, subjects completed sequential [11C]-DASB and [11C]-MRB baseline scans with each tracer, sequential TD-9855, and a follow-up PET scan on or between Days 3 and 7. In the dual-tracer evaluation, subjects completed the first PET scan at baseline, a second PET scan on Day 1 from 7–10 hr (corresponding to Tmax; range 6 to 12 hours; in the ascending single dose studies; Theravance Biopharma US, Inc., data on file) following dosing of TD-9855, and a follow-up PET scan on or between Days 3 and 7. In the dual-tracer evaluation, subjects completed sequential [11C]-DASB and [11C]-MRB baseline scans with each tracer, sequential post-dose scans with each tracer at 7–10 hr post-dose, and one follow-up scan with [11C]-MRB on or between Days 3 and 7. Each subject received one dose of TD-9855, a maximum of 75 milligrams (mg) of radioactivity, and five injections. Only healthy male subjects (age-range of 18–45) were eligible to participate. Key exclusion criteria included no prior exposure to monoamine reuptake inhibitors or stimulants or a history of smoking. Subjects with magnetic resonance imaging (MRI)-incompatible implants and other contraindications for MRI were also excluded.

All adverse events (AEs) were assessed by the investigator and recorded in the case report form, including the dates of onset and resolution, severity, relationship to study drug, outcome, and action taken with study medication after the dosing of TD-9855 through the follow-up visit. Safety was assessed during the entire study by measurement of vital signs, laboratory tests, and evaluation of ECGs.

**PET Imaging and Parameters**

The injected dose of both tracers was typically 14–15 mCi, with mass doses less than 10 μg in all cases. PET images were...
acquired using the High Resolution Research Tomograph (HRRT; Siemens/CTI) with a reconstructed image resolution of approximately 3 mm. Following a transmission scan, \(^{[1]}\text{C}\)-MRB or \(^{[1]}\text{C}\)-DASB was injected intravenously as a 1 min bolus by an infusion pump. List-mode data were acquired for 120 min. Head motion correction was performed using an optical tracking tool (Vicra, NDI Systems) and a rigid tool attached to a swim cap.

Dynamic scan data were reconstructed with corrections for attenuation, normalization, scatter, randoms, dead time, and motion using the MOLAR algorithm (motion-compensation ordered subset-expectation maximization list-mode algorithm for resolution recovery reconstruction) with the following frame motion using the MOLAR algorithm (motion-compensation ordered subset-expectation maximization list-mode algorithm for resolution recovery reconstruction) with the following frame timing: 6 x 30 sec; 3 x 1 min; 2 x 2 min; 22 x 5 min.

**Plasma PK Measurements**

Four 6 mL samples were drawn at 2, 4, 6, and 24 hr post TD-9855 dosing. Three additional 6 mL samples were drawn prior to each PET scan and approximately 60 and 120 min after the beginning of each PET scan. Plasma samples were analyzed for TD-9855 concentrations by a validated LC-MS/MS method. The lower limit of quantification for TD-9855 was 0.05 ng/mL.

**Image Analysis**

The occipital cortex was selected as the reference region for \(^{[1]}\text{C}\)-MRB due to the low density of NET measured in vitro (Schou et al., 2005) and the absence of specific binding in a previous study (Hannestad et al., 2010). The regions of interest (ROIs) selected to assess \(^{[1]}\text{C}\)-MRB specific binding included one cortical region and the paracentral lobule, thalamus, hypothalamus, locus ceruleus, nucleus rubra, and raphe nucleus. The thalamus and cortical ROIs were delineated using the Automated Anatomical Labeling (AAL) template (Tzourio-Mazoyer et al., 2002). The hypothalamus, locus ceruleus, nucleus rubra, and raphe nucleus ROIs were drawn on the template MR image, based on the AAL template (Hannestad et al., 2010).

For \(^{[1]}\text{C}\)-DASB, the cerebellum was used as the reference region. The regions of interest selected to assess \(^{[1]}\text{C}\)-DASB specific binding were the caudate, putamen, amygdala, and thalamus. All regions were delineated using the AAL template (Tzourio-Mazoyer et al., 2002).

**MR and PET Image Processing**

In order to apply the ROIs defined on the template MR image to the PET images to compute regional time-activity curves (TACs), two geometric transforms were estimated:

1) One nonlinear deformation field was estimated between the template MR image and each subject’s MR image (3D MPRAGE) to account for inter-subject anatomical variability. The non-linear deformation was estimated using the BioImage Suite software Version 2.5 (http://www.bioimagesuite.org/).

2) One rigid linear coregistration matrix was estimated between the PET images to compute regional time-activity curves (TACs), with weights equal to 1/SE\(^{-2}\). For \(^{[1]}\text{C}\)-MRB regional BP\(_{\text{ND}}\) values were computed using the multilinear reference tissue model 2 (MRTM2; Ichise et al., 2003). The suitability of MRTM2 to estimate \(^{[1]}\text{C}\)-MRB BP\(_{\text{ND}}\) values was evaluated in a previous study (Hannestad et al., 2010), in which it was shown that MRTM2 BP\(_{\text{ND}}\) estimates are highly correlated with BP\(_{\text{ND}}\) estimated with arterial blood sampling and the multilinear analysis \(y = 0.909 x + 0.078, r^2 = 0.821\), where \(x\) represents the MA1 BP\(_{\text{ND}}\) values and \(y\) represents the MRTM2 BP\(_{\text{ND}}\) values. BP\(_{\text{ND}}\) values and their associated standard errors (SE\(_{\text{BP}_{\text{ND}}} \)) for each ROI were then estimated by performing fits of the regional TACs. Data were fitted starting at time \(t^* = 20\) min. For \(^{[1]}\text{C}\)-DASB, regional BP\(_{\text{ND}}\) values were computed using simplified reference tissue model 2 (SRTM2; Wu and Carson, 2002). SRTM2 is similar to MRTM2, except that it uses the full data set, while MRTM2 uses the period of time \(t > t^*\). When applied to single-voxel TACs, these fits provided the BP\(_{\text{ND}}\) images. Before computing parametric images, the original dynamic images were smoothed with a Gaussian filter with a full width at half maximum of 3 voxels (3.7 mm).

**Computation of Apparent Occupancy**

Apparent occupancy values (r\(_{\text{app}}\)) for individual ROIs and their associated standard errors (SE\(_{\text{app}}\)) were computed for each PET scan after administration of TD-9855 as follows:

\[
r_{\text{app}}(\%) = 100 \times \left(1 - \frac{BP_{\text{ND}}}{BP_{\text{ND}}^\text{baseline}}\right)
\]

and

\[
SE_{\text{app}} = 100 \times \sqrt{SE_{\text{BP}_{\text{ND}}}^2 + \left(\frac{SE_{\text{BP}_{\text{ND}}^\text{baseline}}}{BP_{\text{ND}}^\text{baseline}}\right)^2}
\]

A whole-brain apparent occupancy (r\(_{\text{app}}\)) was computed for each post-drug scan as the weighted average of the regional apparent occupancies:

\[
r_{\text{app}} = \sum_{\text{ROI}} r_{\text{app}} / SE_{\text{app}}^2 + \sum_{\text{ROI}} 1 / SE_{\text{app}}^2
\]

The SE\(_{\text{app}}\) of the r\(_{\text{app}}\) was computed using the error propagation equation (Bevington and Robinson, 2014) as follows:

\[
SE_{\text{app}} = \sqrt{\frac{1}{\sum SE_{\text{app}}^2}}
\]

The EC\(_{50}\) of TD-9855 for NET was estimated by fitting the r\(_{\text{app}}\) values for \(^{[1]}\text{C}\)-MRB, with weights equal to 1/SE\(_{\text{app}}^2\), versus the average plasma concentration of TD-9855 during the scan (C) with a two-parameter model, including the drug EC\(_{50}\) and the tracer maximal apparent occupancy (r\(_{\text{max}}\)):

\[
r_{\text{app}}(\%) = r_{\text{max}} \left(\frac{C}{C + EC_{50}}\right)
\]

Note that either simultaneously fitting all the regional occupancy estimates with weights equal to 1/SE\(_{\text{app}}^2\) or fitting the r\(_{\text{app}}\) estimates with weights equal to 1/SE\(_{\text{app}}^2\) leads to the same EC\(_{50}\), and r\(_{\text{max}}\) estimates up to a constant offset, since the cost functions are identical.

Theoretical maximal occupancy should be 100%; however, to account for the effects of small regional differences in nonspecific binding, the variable r\(_{\text{max}}\) was included. Using the estimate of the
[11C]-MRB $r_{\text{max}}$, the final normalized occupancy estimates for each scan were obtained as $r = \frac{r_{\text{app}}}{r_{\text{max}}}$. The maximal apparent occupancy ($r_{\text{app}}$) would be 100% if the non-displaceable binding were the same in the reference and target tissues. However, it would be greater than 100% if the non-displaceable binding were lower in the target region, and less than 100% if the non-displaceable binding were higher in the target region, relative to the reference region. This sensitivity of $r_{\text{app}}$ to differences in non-displaceable binding is more significant for tracers with low specific binding, such as [11C]-MRB.

The TD-9855 estimated EC$_{50}$ value and the associated standard error obtained from the fit of the occupancy estimates as a function of the plasma concentration were determined.

**Transporter Occupancy Projections after Repeated TD-9855 Administration**

NET occupancy was simulated after repeated TD-9855 administration using a population PK model based upon the PK of TD-9855 observed in single and multiple dose clinical trials (Theravance, data on file). Steady-state TD-9855 plasma concentration versus time profiles were simulated for dose levels of 4mg and 20mg and used in conjunction with the NET occupancy versus plasma concentration model estimates (using $r_{\text{max}} = 100\%$ and EC$_{50} = 1.21 \text{ ng/mL}$) to derive the mean (95% CI) NET occupancy at steady state.

**Results**

**In Vitro Pharmacological Profile of TD-9855**

The in vitro pharmacological profile of TD-9855 was similar at human and rodent monoamine transporters SERT (Table 1). Radiolabelled neurotransmitter uptake studies using cell lines expressing human NET, SERT, and DAT demonstrated that TD-9855 is a potent inhibitor of NET and SERT, but not DAT, with 4-fold higher potency for inhibition of NET over SERT. Similarly, TD-9855 is a potent inhibitor of both [3H]-NE and [3H]-5-HT uptake into rat cortical synaptosomes, with an apparent functional selectivity (10-fold) for NET over SERT, similar to that observed at human transporters. Consistent with the functional inhibition studies, TD-9855 exhibited a high affinity for binding to human NET and SERT, but not DAT (Table 1). Apparent binding affinity values for rat-native NET and SERT in membranes prepared from rat cortices were similar (overlapping confidence intervals) to the corresponding values at human transporters, consistent with a lack of species dependence (Table 1).

**Rat PK/PD Modeling**

The relationship between TD-9855 plasma concentration and NET and SERT central occupancy in rats is presented in Figure 2. Data from all dose levels were pooled in this analysis and depicted by the post-dose time-point, at which the occupancy and plasma concentration were measured irrespective of dose level. A PK/PD model of the time course of plasma concentration and NET/SERT occupancy over time was constructed. The model consisted of a one-compartment oral absorption pharmacokinetic model linked directly to an effect compartment sigmoidal Emax model. PK and PD parameter estimates derived from the effect compartment PK/PD analysis for NET and SERT occupancy are presented in Table 2. The estimated EC$_{50}$ for occupancy was 11.7 ng/mL for NET and 50.8 ng/mL for SERT in rat spinal cords. Accounting for species differences in plasma protein binding (90.2% and 79.1% in rat and human, respectively; Theravance Biopharma US, Inc., data on file), the projected human plasma EC$_{50}$ values were 5.5 ng/mL for NET and 23.9 ng/mL for SERT.

**Phase 1 Human Positron Emission Study**

A total of eight subjects completed the study. The study participants were healthy males, aged 29±5 years, and from diverse...
ethnic and racial backgrounds. Five of the study participants received a single tracer (\([^{11}C]\)-MRB) and three of the study participants received two tracers (\([^{11}C]\)-MRB and \([^{11}C]\)-DASB). Orally administered doses of TD-9855 were 4 mg (n = 3), 10 mg (n = 1), and 20 mg (n = 4). The projected human plasma EC50 values from the rat PK/PD modeling were used to select doses that would provide a range of NET occupancy in human CNS.

Average NET \(BP_{ND}\) images at the level of the thalamus are shown at baseline and at 9.5–11.5 hr, post-administration of either 4 mg or 20 mg of TD-9855, respectively (Figure 3). \([^{11}C]\)-MRB \(BP_{ND}\) for all subjects, in the selected ROIs, are reported in Supplementary Table S1. The regional average of the \(BP_{ND}\) images are highly correlated with the \(BP_{ND}\) values estimated from the analysis of the corresponding regional TACs, except in the locus ceruleus, where the smoothing applied to the dynamic images prior to parametric image computation introduces an underestimation of \(BP_{ND}\) values in this small ROI: regression line equations are \(y = 0.927 x + 0.026, r^2 = 0.964\) for all ROIs except the locus ceruleus, with ROI TAC fit results on the x-axis and parametric images results on the y axis. Time activity curves for the thalamus and occipital cortex, at baseline and the 20 mg dose, are shown in Supplementary Figure S1. Apparent NET occupancy, computed from \(BP_{ND}\) values and their standard errors from regional TAC fits, increased dose-dependently (Table 3). The relationship between estimated NET occupancy \(r_{App}\), estimated across several NET-rich brain regions and corrected for the maximal apparent occupancy (see Methods), and mean plasma concentration of TD-9855 during each post-dose PET scan is depicted in Figure 4. The best fit to the occupancy model indicated an EC50 of 1.21 ± 0.21 ng/mL. Simulation of the NET occupancy after repeated TD-9855 administration (4 mg and 20 mg) is presented in Figure 5. Repeat-dose simulations were based on a TD-9855 population PK model and the estimates of NET EC50 determined after single doses of TD-9855. Due to the long plasma \(t_{1/2}\) of TD-9855 (30–40 hr), accumulation of TD-9855 is observed over time in plasma and thus NET occupancy after repeated administration is higher than that observed after a single dose. Estimates of the NET maximal and trough mean occupancies at steady state indicate a greater than 75% occupancy for the 4 mg dose. Both maximal and trough mean occupancies are predicted to exceed 95% at steady state for the 20 mg dose. Corresponding data for all three dose levels (4, 10, and 20 mg) are presented in Table 4. These estimates indicate that minimal (<10%) variation in peak and trough mean NET occupancy would be anticipated with repeated TD-9855 administration.

Average SERT binding potential images for \([^{11}C]\)-DASB at baseline and at 7.5–9.5 hr post-administration of 20 mg of TD-9855 are shown in Figure 6. \([^{11}C]\)-DASB \(BP_{ND}\) in the selected ROIs are reported in Supplementary Table S2. The average SERT occupancy

| Parameter | \(E_{max}\) (% Occupancy) | EC50 (ng/mL) | \(k_o\) (hr\(^{-1}\)) | \(K01\) (hr\(^{-1}\)) | \(K10\) (hr\(^{-1}\)) | V/F (L/kg) |
|-----------|---------------------------|-------------|----------------|----------------|----------------|-------------|
| SERT      | 79.0 (53)                 | 50.8 (87)   | 11.0 (86)      | 0.777 (108)    | 0.319 (81)     | 54.8 (66)   |
| NET       | 92.0 (19)                 | 11.7 (6.8)  | 1.78 (57)      |                |                |             |

Final parameter estimates are listed with the coefficient of variation (% CV) on each parameter estimate provided in parentheses.
Due to low levels of SERT occupancy (15–30%) at the single dose of 20 mg and the limited number of available data points (n = 3), estimation of an EC50 value for SERT would be subject to significant uncertainty and estimation was not attempted.

Overall, TD-9855 treatment was well tolerated. No deaths, severe AEs, or AEs occurred during the study. No clinically significant abnormalities were noted with respect to the clinical laboratory values, physical examination findings, vital sign measurements, or ECG results.

Discussion

This study describes the application of a preclinical to clinical translational strategy to the development of a novel CNS-penetrant NSRI, TD-9855. The results described establish the functional selectivity of TD-9855 for NET over SERT in both in vitro and in vivo preclinical models and confirm this profile using PET imaging of CNS transporter occupancy in humans. The sequential assessment of transporter occupancy in the same human subjects provided an explicit translation of the selectivity profile into the clinical setting. Collectively, these data establish a relationship between monoamine transporter occupancy and TD-9855 dose. Low doses of TD-9855 should preferentially engage NET, whereas higher doses can yield dual inhibition of NET and SERT. Differential target engagement can inform clinical evaluation in patients with CNS disorders that have distinct underlying mechanisms. To our knowledge a similar translational approach has not been used in the development of single or dual NE and/or 5-HT reuptake inhibitors, although such approaches have been reported in the discovery of other potential CNS therapeutics (Chang et al., 2011).

The modest functional selectivity of TD-9855 for human NE transporters in vitro differentiates it from many other reuptake inhibitors with NE transporter activity. For example, duloxetine and venlafaxine exhibit greater than 10-fold functional selectivity for 5-HT, whereas atomoxetine and esreboxetine exhibit high (≥20- and ≥10 000-fold, respectively) functional selectivity for NE (Wu et al., 2008; Tsuruda et al., 2010). TD-9855 exhibited high affinity binding to both human SERT and NET, although there was a small reduction in apparent NET selectivity between the functional and binding assays, as has been reported previously (Vaishnavi et al., 2004). Assay-dependent differences in transporter potency and binding affinity for monoamine reuptake inhibitors.
inhibitors in vitro are frequently observed, although the mechanisms underlying these are unclear (Tsuruda et al., 2010).

TD-9855 demonstrated in vivo selectivity for NET in the ex vivo evaluation of transporter occupancy, consistent with the NET selectivity observed in the rat in vitro pharmacology studies. The estimates of the equilibration rate constant ($k_{eq}$) were relatively large, indicating a rapid equilibration for TD-9855 within the CNS biophase (CNS equilibration $t_{1/2}$ of ~0.4 hr for NET and ~0.06 hr for SERT). The transporter binding kinetics for TD-9855 are rapid at human NET and SERT in vitro. Association and dissociation rate constants at NET were 0.055 nM^{-1}min^{-1} and 0.069 min^{-1}, respectively, and were not determined at SERT as the binding kinetics were too rapid. These results are consistent with a profile of rapid brain entry and transporter binding kinetics for TD-9855 in rats. Therefore, the minor hysteresis suggested by the time course of NET occupancy versus TD-9855 plasma concentration (i.e., occupancy measured at the 0.5 hr time point was slightly lower than that observed at similar plasma concentrations at ≥2 hr post dose) cannot be explained by significantly-delayed entry of TD-9855 to the brain or slow transporter binding on/off rates (Yassen et al., 2005). It is possible that the observed hysteresis in rats reflects minor delays in the initial brain entry or the time required for distributional equilibrium within the CNS for TD-9855.

Similar to the rat PK/PD studies, the TD-9855 PET study was designed to estimate the CNS occupancy of NET and/or SERT at various time-points post dose. The advantage of this design over single-time point designs (e.g., scanning at the time of maximal plasma concentration) is the ability to evaluate the kinetics of occupancy over time, assess hysteresis in the relationship between occupancy and plasma concentration, and provide confidence in multiple-dose projections of occupancy from a single-dose PET study (Abanades et al., 2011). We observed no significant hysteresis in the relationship between NET occupancy and TD-9855 plasma concentration: i.e., the relationship established on Day 1 was similar to that observed after scanning at time points post-Day 1. Although it is possible that a larger dataset would have yielded the minor hysteresis effect we observed in rats, the pharmacokinetic profile is consistent with rapid penetration of TD-9855 into the human CNS, as would be anticipated for a non-P-gp substrate with high intrinsic permeability properties (Theravance Biopharma US, Inc., data on file).

In humans, the plasma EC$_{50}$ for NET was slightly lower than that estimated from the translational rat PK/PD model (1.21 ng/mL versus 5.5 ng/mL), implying that NET occupancies were higher in humans than predicted from the preclinical model. Species differences in the in vitro binding to rat and human plasma proteins were taken into consideration when translating the rat PK/occupancy relationship to humans; however, these may not be representative of in vivo plasma/protein binding differences. No differences in transporter affinity were assumed during the translation, as NET binding affinity was similar between rat and human under comparable in vitro conditions. Furthermore, the functional inhibition profile of TD-9855 in vitro was not significantly different between species, and similar pharmacological inhibition would be anticipated in humans and rats in vivo.

A key limitation of the current study is that exploration of the SERT occupancy versus plasma concentration relationship was incomplete and an EC$_{50}$ value could not be reliably determined. However, the sequential assessment of transporter occupancy in the same human subjects provided unequivocal evidence of selectivity for NET over SERT after a 20 mg single dose. Preclinical PK/PD modeling predicts ~62% and ~26% occupancy at NET and SERT, respectively, following a single dose of 20 mg. SERT occupancy following repeated administration of a 20 mg dose in humans is predicted to be higher than that observed after a single dose, given the long plasma half-life (30–40 hr) and approximately 3- to 4-fold accumulation. The preclinical PK/PD model predicts ~60% SERT occupancy at 20 mg after repeated dosing. To determine the SERT EC$_{50}$, a PET study incorporating either a higher single dose or repeated dose administration would be needed.

Mechanism-based PK/PD approaches, as used in the development of the current PK/PD models for TD-9855, have been applied in other transporter occupancy studies (Abanades et al., 2011; Bourdet et al., 2012). These approaches provide a description of the full time course of drug concentration in the plasma as it relates to the time course of changing transporter occupancy in the CNS. This allows for a more accurate projection of multiple-dose occupancy from single-dose preclinical and/or clinical PET studies. These approaches are particularly important for compounds that demonstrate some delay in distribution between the plasma and CNS or slow transporter binding
kinetics. An important objective of the PET study was to support dose selection for further clinical development. Multiple factors must be taken into consideration when selecting doses for clinical evaluation, including the doses that will appropriately engage the target(s) as well as the mechanistic basis of the disorder of interest. Dual-monoamine reuptake inhibitors that target multidimensional CNS disorders amplify the challenge of dose selection, because differential target engagement may confer distinct benefits in different CNS disorders and/or comorbidities. Monoamine transporter occupancy studies have been used to understand the relationship between neurotransmitter systems in a disease state and the therapeutic efficacy observed in patients. For example, it is generally accepted that for clinical efficacy of SSRIs in MDD, SERT occupancy should exceed 80% (Meyer et al., 2001, 2004). In contrast, although explored in recent studies (Ding et al., 2014; Takano et al., 2014), the minimum level of NET occupancy required for clinical efficacy in disorders such as ADHD or MDD has not been established. In chronic pain inhibition of NET, but not SERT, alone is sufficient for analgesic efficacy (Rowbotham et al., 2004; Verdu et al., 2008; Hall et al., 2011; Arnold et al., 2012). However, the NET occupancy requirements and the contribution, if any, of SERT to the clinical efficacy of approved dual-reuptake inhibitors is unclear. At the time the studies were conducted.

Supplementary Material

For supplementary material accompanying this paper, visit http://www.ijnp.oxfordjournals.org/

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Statement of Interest

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References

Abanades S, van der Aart J, Barletta JA, Marzano C, Searle GE, Salinas CA, Ahmad JJ, Reiley RR, Pampols-Maso S, Zamuner S, Cunningham VJ, Rabiner EA, Laruelle MA, Gunn RN (2011) Prediction of repeat-dose occupancy from single-dose data: characterisation of the relationship between plasma pharmacokinetics and brain target occupancy. J Cereb Blood Flow Metab 31:944–952.

Adler I. (2013) Monitoring adults with ADHD: a focus on executive and behavioral function. J Clin Psychiatry 71:e18.

Arnold LM, Hirsch I, Sanders P, Ellis A, Hughes B (2012) Safety and efficacy of eseretine in patients with fibromyalgia: a fourteen-week, randomized, double-blind, placebo-controlled, multicenter clinical trial. Arthritis Rheum 64:2387–2397.

Atkinson JH, Slater MA, Williams RA, Zisook S, Patterson TL, Grant I, Wahlgren DR, Abramson I, Garfin SR (1998) A placebo-controlled randomized clinical trial of nortriptyline for chronic low back pain. Pain 76:287–296.

Atkinson JH, Slater MA, Capparelli EV, Wallace MS, Zisook S, Abramson I, Matthews SC, Garfin SR (2007) Efficacy of noradrenergic and serotonergic antidepressants in chronic back pain: a preliminary concentration-controlled trial. J Clin Psychopharmacol 27:135–142.

Basaum AI, Fields HL (1984) Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci 7:309–338.

Berridge CW, Waterhouse BD (2003) The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev 42:33–84.

Bevington P, Robinson D (2014) Data reduction and error analysis for the physical sciences. New York: McGraw-Hill.

Bourdet DL, Tsuruda PR, Obedencio GP, Smith JA (2012) Prediction of human serotonin and norepinephrine transporter occupancy of duloxetine by pharmacokinetic/pharmacodynamic modeling in the rat. J Pharm Exp Ther 341:137–145.

Chang C, Byron W, Lu Y, Jacobsen LK, Badura LL, Sawant-Basak A, Miller E, Liu J, Grimwood S, Wang EQ, Maurer TS (2011) Quantitative PK-PD model-based translational pharmacology of a novel kappa opioid receptor antagonist between rats and humans. AAPS J 13:565–575.

Del CN, Chamberlain SR, Sahakian BJ, Robbins TW (2011) The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. Biol Psychiatry 69:e145–e157.

Ding YS, Lin KS, Logan J (2006) PET imaging of norepinephrine transporters. Curr Pharm Des 12:3831–3845.

Ding YS, Nagawa M, Gallezot JD, Nabulsi N, Lin SF, Ropchan J, Ding YS, Lin KS, Logan J (2006) PET imaging of norepinephrine transporters. Curr Pharm Des 12:3831–3845.

Ding YS, Lin KS, Logan J (2006) PET imaging of norepinephrine transporters. Curr Pharm Des 12:3831–3845.

Ding YS, Nagawa M, Gallezot JD, Nabulsi N, Lin SF, Ropchan J, Ding YS, Lin KS, Logan J (2006) PET imaging of norepinephrine transporters. Curr Pharm Des 12:3831–3845.
Vazey EM, Aston-Jones G (2012) The emerging role of norepinephrine in cognitive dysfunctions of Parkinson's disease. Front Behav Neurosci 6:48.
Verdu B, Decosterd I, Buclin T, Stiefel F, Berney A (2008) Antidepressants for the treatment of chronic pain. Drugs 68:2611–2632.
Wu Y, Carson RE (2002) Noise reduction in the simplified reference tissue model for neuroreceptor functional imaging. J Cereb Blood Flow Metab 22:1440–1452.
Wu D, Pontillo J, Ching B, Hudson S, Gao Y, Fleck BA, Gogas K, Wade WS (2008) Discovery of a potent, selective, and less flexible selective norepinephrine reuptake inhibitor (sNRI). Bioorg Med Chem Lett 18:4224–4227.
Yassen A, Olofsen E, Dahan A, Danhof M (2005) Pharmacokinetic-pharmacodynamic modeling of the antinociceptive effect of buprenorphine and fentanyl in rats: role of receptor equilibration kinetics. J Pharm Exp Ther 313:1136–1149.