Preclinical Pharmacology and Pharmacokinetics of AZD3783, a Selective 5-Hydroxytryptamine 1B Receptor Antagonist

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ABSTRACT

The preclinical pharmacology and pharmacokinetic properties of (2R)-6-methoxy-8-(4-methylpiperazin-1-yl)-N-(4-morpholin-4-ylphenyl)chroman-2-carboxamide (AZD3783), a potent 5-hydroxytryptamine 1B (5-HT1B) receptor antagonist, were characterized as part of translational pharmacokinetic/pharmacodynamic hypothesis testing in human clinical trials. The affinity of AZD3783 to the 5-HT1B receptor was measured in vitro by using membrane preparations containing recombinant human or guinea pig 5-HT1B receptors and in native guinea pig brain tissue. In vivo antagonist potency of AZD3783 for the 5HT1B receptor was investigated by measuring the blockade of 5-HT1B agonist-induced guinea pig hypothermia. The anxiolytic-like potency was assessed using the suppression of separation-induced vocalization in guinea pig pups. The affinity of AZD3783 for human and guinea pig 5-HT1B receptor (Kᵢ, 12.5 and 11.1 nM, respectively) was similar to unbound plasma EC5₀ values for guinea pig receptor occupancy (11 nM) and reduction of agonist-induced hypothermia (18 nM) in guinea pig. Active doses of AZD3783 in the hypothermia assay were similar to doses that reduced separation-induced vocalization in guinea pig pups. AZD3783 demonstrated favorable pharmacokinetic properties. The predicted pharmacokinetic parameters (total plasma clearance, 6.5 ml/min/kg; steady-state volume of distribution, 6.4 l/kg) were within 2-fold of the values observed in healthy male volunteers after a single 20-mg oral dose. This investigation presents a direct link between AZD3783 in vitro affinity and in vivo receptor occupancy to preclinical disease model efficacy. Together with predicted human pharmacokinetic properties, we have provided a model for the quantitative translational pharmacology of AZD3783 that increases confidence in the optimal human receptor occupancy required for antidepressant and anxiolytic effects in patients.

Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter in the central nervous system involved in physiological functions including thermoregulation, modulation of neuronal transmitter release, and anxiety and mood regulation (Delgado et al., 1990; Keane and Soubrie, 1997; Fink and Göthert, 2007). Complex mechanisms have been proposed for the regulation of 5-HT levels in serotonergic synapses and terminal fields, such as release of 5-HT by serotonergic neurons, uptake of 5-HT through serotonin reuptake transporters, and metabolism of 5-HT by monoamine oxidase (Möller and Volz, 1996). These mechanisms have been successfully exploited in developing numerous anxiolytics and antidepressants that increase 5-HT levels.

Currently, the selective serotonin reuptake inhibitors (SSRIs) and serotonin/norepinephrine reuptake inhibitors...
are first-line agents for the treatment of anxiety and depression (Mendlewicz and Lecrubier, 2000; Nutt, 2008). However, therapy with SSRIs or serotonin/norepinephrine reuptake inhibitors requires daily administration over the course of weeks for anxiolytic or antidepressant efficacy to emerge, and it is often accompanied by the appearance of sedation, weight gain, nervousness, and a high incidence of sexual dysfunction (Goldstein and Goodnick, 1998; Sussman, 2008). Increased risk of suicidal ideation in children and adolescents treated with SSRIs has also received attention, although conflicting findings have been reported (reviewed by Hetrick et al., 2010). Despite the success of SSRI therapies, up to 40% of patients with major depressive disorders do not respond (Tsai and Hong, 2003), and the patient’s depressive symptoms often return during maintenance therapy (Byrne and Rothschild, 1998). This combined with the SSRI side effects and the slow onset of SSRI-mediated antidepressant effect drive a significant demand for improved efficacy and tolerability in the treatment of anxiety and depression.

The presynaptic 5-HT$_{1B}$ autoreceptor also regulates 5-HT release, so it has been considered as an alternative target to 5-HT reuptake transporters for antidepressants (Gaster et al., 1998; Hillegaart and Ahlenius, 1998; Roberts et al., 2001; Ahlgren et al., 2004; Dawson et al., 2006). This mechanism also has the potential to provide a rapid onset of clinical efficacy without the liabilities associated with SSRIs (Moret and Briley, 2000; Slussi, 2002). Animal models such as serotonin agonist-induced guinea pig hypothermia (Ma et al., 1988) and guinea pig pup separation-induced vocalization (Hagan et al., 1997; Hudzik et al., 2003; Stenfors et al., 2004; Dawson et al., 2006) were developed to screen and evaluate the pharmacology of serotonergic agents such as 5-HT$_{1B}$ receptor antagonists. Selective 5-HT$_{1B}$ antagonists such as (R)-N-[5-methyl-8-(4-methylpipеразин-1-yl)-1,2,3,4-tetrahydro-2-naphthyl]-4-morpholinobenzamide (AR-A000002) (Hudzik et al., 2003) and 1-[6-(cis-3,5-dimethylpipеразин-1-yl)-2,3-dihydro-5-methoxyindol-1-yl]-1-[2’-methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methane hydrochloride (SB-616234-A) (Dawson et al., 2006) have been shown to elevate serotonergic neurotransmission in vivo and exhibit effects indicative of antidepressant and anxiolytic properties in animals. The discovery of selective, high-affinity 5-HT$_{1B}$ compounds made it possible to develop 5-HT$_{1B}$-specific ligands for positron emission tomography (PET) to obtain non-invasive imaging of a specific binding to the receptor in brain (Pierson et al., 2008; Nabulsi et al., 2010). These tools can provide an understanding of the level of receptor occupancy required for minimal and maximal efficacy and the margin to on-target side effects that is critical for formulating a testable translational pharmacology hypothesis for early human testing. For G protein-coupled receptor (GPCR) antagonists, such as 5HT$_{1A}$ and 5-HT$_{2A}$ and neurokinin 1 antagonists 50 to 87% receptor occupancy was required for therapeutic responses (Grimwood and Hartig, 2009). However, the optimal range of 5-HT$_{1B}$ receptor occupancy in brain by selective 5-HT$_{1B}$ antagonists that is required for antidepressant and anxiolytic efficacy in preclinical models and patients has yet to be established.

Many properties, such as receptor affinity, functional antagonism, physicochemical properties, metabolic stability, and efflux transporter substrate liabilities, will affect the doses and exposures needed to achieve efficacy in preclinical and clinical studies. An ideal 5-HT$_{1B}$ antagonist drug would possess pharmacodynamic and pharmacokinetic properties that enable it to occupy and block the 5-HT$_{1B}$ receptor at levels sufficient for antidepressant and anxiolytic efficacy after once-daily dosing. (2R)-6-methoxy-8-(4-methylpipеразин-1-yl)-N-(4-morpholin-4-ylphenyl)chromane-2-carboxamide (AZD3783) (Fig. 1) is a potent, selective 5-HT$_{1B}$ antagonist that was selected for further study because of its favorable pharmacological and pharmacokinetic profiles. In a recent report on PET studies using a 5-HT$_{1B}$-specific ligand, Varnäs et al. (2011) demonstrated specific and high-affinity binding of AZD3783 to 5-HT$_{1B}$ receptors in human and non-human primate brains. The objective of this study was to characterize the in vitro and preclinical in vivo pharmacodynamic and pharmacokinetic properties of AZD3783 to develop a preclinical translational approach that would enable comparison with PET occupancy results in humans and help guide dose selection for the 5-HT$_{1B}$ antagonist in clinical hypothesis testing for anxiety and mood disorders.

**Materials and Methods**

**Materials**

AZD3783, 6-fluoro-8-(4-methyl-pipеразин-1-yl)-4-oxo-4H-chromene-2-carboxylic acid[4-(4-propionyl-pipеразин-1-yl)-phenyl]-amide (AZD1134), and radiolabeled ligand 5-methyl-8-(4-methyl-pipеразин-1-yl)-4-oxo-4H-chromene-2-carboxylicacid[4-(4-morpholin-4-ylphenyl)-amide] (N-methyl-[^3]H)AZ10419369) were synthesized at AstraZeneca Pharmaceuticals LP (Wilmington, DE). [3H]N-[4-methoxy-3-(4-methylpipеразин-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide (OR25743), a 5-HT$_{1B}$ radiolabeled ligand, was purchased from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). Cryopreserved hepatocytes from human, male cynomolgus monkey, male Beagle dog, and pooled cynomolgus monkey liver microsomes were purchased from CellzDirect (Durham, NC). Pooled human liver microsomes were obtained from BD Gentest (Woburn, MA). Fresh hepatocytes from male Sprague-Dawley rats and liver microsomes from Sprague-Dawley rats and Beagle dogs were prepared within AstraZeneca using standard procedures. Plasma from rat, dog, cynomolgus monkey, guinea pig, and human were obtained from BioReclamation (Hicksville, NY). The 5-HT$_{1B}$ agonist 3-(N-methylpyrrolidin-2-R-ylmethyl)-5-(3-nitropyrindin-2-Yl)-indole (CP135.807) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). The Madin-Darby canine kidney cell line transfected with human MRD1 gene (MRD1-MDCK) was obtained from The Netherlands Cancer Institute (Amsterdam, Netherlands). Dulbecco’s modified Eagle’s medium and fetal bovine serum were obtained from Invitrogen (Carlsbad, CA). The Chinese hamster ovary cells expressing 5-HT$_{1B}$ receptors (10 mg/ml), reagents for the 5-HT$_{1B}$ assays, the Opti-Plates, Soluene 350, and Ultima-Gold scintillation fluid were from PerkinElmer Life and Analytical Sciences (Waltham, MA).

**Animals**

Husbandry, experimental use, and methods of euthanasia met ethical guidelines for the humane care and treatment of laboratory animals. Male Sprague-Dawley rats and Dunkin-Hartley guinea pigs were purchased from Charles River Laboratories, Inc. (Malvern, PA). The body weight of Sprague-Dawley rats, Hartley guinea pigs, Beagle dogs, and cynomolgus monkeys (Macaca fascicularis) ranged from 0.30 to 0.35, 0.2 to 0.4, 10 to 13, and 3 to 8 kg, respectively.

**Fig. 1.** Chemical structure of AZD3783.
Except for guinea pigs in behavior studies, all of the animals were fasted overnight before dosing and fed 2 h after dosing. All animals were individually housed with free access to water and maintained in rooms with constant temperature (approximately 22°C) and a 12-h light/dark cycle. All facilities were approved by the American Association for Accreditation of Laboratory Animal Care, and all testing procedures were performed using protocols approved by the Institutional Animal Care and Use Committee at AstraZeneca R&D Wilmington, in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

**Pharmacology**

**In Vitro Pharmacology Assays.** The 5-HT<sub>1B</sub> receptor binding and [GTP<sup>35S</sup>] functional antagonist assays were modified from previously published procedures (Watson et al., 1996). In the 5-HT<sub>1B</sub> receptor competition binding assays, a predefined quantity of membranes containing human or guinea pig 5-HT<sub>1B</sub> receptors were diluted in assay buffer containing 50 mM Tris-HCl, pH 7.4, 4 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, and 1 mM EDTA. The membranes in the 96-well OptiPlates at 10 μg/well were preincubated in bulk in assay buffer containing 170 pM to 10 μM AZD1134, a potent and very selective 5HT<sub>1B</sub> antagonist synthesized at AstraZeneca, was used to define nonspecific binding at 1 μM final assay concentration. The pharmacology of AZD1134 was well defined, and it demonstrated structural diversity from AZD3783 (Maier et al., 2009). The assay plates were incubated for 1 h at room temperature with shaking. RPQ0011 Ytrrium Silicate-WGA SPA Beads (GE Healthcare) were then added to the plates at 1.5 mg/well and incubated for additional 30 min with vigorous shaking. After the incubation and centrifugation the radioactivity in each well was measured in a scintillation counter (PerkinElmer Life and Analytical Sciences).

In the GTP<sup>35S</sup> functional antagonist scintillation proximity assay, membranes and compound dilutions were the same as those in the 5-HT<sub>1B</sub> receptor binding assay. In this scintillation proximity assay, membranes (15 μg of protein/well), and RPQ0000 WGA PVT beads (50 μg/well) (GE Healthcare) were preincubated in bulk in assay buffer containing 20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM MgCl<sub>2</sub>, and 0.1% bovine serum albumin for 30 min. After adding GTP<sup>35S</sup> (200 PM final assay concentration) and GDP (10 μM final) to the membrane/bead mixture, 150 μL of the mixture was added to 96-well OptiPlates (PerkinElmer Life and Analytical Sciences) containing 2 μL of AZD3783 (170 PM to 10 μM at final concentration). After 5-min preincubation, 50 μL of buffer or 108 nM 5-HT (27 nM final assay concentration at EC<sub>50</sub>) was added. The plates were incubated for 1 h with shaking. After centrifugation the radioactivity in each well was measured in a TopCount counter. Percentage effect with respect to basal (buffer unstimulated) and stimulated (EC<sub>50</sub> of 5-HT) response was determined.

**Guinea Pig Receptor Binding Assay.** The guinea pig receptor occupancy assay was conducted using methods reported by Maier et al. (2009). One to 2 days before the receptor occupancy experiment, the jugular cannula was inserted for intravenous dosing. After the guinea pig was anesthetized with isoflurane, a 2-3 French catheter was inserted into the jugular vein and terminated at the right atrium. The exterior end of the cannula was fed subcutaneously, anchored to the skin dorsal to the shoulder blades, and flushed with 0.9% saline with 100 units/ml heparin to test patency. On the day of the experiment, AZD3783 (0.06, 0.2, 0.6, 2.0, 6.0, and 20.0 μmol/kg s.c.) or vehicle treatment (0.9% saline; volume 1 ml/kg s.c.) was administered to fed animals 30 min before [N-methyl-<sup>3</sup>H]<sub>1B</sub>AZ10419369 (15 μCi/mL i.v. guinea pig). Guinea pigs were euthanized 30 min after radioligand injection. Trunk blood samples were collected, and plasma was analyzed by LC-MS to determine the plasma concentration of AZD3783. The brain was removed and placed on a chilled plate for free-hand dissection into selected brain regions, including the cortex (CTX), striatum/globus pallidus (STR), midbrain including substantia nigra (MID), and cerebellum (CRB). Each tissue region was frozen, weighed, and transferred to scintillation vials containing Scouene 350. After solubilizing the tissue overnight, Ultima-Gold scintillation fluid was added, and total radioactivity was measured using a Tri-Carb scintillation counter (PerkinElmer Life and Analytical Sciences). [N-methyl-<sup>3</sup>H]<sub>1B</sub>AZ10419369 binding was determined for all treatment groups and presented as femtomole per milligram of tissue.

**Blockade of Agonist-Induced Hypothermia in Guinea Pigs.** This experiment was carried out over 2 days. On each day of the experiment, animals to be used were habituated to the test room for at least 1 h before testing. Body weight and baseline rectal body temperatures were determined for each animal, then all subjects were assigned to one of nine treatment groups (n = 6 per treatment group): vehicle + 3 mg/kg agonist (CP135.807) and AZD3783 at 0.006, 0.002, 0.06, 0.2, 0.6, 2, 6, or 20 μmol/kg + agonist. Depending on the treatment group, AZD3783 or vehicle was administered by subcutaneous injection 30 min before the administration of 5-HT<sub>1B</sub> agonist (3 mg/kg i.p.). Previous experiments with CP135.807 (data not presented) had shown that this dose of this 5-HT<sub>1B</sub> agonist would induce approximately a 2 to 3°C reduction in rectal temperature that lasted for several hours and was sensitive to blockade by selective 5-HT<sub>1B</sub> antagonists. One hour after agonist administration, rectal body temperature was measured again. After body temperature measurement, each animal was quickly euthanized to obtain plasma and brain samples. The plasma and brain samples collected were rapidly frozen until analysis. Data were reported as body temperature (°C) or plasma or brain concentration.

**Suppression of Maternal Separation-Induced Vocalizations in Guinea Pig Pups.** The testing apparatus was comprised of a blue-tinted, but see-through, rectangular Rubbermaid (Fairlawn, OH) storeroom container 24.3 inches high × 16 inches wide × 15.8 inches deep. Audio recordings were made using a Sony (Tokyo, Japan) BM-575 Microcassette Dictator and Sony 60 min microcassettes. Testing of the pups was done in a repeated-measures design starting at 4 to 6 days postnatal and continued until 23 to 25 days postnatal with at least 2 days between test days. The litters were transported to the testing laboratory and allowed to acclimate for 1 h before testing. All subjects were given a 5-min (300 s) prescreen to measure the response to maternal separation by recording the number of vocalizations emitted by each subject. After prescreening, four groups, each with 12 pups, were dosed subcutaneously (1 ml/kg) at 0 (vehicle), 0.2, 0.6, and 2.0 μmol/kg AZD3783, respectively. The dose vehicle contained sterile water with 5% lactic acid, pH 4.0. After the postinjection period, the number of vocalizations emitted by each subject was assessed in a 5-min test session.

**Pharmacokinetics**

**P-Glycoprotein Transporter Assay.** Monolayer-based Pgp assays were performed manually in basolateral to apical (B → A) and apical to basolateral (A → B) directions in triplicate (Polli et al., 2001). The MDR1-MDCK cells were seeded onto 12-well Costar Transwell plate (Corning Life Sciences, Lowell, MA) at a density of 3 × 10<sup>5</sup> cells/cm<sup>2</sup> and grown in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum for 3 days. The medium was replaced daily and at 2 h before transport experiments. The transport of AZD3783 was assessed at an initial concentration of 1 μM for 60 min at 37°C. At the end of the experiment, samples from both donor and receiver chambers were analyzed by LC-MS. The apparent permeability values were calculated with equation P<sub>app</sub> = k/L × C<sub>d</sub> × (dQ/dt), where k is the apparent permeability, C<sub>d</sub> is the initial donor drug concentration, and dQ/dt is the amount of drug transported within a given time period. The flux ratio was calculated from P<sub>app</sub> B → A divided by P<sub>app</sub> A → B.

**Plasma Protein Binding.** Plasma protein binding of AZD3783 was determined by equilibrium dialysis in a Spectrum (Rancho Dominguez, CA) equilibrium dialyzer using dialysis membranes with a 12,000- to 14,000-Da molecular mass cutoff against Dulbecco’s phosphate-buffered saline, pH 7.4. Aliquots of 2 ml of plasma were
spiked with AZD3783 to achieve final concentrations of 0.1, 1, and 10 μM with dimethyl sulfoxide <0.5% in plasma. The dialysis was conducted in triplicate overnight at 37°C. At the end of dialysis, aliquots of 100-μl plasma samples were mixed with equal volume of buffer; aliquots of 100-μl buffer dialysate samples were mixed with equal volume of plasma. Two volumes of acetonitrile containing 0.2% formic acid were then added to these samples. After centrifugation for 15 min at 3000 rpm, the supernatant was quantified by LC-MS. The unbound fraction (f_u) was calculated as the concentration of AZD3783 in buffer divided by the concentration in plasma.

Microsome Metabolic Stability Assay. In microsome stability assay, duplicate samples of AZD3783 (1 μM) were incubated at 37°C with liver microsomes (0.5 mg/ml) from human, rat, dog, or cynomolgus monkey in 0.1 M potassium phosphate buffer, pH 7.4. NADPH (1 mM) was added to initiate the incubation. Aliquots of the incubates were removed at 0, 5, 10, 15, 20, and 25 min, respectively, and added to equal volumes of acetonitrile/methanol (1:1, v/v) containing 0.1% formic acid to stop the reaction. After centrifugation for 10 min at 3500 rpm, the remaining AZD3783 in the supernatant was analyzed by LC-MS.

Hepatocytes Metabolic Stability Assay. The hepatocyte metabolic stability of AZD3783 was determined in duplicate at 1 μM with freshly isolated rat hepatocytes or cryopreserved dog, monkey, or human hepatocytes from three individual donors at 10^6 cells/ml. The incubation was carried out at 37°C in Williams E medium, pH 7.4, with 1% insulin transferrin selenium solution (Invitrogen), 2 mM l-glutamine, and 25 mM HEPES. The incubates were removed at 0, 5, 10, 15, 20, and 25 min, respectively, and added to equal volumes of acetonitrile/methanol (1:1, v/v) containing 0.1% formic acid to stop the reaction. After centrifugation for 10 min at 3500 rpm, the remaining AZD3783 in the supernatant was analyzed by LC-MS.

Dog PK. The 5 mM AZD3783 solution was prepared in 25 mM lactic acid containing 3.75% dextrose. The intravenous injection was introduced via tail vein at a dose of 10 μmol/kg. The oral dose was administered by oral gavage at a volume of 6 ml/kg to achieve a target dose of 30 μmol/kg. Aliquots (0.15 ml) of blood samples were collected from the implanted cannulae before dosing and at 15 and 30 min and 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 h postdose for both intravenous and oral routes. Additional samples at 2 and 7 min postdose were also collected for the intravenous doses.

Dosing of AZD3783 in Healthy Male Volunteers. The human PK of AZD3783 was investigated in healthy male subjects in a single ascending dose study that was double-blind, placebo-controlled, and randomized within each dose group. PK parameters from only the 20-mg dose group relevant to the targeted therapeutic exposure are presented here for an evaluation of PK prediction purpose. The study protocol and informed consent documents were approved by the Institutional Review Board. All subjects signed an approved written informed consent before any study-related activities for this clinical study. There were eight subjects in the 20-mg dose group with six randomized to AZD3783 and two to a matching placebo. AZD3783 was administered as capsules under fasted condition. Blood samples for PK evaluation were taken between predose and 72 h postdose. All appropriate safety components were included in the study.

Sample Processes. Rat, dog, and monkey plasma samples were processed by adding 6 volumes of acetonitrile/water (8:2, v/v) containing 0.1% formic acid. After centrifugation for 15 min at 3000 rpm, the supernatants were analyzed by LC-MS. AZD3783 in human plasma samples was extracted in an Oasis HLB μ-eilution 96-well plate (Waters, Milford, MA). The plate was washed with 180 μl of methanol and equilibrated in 180 μl of water before loading with 150 μl of human plasma. The wells were then washed with 180 μl of 30% methanol in water and eluted with 40 μl of acetonitrile/isopropanol (1:1, v/v). A 5-μl aliquot of eluted sample was analyzed by LC-MS.

LC-MS Analysis. For the in vitro samples, the separation was performed using a Shimadzu (Columbia, MD) VP HPLC system with a Phenomenex (Torrance, CA) Synergi Max-RP column (3.5 μm, 3 × 50 mm) coupled to a Micromass Ultima triple quadrupole mass spectrometer (Waters) under positive electrospray ionization mode. The mobile phases included 0.1% formic acid in high-performance
liquid chromatography water (A) and 10% (v/v) methanol in acetonitrile (B). The gradient started with 100% A for 0.3 min, was linearly increased to 95% B in 1.2 min, and held at 95% B for 0.1 min before returning to 100% A. AZD3783 was quantified by integration of ion chromatogram peak area acquired under multiple reaction monitoring with m/z 467/235 transition.

The in vivo AZD3783 plasma extract was separated in a Shimadzu HPLC system with a SepaxHP-Silica 120A analytical column (3 μm, 50 × 2.1 mm) coupled to a Sciex API 3000 mass spectrometer. The isocratic eluting solvent consisted 1:10 (v/v) of 10 mM ammonium formate, pH 4 and acetonitrile/methanol (1:1, v/v). AZD3783 and internal standard D6-AZD3783 were quantified by multiple reaction monitoring under 467/235 transition for AZD3783 and 473/239 transition for D6-AZD3783, respectively.

Data Analysis

In Vitro 5-HT1B Affinity and GTP-γ[35S] Functional Antagonist Potency. Data were analyzed by calculating IC_{50}, and K_i using the Kenakin correction for ligand depletion using following equations:

\[ B = [(K_D + L_T + R_T) - (K_D + L_T + R_T)^2 - 4R_T(L_T)^2]/2 \]  
\[ K_i = (0.5B \times IC_{50} \times K_D) / (L_T \times R_T) + 0.5B(2R_T - L_T) - 0.5B - K_D \]  

where L_T and R_T are the total concentrations of the ligand and receptor, respectively. GTP-γ[35S] functional antagonist potency was defined as the antagonist concentration for 50% inhibition of the stimulated response (EC_{50}).

Guinea Pig Receptor Occupancy Calculation. Specific binding was calculated from total binding for each brain region by subtracting the femtomole per milligram values for the cerebellum (region of nonspecific receptor binding). The in vivo receptor occupancy for AZD3783 was indicated by a reduction in specific binding of [N-methyl-3H]AZ10419369 compared with vehicle controls and represented as a percentage using eq. 3:

\[ \%RO = 100 \times (SB_{saline} - SB_{AZD3783})/SB_{saline} \]  

where %RO is the percentage of receptor occupancy and SB is specific binding. Treatment effects were determined by analysis of variance followed by Dunnett’s post hoc test. Percentage of receptor occupancy sigmoidal curves were fit, and nonlinear regression analysis was used to calculate the ED_{50}, the dose that resulted in 50% receptor occupancy, and the EC_{50}, the plasma exposure value for 50% receptor occupancy, by using Prism (GraphPad Software Inc., San Diego, CA).

Guinea Pig Hypothermia Data. Plasma or brain EC_{50} values for reduction of agonist-induced hypothermia were determined by nonlinear regression analysis using a four-parameter Hill equation in SigmaPlot (Systat Software Inc., San Jose, CA).

Guinea Pig Pup Vocalization Data. The effects on guinea pig pup response to maternal separation were analyzed and reported as number of calls in a 5-min period. The repeated-measures analysis of variance was conducted to assess the main effect of each drug treatment level. Significant effects of drug treatment were followed up.
with Dunnett’s post hoc tests to determine which doses were significantly different from control.

**Estimations of Preclinical PK Parameters.** The PK parameters were estimated by standard noncompartmental pharmacokinetic analysis using WinNonlin version 5.2 (Pharsight, Mountain View, CA). Absolute bioavailability (%F) was determined by comparing AUC values after oral to intravenous administration (Obach et al., 1997) shown in eq. 10:

\[
Y = \alpha (BW)^b
\]  

where \(Y\) represents CL or Vd parameters, BW is body weight, and \(\alpha\) and \(b\) are the coefficient and exponent of the allometric equation, respectively (Hosea et al., 2009). Human CL and Vd were also calculated by applying preclinical PK data to the Oie-Tozer equation (Oie and Tozer, 1979; Obach et al., 1997) shown in eq. 10:

\[
V_d = V_{d\text{animal}} \times \left(1 - f_u\right) \times V_p + \left(1 - f_u\right) \times V_p \times \frac{f_u\text{human}}{f_u\text{animal}}
\]  

where \(f_u\) is the fraction unbound in plasma. The human Vd was also calculated for human PK simulation (Oie and Tozer, 1979; Obach et al., 1997)

where CL was estimated from dog oral PK data.

**Extrapolation of Human PK Parameters from Preclinical In Vivo PK Data.** The allometric scaling of total plasma clearance (CL) and steady-state volume of distribution (Vd) were performed using preclinical data to fit logarithmically with linear regression to eq. 7:

\[
CL_{\text{predict}} = \frac{Q \times CL_{\text{int}}}{Q + CL_{\text{int}}}
\]

where \(Q\) is hepatic blood flow (20, 66, 38, and 44 ml/min/kg for human, rat, dog, and cynomolgus monkey, respectively) and CL' int is the scaled intrinsic clearance determined from eqs. 4 or 5.

\[
Y = a(WB)^b
\]

where \(Y\) represents CL or Vd parameters, BW is body weight, and \(a\) and \(b\) are the coefficient and exponent of the allometric equation, respectively (Hosea et al., 2009). Human CL and Vd were also calculated by applying preclinical PK data to the Oie-Tozer equation (Oie and Tozer, 1979; Obach et al., 1997) shown in eq. 10:

\[
V_d = V_{d\text{animal}} \times \left(1 - f_u\right) \times V_p + \left(1 - f_u\right) \times V_p \times \frac{f_u\text{human}}{f_u\text{animal}}
\]  

where \(V_d\) is the human plasma volume, \(V_p\) is the extracellular fluid volume in human, \(f_u\) is the ratio of binding proteins in extracellular fluid (except plasma) to binding proteins in plasma in human, \(V_r\), “remainder of the fluid,” is the physical volume into which a drug can distribute minus the extracellular space in human, and \(f_u\) values for individual animals were calculated based on eq. 11 (Obach et al., 1997):

\[
f_u = 
\left(\frac{V_{d\text{animal}} - V_{p\text{animal}} - (1 - f_u\text{animal}) \times V_{e\text{animal}}}{(1 - f_u\text{animal}) \times V_{e\text{animal}}}\right)
\]

where \(V_{e\text{animal}}\), \(V_{p\text{animal}}\), and \(V_{d\text{animal}}\) are the volume in human, “remainder of the fluid,” is the physical volume into which a drug can distribute minus the extracellular space in human, and \(f_u\) values for individual animals were calculated based on eq. 11 (Obach et al., 1997):

\[
f_u = 
\left(\frac{V_{d\text{animal}} - V_{p\text{animal}} - (1 - f_u\text{animal}) \times V_{e\text{animal}}}{(1 - f_u\text{animal}) \times V_{e\text{animal}}}\right)
\]

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where \(V_{e\text{animal}}\), \(V_{p\text{animal}}\), and \(V_{d\text{animal}}\) are the volume in human, “remainder of the fluid,” is the physical volume into which a drug can distribute minus the extracellular space in human, and \(f_u\) values for individual animals were calculated based on eq. 11 (Obach et al., 1997):

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\left(\frac{V_{d\text{animal}} - V_{p\text{animal}} - (1 - f_u\text{animal}) \times V_{e\text{animal}}}{(1 - f_u\text{animal}) \times V_{e\text{animal}}}\right)
\]

where \(V_{e\text{animal}}\), \(V_{p\text{animal}}\), and \(V_{d\text{animal}}\) are the volume in human, “remainder of the fluid,” is the physical volume into which a drug can distribute minus the extracellular space in human, and \(f_u\) values for individual animals were calculated based on eq. 11 (Obach et al., 1997):

\[
f_u = 
\left(\frac{V_{d\text{animal}} - V_{p\text{animal}} - (1 - f_u\text{animal}) \times V_{e\text{animal}}}{(1 - f_u\text{animal}) \times V_{e\text{animal}}}\right)
\]

where \(V_{e\text{animal}}\), \(V_{p\text{animal}}\), and \(V_{d\text{animal}}\) are the volume in human, “remainder of the fluid,” is the physical volume into which a drug can distribute minus the extracellular space in human, and \(f_u\) values for individual animals were calculated based on eq. 11 (Obach et al., 1997):

\[
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\left(\frac{V_{d\text{animal}} - V_{p\text{animal}} - (1 - f_u\text{animal}) \times V_{e\text{animal}}}{(1 - f_u\text{animal}) \times V_{e\text{animal}}}\right)
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\[
f_u = 
\left(\frac{V_{d\text{animal}} - V_{p\text{animal}} - (1 - f_u\text{animal}) \times V_{e\text{animal}}}{(1 - f_u\text{animal}) \times V_{e\text{animal}}}\right)
\]

where \(V_{e\text{animal}}\), \(V_{p\text{animal}}\), and \(V_{d\text{animal}}\) are the volume in human, “remainder of the fluid,” is the physical volume into which a drug can distribute minus the extracellular space in human, and \(f_u\) values for individual animals were calculated based on eq. 11 (Obach et al., 1997):

\[
f_u = 
\left(\frac{V_{d\text{animal}} - V_{p\text{animal}} - (1 - f_u\text{animal}) \times V_{e\text{animal}}}{(1 - f_u\text{animal}) \times V_{e\text{animal}}}\right)
\]
specific binding of \([N\text{-methyl}^3\text{H}]\text{AZ10419369}\) in guinea pig brain in a dose-dependent manner (Fig. 3A). Two-way analysis of variance for \([N\text{-methyl}^3\text{H}]\text{-AZ10419369}\) binding showed a significant main effect of AZD3783 treatment \((p < 0.001)\) and brain region \((p < 0.001)\). In all regions containing the 5-HT\(_{1B}\) receptor (CTX, STR, MID), AZD3783 significantly inhibited specific binding with increasing dose, whereas binding in the nonspecific region, the CRB, remained unchanged. Although the binding of AZD3783 was not significantly different from saline at the 0.06 \(\mu\text{mol/kg}\) dose, AZD3783 at doses of 0.6, 6.0, and 20 \(\mu\text{mol/kg}\) significantly blocked specific binding of the radioligand \((p < 0.05)\). AZD3783 receptor occupancy was calculated from the specific binding of \([N\text{-methyl}^3\text{H}]\text{-AZ10419369}\) for each brain region with saline pretreatment group occupancy as the baseline. Maximal occupancy of AZD3783 was 96% in the MID, 85% in the STR, and 71% in the CTX (Fig. 3B). ED\(_{50}\) values for AZD3783 were based on the occupancy values from all experimental animals \((n = 12)\): CTX (0.27 \(\mu\text{M/kg}\)), STR (0.22 \(\mu\text{mol/kg}\)), and MID (0.45 \(\mu\text{mol/kg}\)). They were representative of 50% receptor occupancy across all brain regions. The EC\(_{50}\) values were similar in CTX (18 nM), STR (16 nM), and MID (18 nM). Therefore, the average EC\(_{50}\) in receptor occupancy was estimated to be 17 nM (11 nM unbound), which was consistent to an in vitro affinity \(K_i\) value of 12.5 nM.

**Effect of AZD3783 on 5-HT\(_{1B}\) Agonist-Induced Body Temperature in Guinea Pig.** AZD3783 was shown to reverse the hypothermic effect induced by 5-HT\(_{1B}\) agonist in guinea pig (Fig. 4). The EC\(_{50}\) of AZD3783 on blockade of the 5-HT\(_{1B}\) agonist-induced hypothermia in guinea pig was 29 nM (18 nM unbound) and 25 nM (16 nM unbound) for plasma and brain, respectively, and consistent with the in vitro \(K_i\) and receptor occupancy EC\(_{50}\) values.

**Effects of AZD3783 on Guinea Pig Pup Response to Maternal Separation.** AZD3783 significantly decreased separation-induced vocalizations \((p < 0.0001)\). The guinea pig pups reduced vocalizations at all doses tested \((p < 0.01)\), suggesting the minimum efficacious dose was at or below 0.2 \(\mu\text{mol/kg}\) (Fig. 5).

**Pharmacokinetic Properties**

**Pgp Efflux Liability.** The apparent permeability of AZD3783 in the apical to basolateral direction in the MDCK-MDR1 cell assay was 38 nm/s at the 1 \(\mu\text{M}\) initial concentration, suggesting that AZD3783 would be readily permeable through the blood-brain barrier. Under the same conditions, the flux ratio of \(P_{app\ A\rightarrow B}/P_{app\ B\rightarrow A}\) was 0.9, indicating AZD3783 was not likely a Pgp substrate.

**Plasma Protein Binding.** The plasma protein binding of AZD3783 was determined by equilibrium dialysis at 0.1, 1,
and 10 μM and found to be concentration-independent in that range for all species tested. The average unbound AZD3783 in plasma was 46, 36, and 62% for rat, dog, and guinea pig, respectively. As reported by Varnaš et al. (2011) the unbound AZD3783 in human and monkey plasma was 66 and 73%, respectively.

**In Vitro Metabolic Stability.** The in vitro metabolic stability of AZD3783 was evaluated using both liver microsomes and hepatocytes. The apparent CL_{int} values of AZD3783 were 15, 16, 14, and 81 μl/min/mg protein for human, rat, dog, and monkey liver microsomes, respectively. The CL_{int} values in hepatocytes were 1.6, 8.1, 7.3, and 21 μl/min/10^6 cells for human, rat, dog, and monkey, respectively. The in vitro-in vivo correlation analysis was performed by comparing the observed in vivo clearance with the predicted in vivo clearance calculated from eqs. 4, 5, and 6 (Table 1). The predicted hepatic clearance results for rat, dog, and monkey were consistent with the observed plasma clearance from the preclinical PK studies, indicating that phase I metabolism accounted for the majority of AZD3783 elimination in these species, and the intrinsic clearance parameters from in vitro microsomal or hepatocyte stability assay are predictive of the in vivo clearance.

**Pharmacokinetics of AZD3783 in Rats, Dogs, and Monkeys.** The primary pharmacokinetic parameters and plasma profiles for AZD3783 are summarized in Table 2 and Fig. 6, respectively. The early T_{max} in preclinical species indicated a rapid absorption of AZD3783. The elimination kinetics were monophasic in rat. There was apparent two-phase elimination in dog and cynomolgus monkey. AZD3783 exhibited moderate plasma clearance in rat and dog (approximately 29 and 45% of hepatic blood flow, respectively) with good oral bioavailability, but high clearance in monkey (80% of hepatic blood flow). V_{dss} values ranged from 2.3 l/kg in rat to 5.9 l/kg in monkey (Table 2). AZD3783 also demonstrated a moderate t_{1/2} of 1.4, 3.7, and 4.6 h in rat, dog, and monkey, respectively.

**Human Pharmacokinetics**

**Human Pharmacokinetics Prediction.** Approaches applied to the estimation of human in vivo clearance parameters included in vitro scaling of clearance using eqs. 4, 5, and 6, multiple-species allometric scaling using eq. 7, and dog single-species allometric scaling using eq. 8 (Table 3). The predicted clearances from in vitro scaling (Table 1) were consistent with the observed in vivo plasma clearance for the preclinical species tested (Table 2), suggesting that metabolism is the major elimination pathway in these species and would also probably be in human. Multiple-species allometric scaling yielded a human in vivo clearance of 25 ml/min/kg with exponent of 1.023, which was much greater than 0.75 (Fig. 7). The single-species scaling from dog resulted in a predicted human clearance of 19 ml/min/kg. It seemed from allometric or single-species scaling methods that the predicted clearance would equal or exceed the human liver blood flow, resulting in no bioavailability, which was inconsistent

### Table 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Dose</th>
<th>C_{max}</th>
<th>T_{max}</th>
<th>AUC_{0}</th>
<th>CL_{plasma}</th>
<th>V_{dss}</th>
<th>t_{1/2}</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>IV</td>
<td>10</td>
<td>N.D.</td>
<td>N.D.</td>
<td>8.8 ± 0.9</td>
<td>19 ± 2.8</td>
<td>23 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td>30</td>
<td>9.5 ± 3.8</td>
<td>0.25</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>98</td>
</tr>
<tr>
<td>Dog</td>
<td>IV</td>
<td>2.0</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.9 ± 0.1</td>
<td>18 ± 0.9</td>
<td>4.3 ± 0.4</td>
<td>3.7 ± 0.5</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td>2.0</td>
<td>0.24 ± 0.01</td>
<td>1.00</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>IV</td>
<td>3.0</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.5 ± 0.1</td>
<td>35 ± 0.8</td>
<td>5.9 ± 0.8</td>
<td>4.6 ± 0.6</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

IV, intravenous; PO, oral; N.D., not determined.

* Median number.
with the observed $% F$ in rat and dog or the in vitro-in vivo correlation predicted values in these preclinical species. Therefore, the average clearance of 6.5 ml/min/kg (0.39 l/h/kg) scaled from the human liver microsome and the hepatocyte CLint was adopted as the predicted human CL value (Table 4).

The human Vdss was simulated from multiple-species allometric scaling, dog single-species scaling, and the Oie-Tozer equation (Fig. 8; Table 3). The multiple-species allometric scaling resulted in a predicted human Vdss of 7.5 l/kg with an exponent of 1.190, which was acceptably close to unity. Single-species scaling from dog also yielded a similar Vdss (7.4 l/kg). The Oie-Tozer equation with parameters from rat, dog, and monkey predicted a human Vdss of 7.5 l/kg. To obtain the predicted human CL value, the human Vdss was simulated from multiple-species allometric scaling, dog single-species scaling, and the Oie-Tozer equation (Fig. 8; Table 3). The multiple-species allometric scaling resulted in a predicted human Vdss of 7.5 l/kg with an exponent of 1.190, which was acceptably close to unity. Single-species scaling from dog also yielded a similar Vdss (7.4 l/kg). The Oie-Tozer equation with parameters from rat, dog, and monkey predicted a human Vdss of 7.5 l/kg. To obtain the predicted human CL value, the human Vdss was simulated from multiple-species allometric scaling, dog single-species scaling, and the Oie-Tozer equation.

**Human Pharmacokinetic Data.** After a 20-mg oral administration in healthy human volunteers, AZD3783 was absorbed with a median $T_{\text{max}}$ of 1.5 to 3.1 h. The geometric mean values for $C_{\text{max}}$ and AUC were 79 and 1018 nM · h, respectively. The geometric mean for clearance (CL/F) after oral administration was 0.60 l/h/kg (Table 5). The human PK parameters obtained from this study were consistent with the human PK parameters reported by Varnås et al. (2011) in the AZD3783 human PET study (Table 5). The PK parameters obtained from both human studies, dosed with 20 mg, showed values within 2-fold of the predicted human pharmacokinetic parameters. From the 10 mg/day dose simulation (Fig. 9), the predicted first-day $C_{\text{max}}$ and plasma concentration at 24 h were 53 and 17 nM, respectively, matching closely with the $C_{\text{max}}$ and plasma exposure at 24 h observed in the human PET study with a 10-mg dose (Varnås et al., 2011).

**Predicted Efficacious Dose in Human.** The predicted efficacious dose of AZD3783 in humans was based on two sets of data, the pharmacological data from in vitro and in vivo assays and the predicted human PK parameters. The guinea pig 5-HT$_{1B}$ receptor affinity ($K_i$, 11.1 nM) was shown to correspond to the in vivo receptor occupancy unbound plasma EC$_{50}$ value. These binding values were also consistent with the unbound plasma EC$_{50}$ value for the blockade of 5-HT$_{1B}$ agonist-induced hypothermia and the estimated minimum effective exposure value in reducing separation-induced vocalizations in guinea pig pups. Therefore, it is anticipated that the minimum human unbound plasma exposures ($C_{\text{min}}$) of AZD3783 above its $K_i$ value of 12.5 nM would result in efficacious effects on anxiety and depression. Multiple-dose simulations using the predicted human pharmacokinetic parameters in Table 4 indicate that a 10 mg/day dose should achieve an estimated AZD3783 plasma $C_{\text{min}}$ of 23 nM (14 nM unbound) and $C_{\text{max}}$ of 75 nM (50 nM unbound) at steady state (Fig. 9). Substituting simulated plasma exposure between $C_{\text{min}}$ and $C_{\text{max}}$ for the blockade of 5-HT$_{1B}$ receptor occupancy range of 52 to 78% in human brain at steady state after 10 mg/day dosing (Fig. 9). Human PET study (Varnås et al., 2011) showed that at a dose of 10 mg AZD3783 exposure around $C_{\text{max}}$ resulted in 5-HT$_{1B}$ Receptor occupancy of 61 to 71% in occipital cortex and 62 to 74% in ventral striatum, respectively, confirming the simulated receptor occupancy results.

**Discussion**

Guinea pig was selected for this pharmacology study because of its high homology with human 5-HT$_{1B}$ receptor (Zgombick et al., 1997). This is supported by the similarity of results from the in vitro 5-HT$_{1B}$ receptor binding assays reported here. The in vitro 5-HT$_{1B}$ binding $K_i$ value was in
good agreement with guinea pig unbound plasma EC50 values for receptor occupancy and the blockade of 5-HT1B agonist-induced hypothermic effect. Furthermore, the doses resulting in anxiolytic-like effects in guinea pig pups were in the same range as those showing responses in hypothermia and receptor occupancy tests. Plasma exposure correlated well with AZD3783 exposure in the brain in these studies, indicating that plasma levels could be used as a reasonable approximation of the concentration of compound in the brain. These data also showed that brain 5-HT1B receptor occupancy levels of 50% or higher by AZD3783 were associated with pharmacological and behavioral effects in preclinical models, consistent with the efficacious (50–87%) receptor occupancies previously reported for other GPCR antagonists (Grimwood and Hartig, 2009). A recent PET study (Varnäs et al., 2011) showed that the average AZD3783 plasma concentrations required for 50% 5-HT1B receptor occupancy (Kiplasma) in the brains of monkeys and humans were 26 and 21 nM, respectively. Based on an unbound fraction of 66% for AZD3783 in human plasma, the unbound plasma concentration for AZD3783 required to achieve 50% 5-HT1B receptor occupancy in human brain was estimated to be 14 nM (Varnäs et al., 2011), which also aligned with AZD3783 in vitro 5-HT1B binding Ki (12.5 nM) and preclinical in vivo pharmacology data, such as the unbound plasma EC50 values for blockade of 5-HT1B agonist-induced hypothermia (18 nM) and brain receptor occupancy (11 nM) in guinea pig. The translation of in vitro binding to in vivo occupancy and functional potency in preclinical species and the alignment of these results with human PET Kiplasma values suggest that 50% or greater 5-HT1B Receptor occupancy in the brain will probably be necessary for efficacy against symptoms of anxiety and depression in human.

The estimated absorption rate constant in humans was derived directly from the observed absorption rate constant value in dogs after oral administration with the readily absorbed aqueous formulation. The very short Tmax indicated that absorption of AZD3783 in dogs was rapid. This could be partially the result of the readily absorbed aqueous formulation used in the study. However, it is also possible that the physiological properties and higher pH in dog intestine would result in more efficient absorption of the basic compound (Dressman, 1986; Chiou et al., 2000). Therefore, the predicted human absorption rate constant may be overestimated compared with the observed clinical data. Previous work on AZD3783 (Zhou et al., 2008) indicated similar metabolic pathways across species; N-demethylation was the major metabolic pathway observed in hepatocyte preparations from humans, rats, dogs, and guinea pigs; CYP3A4/5 was the major primary enzyme for the metabolism of AZD3783 with lesser contributions from CYP2C8 and CYP2D6. The extrapolated human clearance values varied depending on the prediction method used. Allometric scaling calculations from rat, dog, and monkey yielded a predicted human clearance of 25 ml/min/kg with an exponent of 1.023, or from dog single-species scaling yielding a predicted human clearance of 19 ml/min/kg, would probably overestimate the human clearance. The predicted in vivo hepatic clearances from liver microsomes and hepatocytes scaled using the well stirred in vitro scaling model were consistent with the observed in vivo plasma clearance in rat, dog, and monkey. Furthermore, similar metabolic pathways between humans and preclinical species also concurred that human clearance can be predicted from human microsome or hepatocyte intrinsic clearance parameters. The human volume of distribution predicted from multiple methods ranged from 4.2 to 7.5 l/kg. Because there was no other confirmation process to evaluate the Vdss value obtained from these methods, the average value was adopted.

This study demonstrated that in vitro binding affinity and preclinical in vivo efficacy of AZD3783 correlated well to the level of 5-HT1B receptor occupancy in the brain, suggesting that like other GPCR antagonists a 50 to 87% receptor occupancy range in human brain would be neces-

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**Table 5**  
Comparison of simulated human PK parameters and the parameters from human subjects after 20-mg oral dose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Predicted</th>
<th>Healthy Male after 20-mg Oral Dosea</th>
<th>AZD3783 Human PET Study at 20-mg Oral Dose®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmax, h</td>
<td>1.4</td>
<td>2.5 (1.5, 3)</td>
<td>2.3, 3</td>
</tr>
<tr>
<td>T1/2, h</td>
<td>13</td>
<td>17 (12, 36)</td>
<td>7.3, 10.7, 12.2</td>
</tr>
<tr>
<td>AUC0-inf, nM/h</td>
<td>1853</td>
<td>1018 (224%)</td>
<td>761, 977, 2684</td>
</tr>
<tr>
<td>Cmax, nM</td>
<td>90</td>
<td>79 (21%)</td>
<td>54, 80, 182</td>
</tr>
<tr>
<td>Cmin, nM</td>
<td>23</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Vdss, l/kg</td>
<td>6.4</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>CL, l/h/kg</td>
<td>0.39</td>
<td>0.60 (19%)</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

a n = 6, values are presented as median (minimum, maximum) value for Tmax and T1/2, geometric mean (CV%) for AUC and Cmax.

® Data are from Varnäs et al. (2011).

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**Fig. 9.** Predicted plasma exposure (solid curve) and 5-HT1B receptor occupancy profile (dotted curve) of AZD3783 in human brain after 10 mg/day oral dosing. Ki is the affinity of AZD3783 for human 5-HT1B receptor; fu is unbound fraction of AZD3783 in human plasma.
sary for an 5-HT1B antagonist to show efficacious therapeu-
tic effect. AZD3783 at 10 mg/d is expected to provide 52
to 78% receptor occupancy coverage at steady state and show a pharmacological effect against anxiety and depres-
sion in patients.

Conclusion

The preclinical pharmacodynamic and pharmacokinetic relationships reported in this study together with the clinical 5-HT1B receptor occupancy data reported by Varna¨s et al. (2011) were incorporated in our translational approach to estimate the AZD3783 dose required for antidepressant effi-
cacy in patients. The in vitro 5-HT1B Receptor affinity (Ki) of AZD3783 in guinea pig closely matched the human affinity value. The Ki values were also consistent with the guinea pig unbound plasma EC50 level for the in vivo receptor occupancy and the blockade of 5-HT1B agonist-induced hypothermia. Doses that resulted in >50% receptor occupancy and were active in hypothermia tests also resulted in the reduction of guinea pig pup separation-induced vocalizations. The predicted human 5-HT1B receptor occupancy of AZD3783 matched closely with the clinical receptor occupancy results. The excellent efficacy and receptor selectivity of AZD3783 combined with its favorable PK properties and good align-
ment of human exposure with predictions from preclinical studies make it an excellent compound for further mechanis-
tic research into the role of 5-HT1B receptor antagonism for the treatment of mood disorders. The approaches used in this article to develop a preclinical translation model for human efficacious dose prediction could be applicable to other 5-HT1B antagonists.

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tor antagonist. 


duction experiments: Zhou and Sobotka-Briner. 

Where or contributed to the writing of the manuscript: Zhang, Zhou, Wang, Maier, Widzowski, Sobotka-Briner, Broekel, Potts, Shenvi, Bernstein, and Pierson.

Conducted experiments: Zhou and Sobotka-Briner. 

Performed data analysis: Zhang, Zhou, Wang, Maier, Widzowski, Sobotka-Briner, Broekel, Potts, and Bernstein. 

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