Translational Evaluation of JNJ-18038683, a 5-Hydroxytryptamine Type 7 Receptor Antagonist, on Rapid Eye Movement Sleep and in Major Depressive Disorder

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ABSTRACT

In rodents 5-hydroxytryptamine type 7 (5-HT7) receptor blockade has been shown to be effective in models of depression and to increase the latency to rapid eye movement (REM) sleep and decrease REM duration. In the clinic, the REM sleep reduction observed with many antidepressants may serve as a biomarker. We report here the preclinical and clinical evaluation of a 5-HT7 receptor antagonist, 3-(4-chlorophenyl)-1,4,5,6,7,8-hexahydro-1-(phenylmethyl)pyrazolo[3,4-e][1,2,3]azepine 2-hydroxy-1,2,3-propanetricarboxylate (JNJ-18038683). In rodents, JNJ-18038683 increased the latency to REM sleep and decreased REM duration, and this effect was maintained after repeated administration for 7 days. The compound was effective in the mouse tail suspension test. JNJ-18038683 enhanced serotonin transmission, antidepressant-like behavior, and REM sleep suppression induced by citalopram in rodents. In healthy human volunteers JNJ-18038683 prolonged REM latency and reduced REM sleep duration, demonstrating that the effect of 5-HT7 blockade on REM sleep translated from rodents to humans. Like in rats, JNJ-18038683 enhanced REM sleep suppression induced by citalopram in humans, although a drug-drug interaction could not be ruled out. In a double-blind, active, and placebo-controlled clinical trial in 225 patients suffering from major depressive disorder, neither treatment with pharmacologically active doses of JNJ-18038683 or escitalopram separated from placebo, indicating a failed study lacking assay sensitivity. Post hoc analyses using an enrichment window strategy, where all the efficacy data from sites with an implausible high placebo response [placebo group Montgomery-Åsberg Depression Rating Scale (MADRS) < = 12] and from sites with no placebo response (MADRS > = 28) are removed, there was a clinically meaningful difference between JNJ-18038683 and placebo. Further clinical studies are required to characterize the potential antidepressant efficacy of JNJ-18038683.

Introduction

Since its identification the 5-HT7 receptor has been the subject of intense research efforts driven by its presence in functionally relevant regions of the brain and by the discovery that several nonselective antipsychotics and antidepressants currently on the market display high affinity for this receptor (Stahl, 2010; Leopoldo et al., 2011; Matthys et al., 2011). The 5-HT7 receptor is positively coupled to adenylate cyclase with a pharmacological profile distinct from all other 5-HT receptor subtypes. In the central nervous system, 5-HT7 receptor is most abundant in the cortex, hippocampus, thalamus, and hypothalamus of both humans and rodents.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); h5-HT, human 5-HT; 5-CT, 5-carboxamidotryptamine; ANOVA, analysis of variance; ASEX, Arizona Sexual Experience Scale; AUC, area under the curve; DOI, (−)-2,5-dimethoxy-4-iodoamphetamine; EEG, electroencephalogram; EMG, electromyogram; FLIPR, fluorescence imaging plate reader; HAMD, Hamilton Rating Scale for Depression; HEK, human embryonic kidney; JNJ-18038683, (3-(4-chlorophenyl)-1,4,5,6,7,8-hexahydro-1-(phenylmethyl)pyrazolo[3,4-e][1,2,3]azepine 2-hydroxy-1,2,3-propanetricarboxylate); MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder; PSG, polysomnogram; REM, rapid eye movement; NREM, non-REM sleep; RO-4368554, 3-benzenesulfonyl-7-(4-methyl-piperazin-1-yl)1H-indole; SB-269970, (2R)-1-{[3-hydroxyphenyl]sulfonfonyl}-2-[2-(4-methyl-1-piperidinyl)ethyl]-pyrrolidine; SSRI, selective serotonin reuptake inhibitor.
of individually sub efficacious doses of citalopram and JNJ-18038683 on REM sleep, immobility time in the tail suspension test, and 5-HT release in the prefrontal cortex of freely moving rats.

After appropriate 1-month Good Laboratory Practice toxicological experiments in rat and dog, we received Investigational New Drug approval from the Food and Drug Administration to conduct a safety, tolerability, and pharmacokinetic study in healthy human volunteers. Two polysomnography studies were carried out in healthy subjects to evaluate the effect of the 5-HT7 receptor antagonist on REM sleep by itself or in combination with citalopram. After this translational study, a multicentered, randomized, double-blind, placebo, active-controlled, parallel-arm study was run to assess the efficacy of JNJ-18038683 in patients with major depressive disorder.

Materials and Methods

All preclinical studies have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) as adopted and promulgated by the National Institutes of Health.

All clinical studies were conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and are consistent with Good Clinical Practices and applicable regulatory requirements. After receiving a complete description of the study and understanding the potential risks and benefits, all healthy subjects/patients gave signed informed consent.

Animals

The tail suspension test and locomotor activity measurements were performed in male C57BL/6J mice weighing 22 to 30 g (The Jackson Laboratory, Bar Harbor, ME). The rat telemetry study (blockade of 5-CT-induced hypothermia) was performed in female Sprague-Dawley rats weighing 300 to 325 g (Charles River Laboratories, Inc.). Sleep/EEG experiments were performed in male Sprague-Dawley rats weighing 400 to 500 g (Harlan, Indianapolis, IN).

Animals were allowed to acclimate for at least 7 days after receipt in the facility before investigations. They were housed in accordance with institutional standards, provided food and water ad libitum, and maintained on a 12-h light/dark cycle (lights on 6:00 AM to 6:00 PM).

Chemicals

JNJ-18038683 was synthesized and prepared as a salt at Jansen Research & Development, LLC. [3H]5-CT was purchased from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). 5-HT and SB-269970 were obtained from Tocris Bioscience (Ellisisville, MO). Citalopram (HBr salt) was purchased from Sigma (St. Louis, MO). For in vitro assays, JNJ-18038683 and SB-269970 was dissolved in dimethyl sulfoxide (stock solution at 10 mM) and further diluted in assay buffer.

For the preclinical studies JNJ-18038683 was formulated in hydroxypropyl methylcellulose (telemetry blockade of 5-HT-induced hypothermia and sleep EEG, oral administration), 0.9% saline (telemetry blockade of 5-HT-induced hypothermia and tail suspension test, intraperitoneal administration), or 5% Pharmasolve (Sigma) and 95% D5W (Baxter, McGaw Park, IL) (5% dextrose solution) (sleep EEG and microdialysis, subcutaneous administration). Citalopram was formulated in 0.9% saline for the tail suspension test (intraperitoneal administration) or 5% Pharmasolve and 95% D5W (5% dextrose solution) for sleep EEG and microdialysis (subcutane-
ous administration). For in vivo preclinical work, SB-269970 was formulated in 5% dextrose.

For clinical studies, JNJ-18038683 was formulated as an oral suspension in 0.5% (w/w) hypromellose solution.

**Primary In Vitro Pharmacology: 5-HT₇ Receptor Binding and 5-HT₃ Receptor Antagonism**

The affinity of JNJ-18038683 for the rat 5-HT₇ recombinant receptor was determined by competition binding assays using [³H]5-CT as described previously (Hagan et al., 2000; Thomas et al., 2000). The assays were performed on membranes prepared from HEK-293 cells stably transfected with the rat 5-HT₇ receptor or membranes prepared from rat thalamus tissue. Sigmoidal inhibition curves were generated and fitted by nonlinear regression analysis (Prism; GraphPad Software, Inc., San Diego, CA). IC₅₀ values (concentration producing 50% inhibition of specific radioligand binding) were calculated. Kᵣ values were derived according to Cheng and Prusoff (1973). Experiments were conducted in triplicate, and at least three separate experiments were run. SB-269970, a standard compound, was included for comparison purposes. Results were later confirmed against the human 5-HT₇ receptor.

In vitro selectivity of JNJ-18038683 versus a panel of monoamine receptors and neurotransmitter uptake sites was performed by using standard radioligand assays as described previously (Schotte et al., 1996). In addition, the selectivity of JNJ-18038683 (1 μM) was evaluated on a commercially available panel of 50 ion channels, transporters, and receptor binding assays at Cerep (Celles L’Evescault, France). Experimental conditions for these assays are described online at www.cerep.fr.

The potency of JNJ-18038683 on the rat 5-HT₇ recombinant receptor was determined with the HitHunter cAMP Assay kit (DisceoRx, Fremont, CA). In brief, HEK-293 cells stably expressing the rat 5-HT₇ receptor were preincubated for 10 min with seven concentrations of JNJ-18038683 (10 nM-100 pM) followed by incubation with 100 nM of the agonist (5-HT). cAMP measurements were determined according to the protocol described in the HitHunter kit. SB-2699970 was used as the reference compound. Results were later confirmed against the human 5-HT₇ receptor. Antagonistic potency values were converted to apparent pKᵣ values by using a modified Cheng-Prusoff correction: apparent pKᵣ = −log IC₅₀/μM + [concentration agonist/EC₅₀].

In addition, in vitro functional assays were run for h5-HT₁B (cAMP measurement), h5-HT₁A (calcium release using FLIPR), h5-HT₃A (calcium release using FLIPR), and h5-HT₃C (calcium release using FLIPR) as described previously (Lesage et al., 1998; Porter et al., 1999).

**Primary In Vivo Pharmacology: Blockade of 5-CT-Induced Hypothermia in Rat**

Rat telemetry experiments were performed as described previously (Hedlund et al., 2004). In brief, telemetry devices were implanted in the peritoneal cavity of rats. After a 7-day recovery period, body temperature was measured noninvasively by radiotelemetry. On the day of the experiment, the baseline was monitored for 60 min before drug injection. Rat core temperature was continuously recorded, before and after injection, and averaged over each 2-h collection period. JNJ-18038683 (0.09, 0.9, and 9.0 mg/kg) was administered orally 6 h before 5-CT (0.1 mg/kg, i.p.) administration or intraperitoneally (0.027, 0.09, 0.18, 0.27, and 0.9 mg/kg) 20 min before 5-CT (0.1 mg/kg, i.p.) administration (n = 5 in each group). Change in core body temperature was calculated for all animals by comparing baseline before dosing to the minimal temperature reached after drug administration. To quantify any statistical difference, one-way analysis of variance (ANOVA) followed by Dunnett’s test was conducted by using Prism software (GraphPad Software, Inc.), and the level of significance was P < 0.05.

**Sleep EEG in Rat**

Sleep EEG experiments in rats were performed as described previously (Bonaventure et al., 2007). Animals were chronically implanted with electrodes for the recording of EEG and electromyogram (EMG) signals by using telemetric devices (Data Sciences International, St. Paul, MN). Polysomnographic waveforms were analyzed per 10-s epoch and classified as wake, nonrapid eye movement (NREM), or REM sleep by using the computer program SleepSign (Kissei Comtec, Nagano, Japan). For each experiment, EEG and EMG signals were recorded for up to 16 h after administration of the tested compounds at the beginning of the light phase (2 h after light onset). Results were then averaged and expressed as mean ± S.E.M. in defined time intervals.

The dose-response experiment with JNJ-18038683 was carried out in 32 animals that were randomly assigned to four treatment conditions (three doses and vehicle; n = 8 per condition). Subsequent statistic analysis of the obtained data were conducted by using Wilcoxon-Mann-Whitney rank sum tests in comparison with the vehicle group. The drug combination experiment (coadministration of citalopram and JNJ-18038683) was performed in a separate group of nine animals that received the four treatment conditions (the two compounds or their corresponding vehicle). To determine whether differences were significant between the four conditions a one-way ANOVA followed by a Newman-Keuls post hoc test was conducted. Differences were determined to be significant if P < 0.05.

The repeated dosing experiment with JNJ-18038683 for 7 days was performed in a separate group of six animals. A paired one-way ANOVA followed by a Dunnett’s multiple comparison post hoc test was executed to assess the differences between vehicle and compound treatment each day.

**Tail Suspension Tests in Mice**

Mice were dosed intraperitoneally 30 min before testing (n = 8 in each group). Experiments were performed as described previously (Bonaventure et al., 2007). The time spent immobile was totaled for the last 4 min of the 6-min test for each animal, averaged for the dose group, and then compared. Statistics were calculated by using Prism software (GraphPad Software, Inc.). The data were presented as the mean ± S.E.M. and evaluated by one-way ANOVA followed by Newman-Keuls multiple comparison test. The level of significance was P < 0.05.

The effect of JNJ-18038683 on locomotor activity was also measured as described previously (Bonaventure et al., 2007). To quantify any statistical difference, one-way ANOVA followed by Newman-Keuls test was conducted by using Prism software (GraphPad Software, Inc.), and the level of significance was P < 0.05.

**Microdialysis in Rat**

Microdialysis in the prefrontal cortex (incisor bar, −3.5 mm, +3.2 mm anterior, 0.8 mm lateral, and 1 mm ventral to Bregma) (Paxinos and Watson, 1997) of freely moving rats (n = 4 in each group) was performed as described previously (Bonaventure et al., 2007). Dialysis samples were analyzed for serotonin, norepinephrine, and dopamine by high-performance liquid chromatography with electrochemical detection. Statistical analyses were performed on the area under the curve (AUC) values by a one-way ANOVA followed by Newman-Keuls multiple comparison test. The level of significance was P < 0.05.

Data were graphed and statistics were calculated by using Prism software (GraphPad Software, Inc.).

**Clinical Studies**

**Polysomnography Studies.** Two clinical studies were performed to investigate the effect of JNJ-18038683 on the human polysomnogram (PSG): study 1 was a blinded, placebo- and comparator-controlled study to investigate the safety, tolerability, pharmacokinetics, and pharmacodynamics of JNJ-18038683 in healthy male subjects, and study 2 was a double-blinded, placebo-controlled, ran-
domized, parallel-group, multiple-dose study to investigate the safety, tolerability, pharmacokinetics, and pharmacodynamics of JNJ-18038683 concomitantly administered with citalopram in healthy male subjects. Both studies were approved by the appropriate local ethics committees before the initiation of any study-related procedure. In total, 48 healthy male subjects who had signed the informed consent form were included.

**Study 1.** Study 1 was performed at Guy’s Drug Research Unit, Quintiles Ltd (London, UK). Initially, 12 subjects (cohort 1) who received a 100-mg loading dose of JNJ-18038683 on day 1 followed by 20 mg of JNJ-18038683 on days 2 and 3, 20 mg of escitalopram on days 1 to 3, and placebo on days 1 to 3 in a three-way crossover study were included. Twelve additional subjects were included in cohort 2 to investigate the effect of JNJ-18038683 at up to 4-fold lower dose levels. Cohort 2 results are not further described but suggest that lower JNJ-18038683 dose levels are efficacious. PSG was recorded three times on separate study days by using Medilog (Belmont, Australia) ambulatory recorders using silver/silver chloride stick-on electrodes placed according to the international 10–20 system. PSG recordings were reviewed by an EEG study scientist while blind to treatment allocation. Results obtained overnight after the last dose administration (days 3–4) were considered primary effect parameters. Between study periods, there was a washout period of at least 14 days. REM sleep after dosing was analyzed by repeated-measures ANOVA on days 1/2 and 3/4.

**Study 2.** Study 2 was performed at QPS Netherlands (Groningen, The Netherlands). Twenty-four subjects received 20 mg of citalopram once daily from days 1 to 17. On day 8, subjects were randomized to receive either 20 mg of JNJ-18038683 once daily (n = 12) or placebo (n = 12) in addition to citalopram. PSG was recorded overnight on days 1 to 1 (baseline), 1 to 2, 7 to 8, 10 to 11, and 17 to 18. PSG was recorded by using TMS (Oldenzaal, The Netherlands) ambulatory recordings using silver/silver chloride stick-on electrodes placed according to the international 10–20 system. PSG recordings were reviewed by an EEG study scientist while blind to treatment allocation. Subjects were hospitalized from days 8 to 22. While hospitalized, regular assessments of safety and tolerability were obtained. Blood samples were collected for the measurement of JNJ-18038683 plasma concentrations at regular time points on days 8 and 11 and for the measurement of S- and R-citalopram on day 17. The following pharmacokinetic parameters were derived from JNJ-18038683 and citalopram plasma concentrations: plasma concentration measured immediately before dosing (C$_{\text{predose}}$); observed maximum plasma concentration after first (day 8, JNJ-18038683 only) and last dose (C$_{\text{max}}$); area under the plasma concentration-time curve from 0 to 24 h after the first (day 8, JNJ-18038683 only) and last dose (AUC$_{0-24h}$); and the ratio of the C$_{\text{max}}$ and AUC$_{0-24h}$ of the last dose over the first dose multiplied by 100% (P$_{\text{rel}}$ JNJ-18038683 only).

REM-onset latencies after dosing were analyzed by ANOVA on days 10/11 and 17/18. The ANOVA used treatment, day, and treatment-by-day interaction as factors and days 7/8 REM latency as the baseline covariate.

### Major Depressive Disorder Study

A multicenter, double-blind, randomized, double-dummy, placebo and active controlled, parallel-design study in subjects with moderate to severe MDD was conducted to evaluate the safety, tolerability, and efficacy of JNJ-18038683 as an antidepressant. A total of 230 men and women diagnosed with moderate to severe MDD (based on a HAMD-17 score ≥25), aged 18 to 60 years, who met the inclusion and exclusion criteria participated in the study. This study consisted of a screening period (up to 1 week), a washout period (7 days or 5 half-lives of the drug to be washed out, whichever was shorter), 7 weeks of treatment, a 1-week follow-up period during which subjects who received escitalopram received a reduced dose and subjects who received JNJ-18038683 received placebo, and a follow-up visit at the end of the follow-up period.

Subjects were randomly assigned to receive a treatment regimen of 20 mg of JNJ-18038683, 20 mg of escitalopram, or placebo in a 1:1:1 fashion. For the JNJ-18038683 treatment group, subjects started at doses of 10 mg once daily for the first week (week 1), which was increased to 20 mg once daily for the remaining 6 weeks (weeks 2–7). The JNJ-18038683 treatment group received placebo during the follow-up period (week 8).

In the escitalopram treatment group, subjects started dosing at 10 mg once daily for the first week (week 1), and during the second week (week 2) the dose increased to 20 mg once daily for the remaining 6 weeks (weeks 2–7). During week 8, the escitalopram treatment group received doses of 10 mg once daily to taper off the medication in accordance with the escitalopram label.

The efficacy analysis set for all analyses was based on the intent-to-treat population, including all randomized subjects who received at least one dose of study treatment and had at least one postbaseline MADRS total score measurement.

The sample size was calculated based on the primary endpoint (change in MADRS total score from baseline to week 7). The desired sample size for each arm was approximated by using a two-sample t-test with a two-sided level equal to 0.05, to produce an 80% power to detect a between-group difference (to placebo) of 4 with a S.D. of 8.4. Assuming a drop-out rate of 5% by the end of week 1 (titration period), a total of 225 (75 per arm) subjects were to be enrolled to ensure the availability of approximately 71 subjects per arm with at least one postbaseline efficacy measurement.

The primary efficacy variable was change from baseline to week 7 in MADRS total score. Key secondary efficacy endpoints included change from baseline to week 7 in the Clinical Global Impressions Scale and week 7 proportion of MADRS responders (≥50% improvement from baseline in MADRS total score). Other secondary efficacy endpoints included change from baseline to week 7 in MADRS-6, HAMD-17, HAMD-6 (O'Sullivan et al., 1997), Epworth Sleepiness Scale, Global Assessment of Function, and Sleep Assessment scores (items 1–4), as well as the week-7 proportion of HAMD-17 responders (subjects who had at least a 50% improvement from baseline).

Safety assessments included reported adverse events, clinical laboratory tests, vital signs measurements, physical examinations, electrocardiogram (ECG) findings, suicide monitoring using the Suicide Tracking Scale, and the Arizona Sexual Experience Scale (ASEX). All safety analyses were performed based on the safety analysis set, which includes all subjects who were randomly assigned to a treatment and received at least one dose of a study drug.

The statistical significance level for the primary endpoint comparison of JNJ-18038683 versus placebo was at 5% (two-sided test). The comparison of escitalopram versus placebo (5%; two-sided) based on the primary endpoint was carried out for the purpose of assay sensitivity; therefore, no multiplicity adjustment was required for these two comparisons.

To control the type I error inflation introduced by the two key secondary efficacy endpoints, a step-down procedure was used without further adjustment of test multiplicity: the statistical test based on the week-7 change in the Clinical Global Impressions Scale score was performed given the significance of the test using the primary endpoint (week-7 change in MADRS score), and the statistical test based on MADRS responders was performed given the significance of the test using the week-7 change in CGI-S score. In addition, for exploratory purposes, both key secondary efficacy endpoints and other secondary endpoints were compared with placebo separately, and no multiplicity adjustment was performed.

### Results

**JNJ-18038683 Is a High-Affinity Relatively Selective 5-HT$_7$ Receptor Antagonist**

The affinity of JNJ-18038683 for the rat and human 5-HT$_7$ receptor binding site was evaluated by competition radiol-
Translational Evaluation of a 5-HT7 Receptor Antagonist

JNJ-18038683 Blocks 5-CT-Induced Hypothermia in Rats

The functional 5-HT7 antagonist activity of JNJ-18038683, given orally or intraperitoneally, was assessed by using the 5-CT-induced hypothermia model in conscious rats (Supplemental Fig. 2). Pharmacokinetic studies in rats had shown that, when given orally, JNJ-18038683 exhibited slow absorption characteristics with a maximum concentration (Cmax) observed approximately 6 h after dosing (data not shown). Thus, JNJ-18038683 (0.09–9.0 mg/kg) was administered orally 6 h before 5-CT (0.1 mg/kg i.p.) administration. JNJ-18038683 antagonized the 5-CT-induced hypothermia in a dose-related manner after either oral or intraperitoneal administration (Supplemental Fig. 2). In this assay, JNJ-18038683 was equipotent (ED50 = 0.40 ± 0.2 mg/kg) to SB-269970 (ED50 = 0.3 ± 0.2 mg/kg; data not shown). The plasma concentration of JNJ-18038683 after oral or intraperitoneal administration of the 0.9 mg/kg dose, which produced a near full blockade of 5-CT-induced hypothermia, was approximately 20 to 40 ng/ml as determined in parallel studies (data not shown).

The results of the in vitro binding studies show that JNJ-18038683 has some affinity for α1 adrenergic and 5-HT2A receptors (Supplemental Table 1). The functional consequences of these were evaluated in vivo, in phenylephrine-induced mydriasis in mice (a model of α1 adrenergic activity) and antagonism of (+)-2,5-dimethoxy-4-iodoamphetamine (DOI)-induced head twitches in mice (a standard test for 5-HT2A antagonism). At plasma concentrations that nearly fully blocked 5-CT-induced hypothermia in rats, JNJ-18038683 did not have any effect on phenylephrine-induced mydriasis or DOI-induced head twitches.

JNJ-18038683 Preclinical Profile as an Antidepressant

REM Sleep-Suppressive Effect in the Rat. Dose-response effects of JNJ-18038683 (1, 3, and 10 mg/kg) on sleep-wake parameters were evaluated in rats orally dosed at the beginning of the light phase. As illustrated in Fig. 1, JNJ-18038683 dose-dependently suppressed REM sleep mainly during the first 4 h after the treatment. The duration of REM sleep was significantly decreased from the dose of 1 mg/kg onward (P < 0.05) during the first 4 h after oral administration (Fig. 1A). Concomitantly, the REM sleep latency tended to be prolonged in a dose-related manner with a significant increase in REM latency occurring only at the highest dose tested (10 mg/kg; P < 0.05; Fig. 1B). These alterations in REM sleep seemed to be state-specific, because no major changes in the duration of either NREM sleep or wake were observed (data not shown). JNJ-18038683 did not induce changes in spontaneous activity, heart rate, blood pressure, or body temperature in rats at doses that clearly affected sleep-wake organization (data not shown).

A separate study was conducted to determine whether repeated administration of JNJ-18038683 for 7 days would result in an adaptation of the EEG sleep response in particular on REM sleep in rats during the course of the treatment and after its discontinuation. JNJ-18038683 was administered for 7 consecutive days (1 mg/kg s.c. per day) at 2 h into
the light phase. EEG and EMG signals were recorded on vehicle control day, on days 1, 3, 5, and 7 of compound administration, and on 2 consecutive recovery days (R1 and R2 after vehicle injection). On the first day of treatment, JNJ-18038683 produced a significant decrease in the time spent in REM sleep during the first 8 h after the injection (Fig. 1C) and a prolongation of the REM sleep latency (Fig. 1D). The REM sleep latency was increased during the 7-day repeated treatment and was normalized on the first recovery day after cessation of treatment. The significant decrease in REM sleep time was maintained during the 7-day repeated treatment, with a rebound occurring on the first recovery day after treatment discontinuation. The NREM sleep latency and the total NREM sleep time were not affected during the entire treatment (data not shown).

Antidepressant-Like Activity in the Mouse Tail Suspension Test. Antidepressant-like activity was investigated in the tail suspension test (Fig. 2). JNJ-18038683 administered intraperitoneally at doses of 0.3, 0.5, and 1 mg/kg significantly decreased the immobility time compared with vehicle-treated mice by 31% (P < 0.05 versus vehicle), 39% (P < 0.01 versus vehicle), and 59% (P < 0.01 versus vehicle), respectively (Fig. 2). JNJ-18038683 (1 mg/kg i.p.) did not significantly change locomotor activity compared with vehicle (P > 0.05 versus vehicle; data not shown).

The Effect of the Combination of Subefficacious Doses of Citalopram and JNJ-18038683 on REM Sleep, 5-HT Transmission, and Antidepressant-Like Behavior. In a previous study performed with SB-269970 we had shown that blockade of 5-HT7 receptor enhanced REM sleep suppression, antidepressant-like behavior, and 5-HT transmission induced by citalopram in rodents (Bonaventure et al., 2007). Therefore, we evaluated the effect of a combination of individually subefficacious doses of citalopram and JNJ-18038683 on REM sleep, immobility time in the tail suspension test, and 5-HT release in the prefrontal cortex of freely moving rats (Fig. 3).

To determine the subefficacious dose of test compounds on REM sleep latency and duration a pilot study was conducted with citalopram (1 mg/kg s.c.) and JNJ-18038683 (0.3 mg/kg s.c.). These doses were found to elicit minimal effects on EEG sleep parameters in rats. Citalopram induced a moderate, but significant, REM sleep inhibition as evidenced by an increase in REM sleep latency (P < 0.05; Fig. 3A) and a decrease in REM sleep duration (P < 0.05, Fig. 3B) during the first 8 h after administration compared with vehicle treatment. JNJ-18038683 had no effect when injected alone
but did potentiate the REM sleep-suppressive effect of citalopram. Compared with citalopram alone, the combination of citalopram with JNJ-18038683 produced an additional delay of the latency to REM sleep (+60 min; \( P < 0.001 \); Fig. 3A) and further reduced the time spent in REM sleep (−12 min; \( P < 0.001 \); Fig. 3B). At the doses tested in this study, citalopram and JNJ-18038683 injected either alone or in combination did not influence the NREM sleep latency and duration or the time spent awake.

A tail suspension test experiment was performed with a combination of subefficacious dose of citalopram and JNJ-18038683 (Fig. 3C). Citalopram (1 mg/kg i.p.) or JNJ-18038683 (0.1 mg/kg i.p.) alone did not significantly change immobility time compared with vehicle-treated mice (Fig. 3C). In contrast, coadministration of citalopram (1 mg/kg i.p.) and JNJ-18038683 (0.1 mg/kg i.p) significantly decreased the immobility time versus vehicle \( (P < 0.001) \). The combination of JNJ-18038683 and citalopram had no effect on locomotor activity.

The effect of various doses of citalopram alone (1, 3, 5, and 10 mg/kg i.p.) or in combination with a fixed dose of JNJ-18038683 (1 mg/kg i.p.) on immobility time was also tested (data not shown). The effect of citalopram on immobility time was significantly enhanced by coadministration of 1 mg/kg JNJ-18038683 \((21 \text{ versus } 49\% , \ P < 0.001; 36 \text{ versus } 70\% , \ P < 0.001; 55 \text{ versus } 80\% , \ P < 0.001; 74 \text{ versus } 84\% , \ P < 0.05)\).

The effect of a combination of subefficacious doses of citalopram and JNJ-18038683 on 5-HT release in cortex was tested by using microdialysis in freely moving rats (Fig. 3D). A dose response of citalopram was tested, and 0.05 mg/kg was determined to be the subefficacious dose on prefrontal 5-HT release. Absolute basal levels of serotonin, dopamine, and norepinephrine in dialysate from the rat frontal cortex (without adjusting for probe recovery) were 0.048 ± 0.003 pg/μl \((n = 16)\), 0.089 ± 0.005 pg/μl \((n = 16)\), and 0.231 ± 0.016 pg/μl \((n = 16)\), respectively.

Subcutaneous injection of JNJ-18038683 (1 mg/kg) did not increase extracellular 5-HT concentration compared with vehicle-treated rats (Fig. 3D). Coadministration of an ineffective dose of citalopram (0.05 mg/kg s.c.) with JNJ-18038683 (1 mg/kg s.c.) resulted in a significant increase in extracellular concentrations of 5-HT \( (P < 0.001) \). Neither citalopram (0.05 mg/kg s.c.), JNJ-18038683 (1 mg/kg s.c.), nor coadministration of citalopram and JNJ-18038683 induced significant change in extracellular concentration of dopamine or norepinephrine (data not shown).

Good Laboratory Practice toxicological studies were conducted in rats and dogs and indicated that JNJ-18038683 has a suitable safety profile to allow testing in humans. JNJ-18038683 was well tolerated in single- and multiple-dose studies of up to 13 weeks in duration. In both rats and dogs, the primary toxicities were neurologic (ptosis and clinical signs of sedation) or gastrointestinal (decreased food consumption, salivation, emesis, fecal changes). In standard tests to assess embryo-fetal safety, genotoxicity, and phototoxicity potential, JNJ-18038683 was also shown to be well tolerated.

During the single-dose and multiple ascending-dose studies, no serious adverse events were reported, and most adverse events were mild, indicating that JNJ-18038683 is well tolerated in healthy volunteers.

**JNJ-18038683 Reduces REM Sleep Duration in Healthy Human Volunteers**

**Study 1.** In the preclinical pharmacology models, the dose for maximal inhibition of REM sleep was measured at plasma concentrations of 20 to 40 ng/ml. Pharmacokinetic modeling indicated that a loading dose of 100 mg of JNJ-18038683 on day 1 followed by two maintenance doses of 20 mg on days 2 and 3 predicted plasma concentrations >20 ng/ml on average throughout the dosing interval.

Twelve subjects (19–41 years) eligible by medical history and screening examination who signed the informed consent form were included in cohort 1. All subjects completed the study. Study treatments were well tolerated overall, and the nature of treatment-emergent adverse events was not different between the different treatment groups.

Both JNJ-18038683 (100/20/20 mg) and citalopram (20 mg) significantly increased the time to the first REM sleep epi-
sode relative to placebo (Table 2). Total REM sleep time was also significantly reduced after both JNJ-18038683 and citalopram (Table 2).

The following JNJ-18038683 plasma pharmacokinetic parameters were measured on day 3 after administration of a loading dose of 100 mg on day 1 followed by 20 mg on days 2 and 3: $T_{\text{max}} = 6$ h; $C_{\text{max}} = 56.7 \pm 14.6$ ng/ml; and $AUC_{0-24h} = 1222 \pm 343$ ng \cdot h/ml.

### Study 2

Twenty-four healthy subjects (18–55 years) eligible by medical history and screening examination who signed the informed consent were included. All subjects completed the study. Overall, the incidence and nature of the adverse events were not significant between the two treatment groups and were mild to moderate in intensity.

Plasma concentrations of JNJ-18038683 accumulated over the 10-day dosing period ($C_{\text{max}}$, 4-fold; $AUC_{0-24h}$, 4.8-fold). Day-17 JNJ-18038683 plasma exposures were comparable with those measured after administration of 100/20/20 mg of JNJ-18038683 in study 1. The peak-trough variation on day 17 was approximately 1.6-fold. In the presence of JNJ-18038683 $C_{\text{max}}$ and $AUC_{0-24h}$ values for S- and R-citalopram seemed relatively increased compared with the placebo condition (Table 3).

REM-onset latency in subjects treated with placebo plus 20 mg of citalopram (group I) increased from $86 \pm 10.44$ min (mean \( \pm \) S.E.M) at baseline to maximally 176.18 \( \pm \) 16.40 min on days 7/8 (Fig. 4A). Relative to days 7/8, the REM-onset latency was somewhat decreased on days 10/11 and 17/18 with --19.4 and 33.4 min, respectively. In subjects treated with 20 mg of JNJ-18038683 plus 20 mg of citalopram (group II) the increase in REM-onset latency from baseline to days 7/8 (citalopram-only treatment) was comparable with subjects assigned to group I: from 69.9 \( \pm \) 7.56 min on days 1/1, 200.33 \( \pm \) 25.79 min on days 10/11 and 214.88 \( \pm \) 32.27 min on days 17/18 in group II subjects (Fig. 4A). Compared with group I, the relatively larger increase in REM-onset latency on days 17/18 in subjects treated with 20 mg of JNJ-18038683 + 20 mg of citalopram was borderline significant ($P = 0.0678$).

In both groups of subjects, total REM time (Fig. 4B) was reduced relatively to baseline on days 1/2 \((22.1 \pm 0.12)$ min with a rebound on days 7/8 (during which period only citalopram was administrated): group I (placebo plus 20 mg of citalopram): 103.18 \( \pm \) 6.36 (days 1/1), 58.73 \( \pm \) 3.40 (days 1/2), and 93 \( \pm \) 5.56 (days 7/8); group II (20 mg of JNJ-18038683 plus 20 mg of placebo): 103.3 \( \pm \) 8.59 (days 1/1), 53.1 \( \pm \) 5.14 (days 1/2), and 80.18 \( \pm \) 6.66 (days 7/8). Compared with combined treatment with placebo plus 20 mg of citalopram, treatment with 20 mg of JNJ-18038683 plus 20 mg of citalopram significantly suppressed total REM duration on days 10/11 (\(-52.46\) min; $P = 0.0071$) and 17/18 (\(-49.5\) min; $P = 0.0136$).

### Depression Study: No Significant Difference from Placebo

Neither JNJ-18038683 nor the active comparator escitalopram demonstrated a statistically significant improvement over placebo in the primary efficacy endpoint (Fig. 5). The mean change from baseline to week 7 in total MADRS score was \(-13.8\) for the placebo group, \(-15.2\) for the JNJ-18038683, and \(-13.5\) for the escitalopram group. In addition, neither JNJ-18038683 nor escitalopram achieved statistical significance over placebo in any secondary measure of efficacy (Supplemental Table 2). This was a failed study because of a lack of assay sensitivity, rather than a negative study.

### Table 2

Duration of REM sleep onset and total time spent in REM sleep after treatment with placebo, citalopram (20 mg), and JNJ-18038683 (100/20/20 mg) for 3 days measured in healthy subjects ($n = 12$).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>REM Onset Latency (min)</th>
<th>Total REM Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predose</td>
<td>63.83 ( \pm ) 4.51</td>
<td>112.33 ( \pm ) 6.25</td>
</tr>
<tr>
<td>Placebo</td>
<td>93.83 ( \pm ) 15.15</td>
<td>98.33 ( \pm ) 7.24</td>
</tr>
<tr>
<td>JNJ-18038683</td>
<td>63.25 ( \pm ) 5.11</td>
<td>104.21 ( \pm ) 7.81</td>
</tr>
<tr>
<td>Days 1/2</td>
<td>60.58 ( \pm ) 3.61</td>
<td>113.00 ( \pm ) 7.69</td>
</tr>
<tr>
<td>Placebo</td>
<td>154.83 ( \pm ) 22.77 (P = 0.0003)</td>
<td>62.5 ( \pm ) 5.62 (P &lt; 0.0001)</td>
</tr>
<tr>
<td>JNJ-18038683</td>
<td>101.33 ( \pm ) 16.14 (P = 0.0207)</td>
<td>68.08 ( \pm ) 6.71 (P &lt; 0.0001)</td>
</tr>
<tr>
<td>Days 3/4</td>
<td>57.67 ( \pm ) 5.64</td>
<td>115.58 ( \pm ) 8.09</td>
</tr>
<tr>
<td>Placebo</td>
<td>188 ( \pm ) 28.65 (P &lt; 0.0001)</td>
<td>69.73 ( \pm ) 10.62 (P = 0.0001)</td>
</tr>
<tr>
<td>JNJ-18038683</td>
<td>85.17 ( \pm ) 7.07 (P &lt; 0.00097)</td>
<td>90.75 ( \pm ) 7.57 (P = 0.0256)</td>
</tr>
</tbody>
</table>
rhea, vomiting, back pain, upper respiratory tract infection, palpitations, and fatigue. The only reported case of euphoric mood occurred in the placebo group.

There were no clinically significant changes in laboratory values or physical examination findings in the three treatment groups.
like behavior, and REM suppression induced by citalopram.

JNJ-18038683 enhanced 5-HT transmission, antidepressant-like behavior, and REM suppression induced by citalopram in rodents. Nonclinical safety studies indicated that JNJ-18038683 has a suitable safety profile to allow testing in humans. JNJ-18038683 was well tolerated in humans, and the effect of 5-HT7 blockade on REM sleep translated from rodent to humans, demonstrating target engagement. The augmentation of citalopram effect on REM sleep by JNJ-18038683 was also observed in humans, but citalopram plasma concentration was also increased. Finally, the compound was evaluated in a depression clinical trial, but the lack of separation observed with the positive control group indicated a failed study, thus rendering interpretation of the lack of separation in the JNJ-18038683 group data inconclusive.

The recent availability of selective 5-HT7 receptor antagonists and 5-HT7 receptor knockout mice has considerably advanced the understanding of the physiological function of this receptor in rodents (Leopoldo et al., 2011; Matthys et al., 2011). SB-269970 is the most commonly used compound in the literature, but it has a relatively short half-life in preclinical species and poor drug-like properties (Hagan et al., 2000). We set out to identify a suitable 5-HT7 receptor antagonist for clinical studies. After a high-throughput screening campaign and a medicinal chemistry effort, JNJ-18038683 was synthesized and characterized. In vitro, the JNJ-18038683 has similar affinity/potency for the human and rat 5-HT7 receptor compared with SB-269970. The functional 5-HT7 antagonist activity of JNJ-18038683 was demonstrated by using the 5-CT-induced hypothermia model in conscious rats. JNJ-18038683 efficacy seems to correlate well with the Cmax, thus demonstrating a pharmacokinetic/pharmacodynamic relationship. JNJ-18038683 was equipotent to SB-269970 after intraperitoneal administration. JNJ-18038683 was also found to be efficacious after oral administration of a low dose in this model. The results of the in vitro binding studies show that JNJ-18038683 has moderate affinity for α1 adrenergic 5-HT2A and 5-HT6 receptors. However, the in vivo functional consequences of this cross-reactivity at α1 and 5-HT2A receptor were minimal, and a margin more than 50-fold was established by using the antagonism of phenylephrine-induced mydriasis and antagonism of DOI-induced head twitch models, respectively. The 5-HT6 receptor has not been associated with any clinical liability, and several selective 5-HT6 receptor ligands entered clinical development as potential antidepressant, antipsychotic, and antiobese drugs (Marazziti et al., 2011). A noteworthy example, 3-benzensulfonyl-7-(4-methyl-piperazin-1-yl)1H-indole (RO-4368554), a selective 5-HT6 receptor antagonist, has no effect on REM sleep in rats (Moraity et al., 2008).

Mean heart rate was slightly elevated in the JNJ-18038683 group as measured by both vital signs and ECG. Mean supine-to-standing diastolic blood pressure decreased slightly in the JNJ-18038683 group, but no subject in the JNJ-18038683 group met the criteria for orthostatic hypotension as predefined in the statistical analysis plan (decrease in systolic blood pressure > 20 mm Hg or diastolic BP > 10 mm Hg and an increase in heart of >15 bpm) at any time point.

Mean changes from baseline in suicide tracking scores were similar for all treatment groups. Mean (ASEX) scores for men and women in the placebo and JNJ-18038683 treatment groups were decreased compared with baseline, and mean ASEX scores for men and women in the escitalopram group were increased compared with baseline (higher score indicates increased sexual dysfunction).

**Discussion**

It has been proposed that the selective blockade of 5-HT7 receptors may be a novel therapeutic approach for achieving antidepressant action (Stahl, 2010). Here, we report the preclinical and clinical characterization of JNJ-18038683, the first relatively selective small-molecule 5-HT7 receptor antagonist with suitable drug-like properties for examination in humans. In rodents JNJ-18038683 increased the latency to REM sleep and decreased REM duration. The compound was also effective in the mouse tail suspension test. In addition, JNJ-18038683 enhanced 5-HT transmission, antidepressant-like behavior, and REM suppression induced by citalopram in rodents. Nonclinical safety studies indicated that JNJ-18038683 is well tolerated in humans, and the effect of 5-HT7 blockade on REM sleep translated from rodent to humans, demonstrating target engagement. The augmentation of citalopram effect on REM sleep by JNJ-18038683 was also observed in humans, but citalopram plasma concentration was also increased. Finally, the compound was evaluated in a depression clinical trial, but the lack of separation observed with the positive control group indicated a failed study, thus rendering interpretation of the lack of separation in the JNJ-18038683 group data inconclusive.

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Despite some encouraging preclinical data, there is currently no suitable 5-HT7 positron emission tomography tracer available for clinical studies (Andries et al., 2011). Therefore, we decided to use the effect of JNJ-18038683 on REM sleep as a biomarker for target engagement in humans. Consistent with the data reported for SB-269970 and other 5-HT7 receptor antagonists (Hagan et al., 2000; Thomas et al., 2003), JNJ-18038683 was found to increase REM sleep latency and decrease REM duration after oral administration in rats. In agreement with our data obtained with SB-269970 (Bonaventure et al., 2007) and in 5-HT7 knockout mice (Shelton et al., 2009), JNJ-18038683 was also found to enhance REM suppression induced by citalopram in rodents. Furthermore, total REM sleep time was decreased during the 7-day repeated treatment in rats, with a rebound on the first recovery day.
after treatment cessation. Likewise, REM sleep rebound occurs after SSRIs abrupt discontinuation in volunteers and depressed patients (Sharpley and Cowen, 1995). In healthy volunteers, JNJ-18038683 was found to increase REM latency and decrease REM sleep duration, demonstrating that the effect of 5-HT7 blockade on REM sleep translated from rodent to humans similarly to other antidepressants, in particular SSRIs (Sharpley and Cowen, 1995). The 5-HT7 receptor antagonist was more effective in decreasing REM sleep duration than increasing REM latency in both rats (Fig. 1, A and B) and humans (Table 2). JNJ-18038683 also enhanced REM suppression induced by citalopram in humans, again and B) and humans (Table 2). JNJ-18038683 also enhanced REM suppression induced by citalopram in humans, again.

In conclusion, we successfully demonstrated that the effect of JNJ-18038683 on REM sleep translated from rodents to humans. Because of the failed MDD study caused by assay sensitivity, it is not possible to establish a definitive conclusion regarding the efficacy of JNJ-18038683 in the treatment of MDD. More clinical studies are needed to assess the clinical potential of 5-HT7 antagonists.

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Participated in research design: Bonaventure, Dugovic, Kramer, De Boer, Sands, and Lovenberg.
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Contributed new reagents or analytic tools: Chai, Dvorak, and Carruthers.
Performed data analysis: Bonaventure, Dugovic, Kramer, De Boer, Singh, Wilson, Bertelsen, Di, Shelton, Aluisio, and Drinenburg.
Wrote or contributed to the writing of the manuscript: Bonaventure, Dugovic, Kramer, De Boer, Singh, and Lovenberg.

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