Characterization of JNJ-42847922, a Selective Orexin-2 Receptor Antagonist, as a Clinical Candidate for the Treatment of Insomnia

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ABSTRACT

Dual orexin receptor antagonists have been shown to promote sleep in various species, including humans. Emerging research indicates that selective orexin-2 receptor (OX2R) antagonists may offer specificity and a more adequate sleep profile by preserving normal sleep architecture. Here, we characterized JNJ-42847922 (5-(4,6-dimethyl-pyrimidin-2-yl)-hexahydropyrrrolo [3,4-c]pyrrol-2-yl)-(2-fluoro-6-[1,2,3]triazol-2-yl)phenyl)methanone), a high-affinity/potent OX2R antagonist. JNJ-42847922 had an approximate 2-log selectivity ratio versus the human orexin-1 receptor. Ex vivo receptor binding studies demonstrated that JNJ-42847922 quickly occupied OX2R binding sites in the rat brain after oral administration and rapidly cleared from the brain. In rats, single oral administration of JNJ-42847922 (3–30 mg/kg) during the light phase dose dependently reduced the latency to non-rapid eye movement (NREM) sleep and prolonged NREM sleep time in the first 2 hours, whereas REM sleep was minimally affected. The reduced sleep onset and increased sleep duration were maintained upon 7-day repeated dosing (30 mg/kg) with JNJ-42847922, then all sleep parameters returned to baseline levels following discontinuation. Although the compound promoted sleep in wild-type mice, it had no effect in OX2R knockout mice, consistent with a specific OX2R-mediated sleep response. JNJ-42847922 did not increase dopamine release in rat nucleus accumbens or produce place preference in mice after subchronic conditioning, indicating that the compound lacks intrinsic motivational properties in contrast to zolpidem. In a single ascending dose study conducted in healthy subjects, JNJ-42847922 increased somnolence and displayed a favorable pharmacokinetic and safety profile for a sedative/hypnotic, thus emerging as a promising candidate for further clinical development for the treatment of insomnia.

Introduction

Orexin-A and orexin-B, also known as hypocretin-1 and hypocretin-2, are neuropeptides produced by perifornical and lateral hypothalamic neurons that project throughout the brain and bind two different G protein-coupled receptors, orexin-1 receptor (OX1R) and orexin-2 receptor (OX2R) (de Lecea et al., 1998; Sakurai et al., 1998). Impairment of the orexin system at the OX2R level in an inherited canine model of narcolepsy was reported 1 year after its initial discovery (Lin et al., 1999). At the same time, a narcoleptic phenotype was observed in mice lacking the gene coding for orexin, identifying orexin as a key mediator for the maintenance of wakefulness (Chemelli et al., 1999). The following year, Peyron and colleagues (2000) demonstrated that there was a near complete loss of central orexin production in human narcolepsy, as measured by orexin immunoreactivity in postmortem brain slices. The salient narcoleptic phenotype of orexin knockout (KO) mice and the altered orexin signaling in narcoleptic patients provided both genetic and clinical proof of the critical role of orexin receptors (OXRs) for sleep regulation. It was then hypothesized that pharmacological blockade of the OXRs would be a novel approach for the treatment of insomnia. Less than 10 years after the elucidation of the role of the orexin system, the

ABBREVIATIONS: CHO-K1, Chinese hamster ovary K1; DORA, dual orexin receptor antagonist; EEG, electroencephalogram; EMPA, N-ethyl-2-[(6-methoxy-pyridin-3-yl)-(toluene-2-sulfonyl)-amino]-N-pyridin-3-ylmethyl-acetamide; HEK-293, human embryonic kidney 293; JNJ-10397049, N-[2,4-dibromophenyl]-N’-[4-(5S,5R)-2,2-dimethyl-4-phenyl-1,3-dioxan-5-yl]-urea; JNJ-42847922, 5-(4,6-dimethyl-pyrimidin-2-yl)-hexahydropyrrrolo [3,4-c]pyrrol-2-yl)-(2-fluo-6-[1,2,3]triazol-2-yl-phenyl)-methanone; KO, knockout; LSN2424100, N-biphenyl-2-yl-4-fluoro-N-(1H-imidazol-2-yl)methanesulfonamide HCl; MK-1064, 5-chloro-N-[5-(6-dimethoxypyridin-2-yl)methyl]-5-methyl-4-thiazolyl-2-yl)-2,3-bipyrindine)-4-carboxamide; NREM, non-REM; OX1R, orexin receptor; OX2R, orexin-1 receptor; OX2R, orexin-2 receptor; PE-6, (2-(2H-1,2,3-triazol-2-yl)phenyl)(2R,5R)-5-[(8-fluoroquinolin-4-yl)oxy]-2-methyl(piperidin-1-yl)methanone; REM, rapid eye movement; SB-649868, N-([2S]-1-[5-(4-fluorophenyl)-2-methyl-4-thiazolyl]carbonyl)-2-piperidinyl)methyl-4-benzofurancarboxamide; SB-674042, 1-[5-(2-fluoro-phenyl)-2-methyl-thiazol-4-yl]-1-[S]-2-[5-(phenyl-1,3-dioxadiazol-2-yl)methyl-pyrrolidin-1-yl]-methanone; 2-SORA 19, (Z)-2-(2H-1,2,3-triazol-2-yl)phenyl)[4-(5-chloro-2-methylpyrimidin-4-yl)-7-methyl-1,4-diazepan-1-yl]methanone; TCS OX229, (2S)-1-[3,4-dihydro-6,7-dimethoxy-(1H)-isooquinolinyl]-3,3-dimethyl-2-[(4-pyridinyl)methyl]amino]-1-butanone hydrochloride; WT, wild-type.
first clinical data on almorexant, a dual OXR antagonist (DORA), were disclosed and confirmed the validity of this new mechanism of action for the treatment of insomnia (Brisbare-Roch et al., 2007). Only a few years later, at least three other DORAs (SB-649868 [N-(2S)-1-(5-(4-fluorophenyl)-2-methyl-4-thiazolyl)carbonyl]-2-piperidinyl[methyl]-4-benzofurancarboxamide], suvorexant, and filorexant) reached clinical phase II for the treatment of sleep-related disorders (reviewed in Hoey and Jacobson, 2013). In August 2014, the U.S. Food and Drug Administration approved Belsomra (Merek, Kenilworth, NJ; suvorexant) for the treatment of primary insomnia.

Taking advantage of newly developed selective OX1R and OX2R antagonists, we dissected the contribution of each OXR and demonstrated that, in rodents, antagonism of OX2R alone was sufficient to induce and prolong sleep (Dugovic et al., 2009). In agreement with these data, Mang and colleagues (2012) reported that almorexant did not induce sleep in mice lacking the OX2R. Furthermore, we showed that the addition of OX1R blockade to OX2R antagonism elicited a dysregulation of rapid eye movement (REM) sleep by shifting the balance in favor of REM sleep at the expense of non-REM (NREM) sleep that may increase the risk of narcolepsy-like events (Dugovic et al., 2014).

Based on these preclinical data, we developed a selective OX2R antagonist with suitable drug-like properties for clinical evaluation. We optimized the compound for selectivity over the OX1R (greater than 50-fold), oral bioavailability, rapid absorption, and short half-life to avoid next-day residual effects. A separate paper describes the new OX2R antagonist JNJ-42847922 (5-(4,6-dimethyl-pyrrolidin-2-yl)-hexahydro-pyrrolo[3,4-c]pyrrol-2-yl)-(2-fluoro-6-[1,2,3]triazol-2-yl-phenyl)-methanone) (Fig. 1) and its synthesis, structure activity relationship, pharmacokinetics in preclinical species, and acute dose-response effects on sleep in rats during the dark phase (Letavic et al., 2015). Here, we report a comprehensive characterization of JNJ-42847922, a novel selective OX2R antagonist.

In vitro affinity and potency for the human and rat OX2R were determined by radioligand binding and in vitro functional assays. In vivo target engagement was measured in rat brain using ex vivo receptor occupancy after oral administration. To confirm the mechanism of action, the acute dose-response effects of the OX2R antagonist on sleep were tested in rats and in OX2R KO and corresponding wild-type (WT) mice. The maintenance of the sleep response upon repeated administration of JNJ-42847922 was also assessed in rats. The effect of the compound on motor coordination and alcohol-induced ataxia was investigated in rats using the rotarod test. Potential abuse liability was evaluated by measuring changes in nucleus accumbens dopamine levels using microdialysis in freely moving rats, and by using the conditioned place preference test in mice. Following appropriate 1-month Good Laboratory Practice toxicological studies in rat and dog, we conducted a single ascending dose to evaluate the safety, tolerability, and pharmacokinetics of JNJ-42847922 in healthy subjects. Sleepiness was assessed by the Stanford Sleepiness Scale.

Materials and Methods

All animal procedures performed in this study were in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the U.S. National Institutes of Health (publication no. 80-23, revised 1996) and the guidelines of the Institutional Animal Care and Use Committee. Animals were housed individually under controlled conditions with a 12-hour light/dark schedule and temperature of 22 ± 2°C. Food and water were provided ad libitum. Experiments were performed after animals had acclimated for at least 1 week unless stated otherwise.

In Vitro Radioligand Binding Assays. Human or rat OX2R binding was measured in competitive radioligand binding assays using [3H]EMPA ([3H]-N-ethyl-2-[(6-methoxy-pyridin-3-yl)-(toluene-2-sulfanyl)-amino]-N-pyridin-3-ylmethyl-acetamide; 2 nM, specific activity 27 Ci/mmol) (Malherbe et al., 2009). Cell membranes were prepared from a stable pool of human embryonic kidney 293 (HEK-293) cells transfected with the human OX2R or Chinese hamster ovary K1 (CHO-K1) cells transfected with the rat OX2R. Dilutions of test compounds were made in Dulbecco’s phosphate-buffered saline from 10 mM stocks dissolved in dimethylsulfoxide. After a 60-minute incubation at room temperature, binding reactions were filtered. The membranes were counted in a scintillation counter. Nonspecific binding was determined in the presence of 10 μM almorexant [(2R)-2-[(IS)-6,7-dimethoxy-1-[2-[4-(trifluoromethyl)phenylethyl]yl]-3,4-dihydroisoquinolin-2(1H)-yl]-N-methyl-2-phenylethylamine].

Affinities of compounds for the human or rat OX2R were measured using [3H]SB-674042 ([3H]-1-(5-(2-fluoro-phenyl)-2-methyl-thiazol-4-yl)-1-(S)-(2-(5-phenyl-[1,3,4]oxadiazol-2-ylmethyl)-pyrrolidin-1-yl)-methanone) as a tracer (4 nM, specific activity 35 Ci/mmol) (Langmead et al., 2004). Cell membranes were prepared from clonal CHO-K1 cells transfected with the human OX1R or clonal HEK-293 cells transfected with the rat OX1R. Nonspecific binding was determined with 10 μM almorexant. The Ki of the test compounds was calculated based on nonlinear regression (one-site competition) using GraphPad Prism (GraphPad Software, San Diego, CA).

The selectivity of JNJ-42847922 was evaluated in a large panel of binding assays, including adenosine (A1, A2A, A3), adrenergic (α1, α2, α1), angiotensin (AT1), dopamine (D1, D2), bradykinin (B2), cholecystokinin (CCKA), galanin (GAL2), melanotin (ML2), muscarinic (M1, M2, M3), neuropeptide Y (NPY), neurtin (NT1), neurokinin (NK1, NK2), opiate (μ, κ, δ), serotonin (5-HT1A, 5-HT1B, 5-HT2A, 5-HT3, 5-HT6, 5-HT7), somatostatin, vasopressin (V1a), norepinephrine transporter, dopamine transporter, and ion channels (sodium, calcium, potassium, and chloride). These assays were performed by Eurofins (Celle L/Evescault, France).

In Vitro Functional Assays (Calcium Mobilization Assays). Functional antagonism of OX2R has been shown in cellular assays by binding to the agonist response by a dose range of the antagonists. The cells were plated in black 96-well tissue culture plates with clear bottoms at 50,000 cells/well and grown overnight at 37°C in 5% carbon dioxide (CO2). Dilutions of the antagonist were prepared in Hanks’ balanced salt solution from 10 mM dimethylsulfoxide stocks.
whereas dilutions of OX peptides (OX-A for OX1R assays, OX-B for OX2R assays) were prepared in Hank’s balanced salt solution + 0.1% bovine serum albumin. On the day of the assay, a 2 × dye-loading solution (BD Calcium Assay Kit; BD Biosciences, Franklin, NJ) was added to the cells and incubated for 45 minutes at 37°C in 5% CO2. Dilutions of the test compounds were added, and the cells were incubated at room temperature for 15 minutes. The cell plate was then transferred to the Fluorometric Imaging Plate Reader Tetra instrument (Molecular Devices, Sunnyvale, CA), which adds the OXR agonist and monitors changes in fluorescence reflecting intracellular calcium levels.

Results were calculated using GraphPad Prism. Raw data from the Fluorometric Imaging Plate Reader Tetra were exported as the difference between maximum and minimum fluorescence observed for each well. A nonlinear regression was used to determine the agonist EC50 and antagonist IC50 for each plate, then the antagonist Kd was calculated according to Cheng and Prusoff (1973).

**Ex Vivo Receptor Occupancy Assays in Rats.** Experiments were performed as previously described (Dugovic et al., 2009) in male Sprague-Dawley rats (300–400 g; Charles River Laboratories, San Diego, CA). Animals were treated orally with JNJ-42847922 or vehicle. Time and dose dependency were evaluated. The animals were euthanized using CO2 and decapitated at different time points after drug administration (n = 3 per time point or dose regimen). Brains were rapidly frozen on powdered dry ice and stored at −80°C before sectioning. Plasma samples were also collected for bioanalysis (liquid chromatography–mass spectrometry–spectrometry) [LCSM]. Tissues were sliced to verify the probe placement.

**In Vivo Microdialysis in Rats.** Rats were rapidly frozen on powdered dry ice and stored at −80°C for power spectra were divided into four frequency bands: delta (1–4 Hz), theta (4–10 Hz), sigma (10–15 Hz), and beta (15–30 Hz). For each experiment, all signals were recorded for up to 6 hours after administration of the tested compounds.

Analysis of sleep parameters included latency to NREM sleep (defined as the time interval to the first six consecutive NREM epochs) and REM sleep (the first two consecutive REM epochs post-treatment), the duration of NREM and REM sleep, and bout analysis (number and duration) for each vigilance state. Locomotor activity counts and body temperature were analyzed into 1- and 15-minute bins, respectively, and averaged into 2-hour periods for each animal. Results were averaged and expressed as the mean ± S.E.M. in defined time intervals. To determine whether differences were significant at a given interval, one-way analysis of variance followed by Dunnett’s multiple comparison post-hoc test was performed for experiments in rats, and Student’s t test was performed for experiments in mice. A significance threshold of P < 0.05 was used.

**Ex Vivo Receptor Occupancy Assays in Rats.** Experiments were performed as previously described (Dugovic et al., 2009) in male Sprague-Dawley rats (300–320 g) from Charles Rivers (San Diego, CA), as previously described (Galici et al., 2011), using a guide cannula (Eicom, Kyoto, Japan) in the nucleus accumens core (incisor bar set at −3.5 mm; nucleus accumens: +1.8 mm anterior, 1.6 mm lateral, and 6.0 mm ventral to bregma) (Paxinos and Watson, 2005). Microdialysis probes (artificial cellulose; molecular weight cutoff, 50,000; 2-mm active membrane length; Eicom) were implanted the afternoon prior to sample collection and perfused with artificial cerebral spinal fluid (147 mM NaCl, 4.0 mM KCl, 0.85 mM MgCl2, 2.3 mM CaCl2) at a rate of 1 μl/min. The following morning, three 30-minute baseline samples were collected prior to drug injections. Animals were then treated with JNJ-42847922 (30 mg/kg p.o.) or vehicle. Samples were collected every 30 minutes for 240 minutes postdosing into a 96-well plate containing 7.5 μl of antioxidant (0.1 M acetic acid, 1 mM oxalic acid, and 3 mM L-cysteine in ultra-pure water) maintained at 4°C. One hour prior to the end of the experiment (t = 180 minutes), each animal received an injection of amphetamine (0.3 mg/kg s.c.) as a positive control. Microdialysis samples were analyzed for dopamine by high-performance liquid chromatography with electrochemical detection (Eicom) as previously described (Galici et al., 2011). Following the experiment, the brains were removed and sliced to verify the probe placement.

The concentration of dopamine in each sample was calculated from the peak area of the chromatographic signal and the slope from the corresponding standard curve. The percent change from baseline value was calculated from the mean basal value of each neurotransmitter for each animal and is presented in the figures as the mean ± S.E.M. Data were graphed using GraphPad Prism, and statistical analysis was performed using unpaired t test with a significance threshold of P < 0.05.

**Conditioned Place Preference in Mice.** Experiments were conducted in male DBA/2 mice (30–35 g; Jackson Laboratory, Sacramento, CA). The conditioning apparatus consisted of two chambers, identical in size and texture, divided by a Plexiglass partition with a door that could be opened or closed. One of the chambers was decorated with all four walls and the floor with a black and white cow pattern and scented with lemon extract (200 μl; Kroger Co., Cincinnati, OH) as an olfactory cue. The other chamber was decorated on all four walls and the floor with a black and white checker pattern and the scent of almond extract (200 μl; Kroger Co.). The conditioning apparatus automatically recorded the location of the animal via a grid of photobeams (8 photobeams along its length and 4 along its width, evenly spaced). On the first day, during habituation to the apparatus, animals were placed in one of the chambers (chosen randomly) and given free access to both chambers (door open) for 30 minutes. Over the next 4 days, animals were conditioned twice a day. In the morning, animals received a treatment of vehicle and were immediately confined to the initially most preferred conditioning chamber (as determined during the habituation) with the door closed for 30 minutes. In the afternoon, animals received either JNJ-42847922 (10 mg/kg i.p.), zolpidem (10 mg/kg i.p.), or vehicle (20 or 45% hydroxypropyl β-cyclodextrin, 10 ml/kg i.p.) as a control, and were immediately confined to the opposite conditioning chamber (door closed) for 30 minutes. For historical reason, the intraperitoneal route of administration was used for this test. OX2R occupancy after administration of 10-mg/kg (i.p.) JNJ-42847922 was also determined in mice to demonstrate target engagement. Animals received the same treatment each
afternoon. As a positive control, an additional group of animals was conditioned in a similar manner, receiving saline (10 mL/kg i.p.) in the morning and amphetamine (2 mg/kg i.p.) in the afternoon. On the sixth day, animals received a test for conditioned place preference. Animals were placed in one of the chambers (chosen randomly), with the door open, and the time spent in each chamber was recorded over 15 minutes.

The time spent in the drug-paired chamber was analyzed with a two-way analysis of variance for test (comparing time during the habituation session to time during the conditioned place preference test) and group, with repeated measures on time. A Dunnett post-hoc test with a significance threshold of P < 0.05 was used to compare each group to the time spent by the vehicle group in the drug-paired chamber during the place preference test.

**Rotarod Test in Rats.** Male Sprague-Dawley rats (400–450 g; Harlan Laboratories) were trained on the rotarod (Stoelting, Wood Dale, IL) a day prior to testing. On the day of the experiment, JNJ-42847922, zolpidem, or vehicle (30% sulfobutyl ether β-cyclodextrin) was administered orally, and ethanol was coadministered intraperitoneally; the test was then conducted 15 minutes post-treatment. The time that the animals were able to remain on the rotating drum was recorded. For rotarod testing, the latencies were given a 60-second cutoff time. Statistical analysis was performed using a Kruskal-Wallis test with Dunnett’s multiple comparisons using a significance threshold of P < 0.05.

**Single Ascending Dose Study in Humans.** The study was conducted in accordance with the Declaration of Helsinki and approved by a local ethics committee. A double-blind, randomized, placebo-controlled, single ascending dose study was conducted at a phase 1 unit in Germany between May and August 2011. Eligible subjects were healthy males between 18 and 55 years of age without sleep apnea, heavy snoring, or regular use of sleep medications. Subjects in each cohort were randomized to receive a single dose of either JNJ-42847922, formulated as an oral suspension, or placebo. Subjects slept in the unit the evening prior to dosing, were fasted overnight, and received study drug the morning of the following day. A starting dose of 10 mg was administered to three subjects (JNJ-42847922, n = 2; placebo, n = 1). Subsequent cohorts (n = 6 active, 3 placebo) received doses of 10, 20, 40, and 80 mg, based upon review of safety, tolerability, and plasma pharmacokinetic data for each cohort. Assessments were performed while subjects remained in the clinic at least 72 hours after dosing, as well as at a follow-up visit 1–2 weeks after dosing.

Safety assessments included collection of adverse events, physical and neurologic examinations, vital signs, 12-lead and continuous ECGs, pulse oximetry, and blood and urine safety laboratories. Venous blood samples were collected for determination of plasma JNJ-42847922 levels at various time points through 72 hours after dosing. Plasma samples were analyzed using a validated, specific, and sensitive liquid chromatography/mass spectrometry/mass spectrometry method. The Stanford Sleepiness Scale was used to collect subjects' ratings of somnolence at 2, 8, and 12 hours after dosing.

**Chemicals.** Almorexant, EMPA, SB-674042, JNJ-10397049, and JNJ-42847922 were synthesized at Janssen Research & Development (Torrance, CA). Zolpidem tartrate was obtained from Aarti Drugs Ltd. (Tarapur, Maharashtra). Peptides were obtained from Bachem (Torrance, CA).

**Results**

**JNJ-42847922 Is a Selective High-Affinity OX2R Antagonist.** JNJ-42847922 showed high-affinity binding to the human and rat OX2R, with pKᵦ values of 8.0 and 8.1, respectively (Table 1). The binding selectivity of JNJ-42847922 at the OX2R compared with the OX1R was substantial (approximately 2 log units). In a panel of 50 receptors, ion channels, and transporter assays, JNJ-42847922 at 1 μM had no significant affinity for any receptor/transporter/ion channel (<50% inhibition at 1 μM) other than the OX2R. The functional antagonism of JNJ-42847922 for the human or rat OX2R assessed by measuring changes in intracellular calcium in cell culture assays in response to an EC₅₀ dose of OX-A showed that the high-affinity OX2R binding of JNJ-42847922 was reflected in potent functional activity (Table 1). The pKᵦ values correlated well with the pKᵦ values for the human and rat OX2R. The binding selectivity of JNJ-42847922 at the OX2R compared with the OX1R was confirmed at the functional level (Table 1).

**JNJ-42847922 Crosses the Blood-Brain Barrier and Occupies the OX2R in Rat Brain after Oral Administration.** Time dependency and dose dependency of in vivo occupancy of OX2R assessed by ex vivo receptor binding autoradiography of [³H]EMPA in rat cortex brain tissue sections after oral dosing are shown in Fig. 2. Corroborating plasma and brain concentrations are presented in Table 2.

Orally administered JNJ-42847922 (30 mg/kg) inhibited [³H]EMPA binding to the rat cortex, indicating sufficient oral bioavailability and brain penetration. JNJ-42847922 OX2R occupancy reached maximal levels at 60 minutes (74 ± 6%), followed by a rapid decline to 40% at 4 hours and no occupancy at 24 hours (Fig. 2A). Overall, there was a strong relationship between receptor occupancy level and plasma/brain concentration, i.e., receptor occupancy levels dropped when plasma/brain levels decreased. In vivo OX1R occupancy was also determined in the same experiment to ensure selectivity for the OX2R. Oral administration of JNJ-42847922 (30 mg/kg) minimally inhibited [³H]SB-674042 binding to the rat tenia tecta, indicating low OX1R occupancy (<20%; data not shown).

For the dose-response analysis, ex vivo receptor occupancy was measured 15 minutes after dosing (Fig. 2B). This time point was the apparent time to maximum plasma drug concentration (tₘₐₓ) of JNJ-42847922 attained after the 30-mg/kg dose (Table 2). The measured ED₅₀ dose was 3 mg/kg, and OX2R occupancy was approximately 74% at the highest dose of 60 mg/kg (Fig. 2B). The ED₅₀ dose of 3 mg/kg in this study corresponded to a calculated plasma exposure of 171 mg/L. Occupancy of the OX1R by JNJ-42847922 was negligible (<20% at 30 mg/kg; data not shown). The unbound plasma concentration of JNJ-42847922 (rat plasma unbound fraction of 0.056) (Letavic et al., 2015) corresponding to 50% receptor occupancy in the rat was 9.58 ng/mL. In humans, JNJ-42847922 has a similar affinity for OX2R compared with rats. Therefore, based on a mean unbound plasma fraction of 0.029, the predicted total human plasma concentration for a 50% level of OX2R occupancy would be 330 ng/mL.

OX2R occupancy was also determined in mice to demonstrate target engagement (data not shown). After intraperitoneal

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>In vitro binding affinity (pKᵦ values) and in vitro pKᵦ values for intracellular calcium responses of JNJ-42847922 at human and rat OX1R and OX2R</td>
</tr>
<tr>
<td>The values are expressed as the average ± S.D., and the number of triplicate experiments is indicated in parentheses.</td>
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<tr>
<td>Affinity pKᵦ</td>
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<tr>
<td>hOX2R</td>
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<td>hOX1R</td>
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<td>rOX2R</td>
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<td>rOX1R</td>
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H, human; r, rat.
Characterization of JNJ-42847922, a Selective OX2R Antagonist

JNJ-42847922 Dose-Dependently Induces and Prolongs Sleep in Rats. Acute effects of JNJ-42847922 on sleep-wake patterns in rats were investigated during either the dark (active) phase or the light (rest) phase in two separate studies. In the first study (Letavic et al., 2015), rats were orally dosed at the onset of the dark phase (1, 3, 10, and 30 mg/kg), and in the second study (present data), rats were orally dosed at 2 hours into the light phase (3, 10, and 30 mg/kg). Based on our observation that the lowest dose tested was not effective in the dark phase (when the hypnotic activity of OXR antagonists is preferentially revealed), this dose of 1 mg/kg was not evaluated in the light phase. Oral administration of JNJ-42847922 during either the dark phase or the light phase produced a dose-dependent reduction in NREM sleep latency and an increase in NREM sleep time in the first 2 hours (dark phase effects shown in Fig. 3). JNJ-42847922 displayed efficacy from the dose of 3 mg/kg onward for both sleep induction (decreased NREM sleep latency) [F(3, 21) = 42,847, P < 0.001] (Fig. 3A) and sleep promotion (increased NREM sleep time) [F(3, 21) = 7.89, P = 0.001] (Fig. 3B). Whereas the dose-response effects were more apparent during the dark phase than during the light phase, the time spent in NREM sleep was still gradually prolonged from 3 to 30 mg/kg. The increased NREM sleep time that was measured with the highest dose tested (30 mg/kg) during the light phase was the result of a prolongation of NREM bout duration (compound: 2.83 ± 0.16 minutes versus vehicle: 2.25 ± 0.14 minutes; P < 0.05 based on paired Student's t test) with no change in the number of NREM bouts (compound: 28.9 ± 1.9 versus vehicle: 29.4 ± 2.4), suggesting an enhanced sleep consolidation. There was no significant effect on REM sleep latency or REM sleep duration at doses up to 30 mg/kg (Fig. 3, B and D). REM sleep latency was significantly reduced and REM sleep time was significantly increased only at the dose of 60 mg/kg tested during the dark phase (data not shown). Sleep architecture was preserved at all doses tested in the light phase, as indicated by the maintenance of the REM/total sleep ratio at 3 mg/kg (12.1%), 10 mg/kg (11.0%), and 30 mg/kg (10.9%) as compared with vehicle treatment (10.8%).

TABLE 2

Ex vivo OX2R occupancy with JNJ-42847922 (30 mg/kg p.o.) in rat cortex

<table>
<thead>
<tr>
<th>Time</th>
<th>% OX2R RO</th>
<th>Brain Concentration</th>
<th>Plasma Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>49 ± 5</td>
<td>205 ± 30</td>
<td>1119 ± 296</td>
</tr>
<tr>
<td>0.25</td>
<td>62 ± 1</td>
<td>646 ± 153</td>
<td>2330 ± 443</td>
</tr>
<tr>
<td>0.5</td>
<td>68 ± 1</td>
<td>512 ± 106</td>
<td>1844 ± 298</td>
</tr>
<tr>
<td>1</td>
<td>74 ± 6</td>
<td>1040 ± 388</td>
<td>2107 ± 109</td>
</tr>
<tr>
<td>2</td>
<td>43 ± 4</td>
<td>98 ± 9</td>
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<td>40 ± 4</td>
<td>42 ± 7</td>
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<td>6</td>
<td>23 ± 4</td>
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<tr>
<td>24</td>
<td>0 ± 0</td>
<td>BLLOQ</td>
<td>BLLOQ</td>
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</table>

BLLOQ, below the lowest level of quantification; RO, receptor occupancy.
that the increased sleep time did not impact sleep intensity as evidenced by consistent NREM delta power and REM theta power across vehicle and compound treatment days (data not shown). Concomitantly, locomotor activity was significantly diminished only during the JNJ-42847922 treatment period \( \left[ F(5, 40) = 6.25, P < 0.001 \right] \) (Fig. 4F). The values of all sleep parameters and locomotor activity returned to control levels after discontinuation of the treatment.

**JNJ-42847922 Has No Effect on Sleep Parameters in Mice Lacking the OX2R.** First, spontaneous sleep-wake states were evaluated in two groups of OX2R KO and corresponding WT mice during a 24-hour baseline recording period, and the results are presented in Table 3. As compared with WT, OX2R KO mice spent less time in wake (\( -64 \) minutes) and more time in NREM sleep (\( +56 \) minutes) during the dark phase only. REM sleep amounts were slightly higher during the dark phase (\( +8 \) minutes) but lower during the light phase (\( -7 \) minutes). Next, the effects of JNJ-42847922 (30 mg/kg) on sleep parameters were investigated in WT and OX2R KO mice after oral administration at the onset of the dark phase (Fig. 5). In accordance with the differences observed in baseline conditions, OX2R KO mice showed a shorter NREM latency and higher amounts of NREM sleep as compared with WT following vehicle treatment. In WT mice, JNJ-42847922 displayed efficacy for both sleep induction (decreased NREM sleep latency) and sleep promotion (increased NREM sleep time) in the first 2 hours after compound administration (Fig. 5, A and C). These effects on NREM sleep in mice were similar to those observed in rats. As in rats, administration of JNJ-42847922 in WT mice had little impact on REM sleep latency and REM sleep duration (Fig. 5, E and G). In contrast, JNJ-42847922 did not affect any sleep parameters in OX2R KO mice. Specifically, there were no significant differences between compound and vehicle conditions in NREM sleep latency or duration (Fig. 5, B and D) or REM sleep latency or duration (Fig. 5, F and H). Interestingly, both the NREM latency and the NREM sleep duration were comparable in vehicle-treated OX2R KO mice and in WT mice treated with the OX2R antagonist. These results demonstrate that the mechanism of action for the sleep promotion elicited by JNJ-42847922 is mediated via the selective blockade of the OX2R.

**JNJ-42847922 Does Not Affect Dopamine Release in the Rat Nucleus Accumbens.** In vivo microdialysis was used to assess the effect of JNJ-42847922 on extracellular concentrations of dopamine in the nucleus accumbens of freely moving rats (Fig. 6A). Oral administration of 30 mg/kg JNJ-42847922 had no effect on extracellular dopamine release in rat nucleus accumbens (\( P > 0.05 \)). Amphetamine, a common drug of abuse known to increase dopamine release in the nucleus accumbens, was administered as a positive control at the end of the study. Administration of amphetamine (0.3 mg/kg s.c.) increased dopamine release by approximately 300% above baseline levels (Fig. 6A).

**JNJ-42847922 Does Not Produce Place Preference in Mice after Subchronic Conditionings.** Mice were conditioned to zolpidem (10 mg/kg i.p.), JNJ-42847922 (10 mg/kg i.p.), or vehicle using a place preference model (Fig. 6B). Amphetamine (2 mg/kg i.p.) was also included as a positive control. A two-way analysis of variance revealed a significant
interaction between test and group \(F(3,56) = 4.43, P = 0.007\). Dunnett’s post-hoc tests revealed that both the zolpidem and amphetamine groups spent significantly more time in the drug-paired chamber during the place preference test than the vehicle group \(P < 0.05\), indicating a place preference for these drugs. JNJ-42847922 was not significantly different

**TABLE 3**
Basal duration of wake, NREM, and REM sleep during the light phase and dark phase in WT and OX2R KO mice
Data are the means ± S.E.M. (N = 10 WT, N = 10 OX2R KO) expressed in minutes.

<table>
<thead>
<tr>
<th></th>
<th>Wake Duration</th>
<th>NREM Duration</th>
<th>REM Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light Phase</td>
<td>Dark Phase</td>
<td>Light Phase</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td></td>
<td>min</td>
</tr>
<tr>
<td>OX2R WT</td>
<td>233.4 ± 12.6</td>
<td>472.5 ± 14.7</td>
<td>431.3 ± 11.6</td>
</tr>
<tr>
<td>OX2R KO</td>
<td>255.0 ± 5.8</td>
<td>408.6 ± 11.9**</td>
<td>416.2 ± 6.4</td>
</tr>
</tbody>
</table>

*P < 0.05 and **P < 0.01 versus WT based on unpaired Student’s t test.
from the vehicle group ($P = 0.4$), indicating a lack of place preference or aversion. OX2R occupancy was determined in mice after intraperitoneal administration of JNJ-42847922 to demonstrate target engagement (see above). The levels of receptor occupancy and plasma exposure values obtained after intraperitoneal administration (10 mg/kg) are similar to the values measured in rat after administration of a 30-mg/kg p.o. dose.
JNJ-42847922 Has No Effect on Motor Coordination or on Alcohol-Induced Ataxia in Rats. The effects of JNJ-42847922 on ataxia in rats were evaluated using the rotarod apparatus. Animals were trained to stay on the rotarod apparatus for 60 seconds (i.e., acquisition criteria) for a maximum of three trials. When tested 24 hours later, JNJ-42847922 (30 mg/kg p.o.) had no effect on motor coordination in rats at a dose that induced sleep (Fig. 6C) \( (P < 0.05) \).

The effects of JNJ-42847922 on alcohol-induced ataxia in rats were also evaluated using the rotarod apparatus. JNJ-42847922 (30 mg/kg p.o.) did not modify the ataxic effects of alcohol (1 g/kg i.p.), whereas zolpidem (10 mg/kg i.p.) exacerbated the effect of alcohol (Fig. 6D). These data indicate that JNJ-42847922 had no myorelaxant effect and no effect on alcohol-induced ataxia.

Toxicology. Toxicological studies up to 1 month in duration under good laboratory practice conditions were conducted in rats and dogs and indicate that JNJ-42847922 had a suitable safety profile to allow testing in humans. In both species, JNJ-42847922 was well tolerated in single- and multiple-dose studies of up to 1-month duration. JNJ-42847922 had no genotoxicity potential in the standard genotoxicity test battery and was well tolerated in a dog cardiovascular safety study.

JNJ-42847922 Displays a Suitable Pharmacokinetic Profile in Humans and Promotes Somnolence in a First-in-Human Trial. Plasma pharmacokinetics of JNJ-42847922 were characterized by rapid absorption followed by an apparently monophasic decline, with mean \( t_{\text{max}} \) for each dose ranging from 0.33 to 0.5 hour and a half-life of approximately 2 hours. The \( C_{\text{max}} \) and area under the curve values were dose-dependent but changed in a manner somewhat less than dose-proportional (Fig. 7; Table 4).

Evidence of somnolence, the expected pharmacodynamic effect of JNJ-42847922, was apparent at all doses. Somnolence was reported as an adverse event for 22 of 26 subjects (85%) who received JNJ-42847922, compared with 3 of 13 subjects (23%) who received placebo. The incidence of somnolence appeared to be dose-dependent, reaching 100% at 40 mg (Fig. 8). The time of onset of the first somnolence adverse events ranged from 10 to 40 minutes after dosing, with a mean of 20 minutes for both actively treated and placebo subjects. Additionally, actively treated subjects in all cohorts had significantly higher Stanford Sleepiness Scale scores, reflecting greater sleepiness, compared with placebo at the first assessment time point of 2 hours after dosing (analysis of covariance with baseline as covariate, \( P = 0.023 \) for 10 mg, \( P = 0.017 \) for 20 mg, \( P < 0.0001 \) for 40 and 80 mg). No significant treatment differences were observed at 8 and 12 hours after dosing.

Fig. 6. Nonclinical secondary pharmacodynamic studies performed with JNJ-42847922. All data are presented as means ± S.E.M. (A) Effects of JNJ-42847922 (30 mg/kg p.o.) on extracellular dopamine levels from rat nucleus accumbens \( (N = 5) \). Amphetamine (0.3 mg/kg s.c.) was administered as a positive control at the end of the experiment. (B) Effects of JNJ-42847922 (10 mg/kg i.p., \( N = 17 \)), vehicle \( (N = 13) \), or zolpidem (10 mg/kg i.p., \( N = 18 \)) in the conditioned place preference model in mice; amphetamine (2 mg/kg i.p., \( N = 12 \)) was administered as a positive control. (C) Effects of JNJ-42847922 (30 mg/kg p.o.) or vehicle on rat motor coordination in the rotarod apparatus \( (N = 8 \text{ animals per group}) \). (D) Effects of JNJ-42847922 (30 mg/kg p.o.), vehicle, or zolpidem (10 mg/kg p.o.) coadministered with alcohol (1 g/kg i.p.) on motor coordination in the rotarod apparatus \( (N = 8 \text{ animals per group}) \). * \( P < 0.05 \) compared with vehicle.
There were no deaths, serious adverse events, or discontinuations due to an adverse event, and all events were considered mild or moderate in severity. Overall, 24 of 26 subjects (92%) who received JNJ-42847922 and 8 of 19 placebo-treated subjects (42%) had at least one adverse event, including somnolence. The most frequently reported adverse events other than somnolence were headache (12% for JNJ-42847922 versus 0% for placebo) and dizziness/postural dizziness (12% for JNJ-42847922 versus 8% for placebo). One subject administered 80 mg of JNJ-42847922 experienced a single brief episode of sleep paralysis (awareness of environment with inability to move, while transitioning from wakefulness to sleep) shortly after dosing. There were no clinically significant observations in any other safety evaluations for actively treated subjects.

**Discussion**

In the present report, we characterized a novel selective OX2R antagonist as a clinical candidate for the treatment of insomnia. We demonstrated that the compound had excellent in vitro affinity/potency/selectivity for the OX2R and promoted sleep after oral administration in rodent models. In healthy subjects, JNJ-42847922 displayed a favorable pharmacokinetic and safety profile as well as a strong pharmacodynamic effect.

Only a few selective OX2R antagonists have been reported in the literature. The first one to be reported was TCS OX229 [(2S)-1-(3,4-dihydro-6,7-dimethoxy-2(1H)-isoquinolinyl)-3,3-dimethyl-2-[(4-pyridinylmethyl)amino]-1-butanoate hydrochloride], a tetrahydroisoquinoline with moderate affinity but good selectivity for the OX2R (Hirose et al., 2003). Our evaluation of this molecule revealed poor pharmacokinetic properties and lack of target engagement after systemic administration in rodents (data not shown). A year later, we reported the synthesis of JNJ-10397049, a phenyl-dioxanyl urea (McAtee et al., 2004). JNJ-10397049 crossed the blood-brain barrier, engaged the OX2R, and induced sleep in rats after systemic administration (Dugovic et al., 2009). However, further development of that molecule was stopped due to poor drug-like properties, including low oral bioavailability, poor solubility, and cytochrome P450 interactions. EMPA, an acetamide, was disclosed by Roche in 2009 and was shown to be a highly selective molecule for OX2R (Malherbe et al., 2009). This molecule was also used as a radioligand for the OX2R. Eli Lilly has also recently reported the development of LSN2424100 [N-biphenyl-2-yl-4-fluoro-N-(1H-imidazol-2-ylmethyl) benzensulfonylamide HCl], a sulfonamide (Fitch et al., 2014). Interestingly, this selective OX2R antagonist displayed antidepressant-like activity in rodents (Fitch et al., 2014). Last, MK-1064 (5′-chloro-N-[(5,6-dimethoxypyridin-2-yl)methyl]-2,2′-terpyridine-3′-carboxamide) and MK-3897 [N-[(5,6-dimethoxypyridin-2-yl)methyl]-5-methyl-5-(thiazol-2-yl)-2,3′-bipyrindine-4-carboxamide] were disclosed by Merck and are potential clinical candidates (Roecker et al., 2014a,b).

In the present study, we conducted an extensive preclinical characterization of this novel OX2R antagonist. In vitro, JNJ-42847922 was a high-affinity OX2R antagonist with an approximately 2-log selectivity ratio versus the OX1R. It had a remarkable intrinsic selectivity for the OX2R over a panel of receptors and enzymes. Taking advantage of the OX2R KO mice, we clearly demonstrated that the mechanism of action for the sleep-promoting effect occurred via selective blockade of OX2R. We also showed that both the reduced sleep onset and the increased sleep duration were maintained upon 7-day repeated dosing, and that upon discontinuation, there was no rebound effect on any of the sleep parameters measured.

Various DORAs have been shown to promote both NREM and REM sleep in animals and humans (Hoyer and Jacobson, 2016). Insomnics who used a DOR are more likely to fall asleep within 15 minutes and to sleep longer than control subjects, with a higher quality of sleep (Kupfer et al., 2010). The clinical trial carried out by the present study showed that JNJ-42847922 increased the sleep duration in the morning by 25% and also promoted REM sleep. Given these data, we believe that JNJ-42847922 is a novel OX2R antagonist suitable for the treatment of insomnia.

**Fig. 7.** Plasma concentrations of JNJ-42847922 (means ± S.E.M.) at various time points after a single oral dose in humans. Doses were 10 mg (●), 20 mg (■), 40 mg (○), and 80 mg (□). The inset shows the plasma concentrations at the different doses. The x-axis depicts the natural log of each subject's Cmax (□) and area under the curve (○) value for each dose, plotted versus the natural log of each dose on the y-axis. Linear regression with 99% confidence limits was performed for Cmax and area under the curve. R2 values for goodness of fit were 0.86 and 0.71, respectively.

**TABLE 4**

Plasma pharmacokinetics of JNJ-42847922 after single oral doses of 10–80 mg in the morning in healthy male subjects.

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>N</th>
<th>Cmax (ng/mL)</th>
<th>tmax (h)</th>
<th>AUC0-24h (ng·h/mL)</th>
<th>t1/2 (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>8</td>
<td>309 ± 74</td>
<td>0.33</td>
<td>802 ± 275</td>
<td>2.02 ± 0.26</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>556 ± 104</td>
<td>0.42</td>
<td>1616 ± 607</td>
<td>2.11 ± 0.47</td>
</tr>
<tr>
<td>40</td>
<td>6</td>
<td>743 ± 149</td>
<td>0.42</td>
<td>1807 ± 533</td>
<td>2.02 ± 0.30</td>
</tr>
<tr>
<td>80</td>
<td>6</td>
<td>1208 ± 291</td>
<td>0.50</td>
<td>3655 ± 1064</td>
<td>2.44 ± 0.46</td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. Cmax, area under the curve.
MK-1064 (Roecker et al., 2014a), MK-3697 (Roecker et al., 2014b), antagonists from Merck: compound 18 (Mercer et al., 2013), REM sleep has been shown with a number of selective OX2R dark phase (Etori et al., 2014). Efficacy on both NREM and effects on REM sleep when administered in mice during the reported to primarily increase NREM sleep time with minimal on day 4 during the subchronic treatment. Similarly, a newly conditions, small nonsignificant REM sleep-promoting effects Renger, 2014). At all doses of JNJ-42847922 tested in acute contrast, higher levels of receptor occupancy were required for degree of receptor occupancy with the OX2R antagonist JNJ- 1,4-diazepan-1-yl)methanone] (Roecker et al., 2015) in rodent evaluation, indicating that 50% OX2R occupancy was suffi- latency and increased NREM sleep duration, whereas REM sleep was minimally affected. The lowest effective dose was 3 mg/kg administered either at the beginning of the light phase (present study) or at dark onset (Letavic et al., 2015). This dose corresponds to the ED_{50} value in the receptor occupancy evaluation, indicating that 50% OX2R occupancy was suffi- cient to promote sleep. Sleep efficacy was obtained at the same degree of receptor occupancy with the OX2R antagonist JNJ-10397049 at a dose of 3 mg/kg s.c. (Dugovic et al., 2009). In contrast, higher levels of receptor occupancy were required for sleep-promoting effects with DORAs: 90% OX2R occupancy with almorexant at 30-mg/kg dosage (Dugovic et al., 2009) and 70–80% receptor occupancy with suvorexant (Winrow and Renger, 2014). At all doses of JNJ-42847922 tested in acute conditions, small nonsignificant REM sleep-promoting effects were observed. However, significant levels were reached only on day 4 during the subchronic treatment. Similarly, a newly developed selective OX2R antagonist, compound 1m, has been reported to primarily increase NREM sleep time with minimal effects on REM sleep when administered in mice during the dark phase (Etori et al., 2014). Efficacy on both NREM and REM sleep has been shown with a number of selective OX2R antagonists from Merck: compound 18 (Roecker et al., 2013), MK-1064 (Roecker et al., 2014a), MK-3697 (Roecker et al., 2014b), compound PE-6 [(2-(2H-1,2,3-triazol-2-y)phenyl)(2R,5R)-5-((8- fluoroquinolin-4-yl)oxy)-2-methylpipеридин-1-yl)methanone] (Raheem et al., 2015), and 2-SORA 19 [(R)-(2-(2H-1,2,3- triazol-2-yl)phenyl4-(5-chloro-2-methylpyrimidin-4-yl)-7-methyl-1,4-diazepan-1-yl)methanone] (Roecker et al., 2015) in rodent models. However, dose-response effects have not been report- ed with these compounds, and the single dose tested might have been high enough to promote both sleep states. Al- though JNJ-42847922 did achieve mainly NREM sleep efficacy at doses up to 30 mg/kg, we found a significant reduction in REM sleep latency and increased REM sleep time at the high dose of 60 mg/kg administered at dark onset in rats, with which 74% receptor occupancy was attained. These data confirm that selective OX2R blockade primarily promotes NREM sleep, as opposed to the DORAs SB-649868 and suvorexant that have been reported to predominantly promote REM sleep (Dugovic et al., 2014; Etori et al., 2014). Importantly, specificity of OX2R antagonists may favorably preserve sleep architecture as evidenced by the maintenance of the REM/total sleep ratio with JNJ-42847922 (present study) and JNJ-10397049 (Dugovic et al., 2009) even at doses that also promote REM sleep, in contrast to several dual OX1/2R antagonists (Hoyer and Jacobson, 2013; Dugovic et al., 2014; Etori et al., 2014).

We also evaluated the potential abuse liability of this mechanism of action using neurochemistry and conditioned placed preference. The neurochemistry experiment showed that JNJ-42847922 did not increase dopamine release in nucleus accumbens. In the conditioned place preference test, JNJ-42847922 did not produce place preference, unlike zolpidem, indicating that the compound lacks intrinsic motivational properties. The rotarod test was also used to assess the effect of JNJ-42847922 on motor coordination. At 30 mg/kg, a dose 10-fold higher than the minimal effective dose to induce sleep, the compound did not alter motor coordination. In addition, the compound did not poten- tiate the ataxic effect of alcohol in this test. This is in sharp contrast to the well known zolpidem-induced ataxia, highlight- ing the safety advantage of this new mechanism of action versus the traditional GABAAergic mechanism. Therefore, JNJ-42847922, similar to other OXR antagonists, pro- motes sleep without causing motor impairment or alcohol interaction (reviewed in Hoyer and Jacobson, 2013; Winrow and Renger, 2014).

JNJ-42847922 did show great promise as a development candidate for the treatment of insomnia. A clear, consistent, and rapid hypnotic-like effect was observed with single-dose administration of 10–80 mg in healthy subjects. JNJ-42847922 was safe and well tolerated, and had a favorable pharmacokinetic profile for a sedative/hypnotic, characterized by rapid absorption and a short half-life of approximately 2 hours. The human pharmacokinetic profile is in line with the prediction based on the pharmacokinetic properties in mouse, rat, dog, and monkey (Letavic et al., 2015). Specifically, the compound had a relatively short duration of action in all preclinical species and moderate to low bioavailability. Remarkably, the plasma concentration obtained in humans at the 20-mg dose (∼550 ng/ml) is in the range of the plasma concentration required to occupy 50% of OX2R in rat brain occupancy studies (∼400 ng/ml). Preliminary EEG polysomnography data obtained in a phase 1b study showed that treatment with JNJ-42847922 re- sulted in significant improvement in both sleep onset and total sleep duration in patients with comorbid insomnia related to major depressive disorder (http://ir.minervaneur- osciences.com/). Further details will be disclosed in a future manuscript.

In conclusion, JNJ-42847922 displayed a favorable pharmacokinetic and safety profile in concert with a strong pharmaco- dynammic effect on sleep induction. The data indicate multiple points of differentiation from nonbenzodiazepine sedative-hypnotics such as zolpidem, as well as clear distinc- tion on sleep architecture and achievable efficacy from DORAs such as suvorexant. Thus, JNJ-42847922 is a prototype of the selective orexin-2 receptor antagonists emerging as a promis- ing new class of nonsedative hypnotics for further clinical development in the treatment of insomnia.
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Wrote or contributed to the writing of the manuscript: Bonaventure, Halter, Dugovic.

References


Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22:3099–3108.


