

JPET-AR-2021-000719

**The dopamine D1 receptor positive allosteric modulator mevidalen (LY3154207) enhances wakefulness in the humanized D1 mouse and in sleep deprived healthy male volunteers**

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**Journal- The Journal of Pharmacology and Experimental Therapeutics**

<http://jpet.aspetjournals.org/content/ifora>

**Running Title:** D1PAM enhances wakefulness in male hD1 mice and humans.

**Keywords:** Mevidalen (LY3154207), dopamine, wakefulness, humanized D1 mouse, sleep.

**Word Counts:**

Abstract: 223

Introduction: 751

Discussion: 1699

**Figure Count: 6**

**Total Pages: 38**

**Abbreviations:**

AAALAC	Association for Accreditation of Laboratory Animal Care
AE	adverse event
AUC	area under the concentration versus time curve
BP	blood pressure
CI	confidence interval
CL/F	apparent clearance
C <sub>max</sub>	maximum concentration
CRU	Clinical Research Unit
CV	coefficient of variation
D1	Dopamine receptor 1
D1PAM	Dopamine Receptor 1 Positive Allosteric Modulator
DA	dopamine
ECG	electrocardiogram
EEG	electroencephalogram
ERP	event-related potential
H3	Histamine 3 receptor
hD1	human Dopamine receptor 1
ICH	International Conference on Harmonisation of Technical Requirements for

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	Registration of Pharmaceuticals for Human Use
KSS	Karolinska Sleepiness Scale
LC/MS/MS	liquid chromatography with tandem mass spectrometry
LLOQ	lower limit of quantification
LS	least squares
MMRM	mixed-effects model repeated measures
MSLT	Multiple Sleep Latency Test
NIH	Nation institute of Health
NREM	Non-REM sleep
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PSG	polysomnography
QC	quality control
REM	rapid eye movement sleep
RSD	relative standard deviation
RT	reaction time
SAE	serious adverse event
SD	standard deviation
SPL	sound pressure level
SR	Sleep restriction
t <sub>1/2</sub>	half-life
TEAE	treatment-emergent adverse event
t <sub>max</sub>	time to maximum concentration
VAS	visual analog scale
V <sub>d</sub> /F	apparent volume of distribution
WASO	wake time after sleep onset
ZT	Zeitgeber Time

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## Abstract

Dopamine (DA) plays a key role in several central functions including cognition, motor activity and wakefulness. While efforts to develop D1 receptor agonists have been challenging, a positive allosteric modulator (PAM), represents an attractive approach with potential better drug-like properties. Our previous study demonstrated an acceptable safety and tolerability profile of the D1 PAM mevidalen (LY3154207) in single and multiple ascending dose studies in healthy volunteers (Wilbraham et al., 2020). Herein, we describe the effects of mevidalen on sleep and wakefulness in the humanized dopamine D1 mice (hD1) and in sleep deprived healthy male volunteers. Mevidalen enhanced wakefulness (latency to fall asleep) in the hD1 mouse in a dose dependent (3-100 mg/kg, PO) fashion when measured during the light (Zeitgeber Time 5) and predominantly inactive phase. Mevidalen promoted wakefulness in mice following prior sleep deprivation and delayed sleep onset by 5.5 and 15.2-fold compared to vehicle treated animals, after the 20 and 60 mg/kg PO doses respectively, when compared to vehicle treated animals. In humans, mevidalen demonstrated a dose-dependent increase in latency to sleep onset as measured by the multiple sleep latency test and all doses (15, 30, 75 mg) separated from placebo at the first 2-hour post dose time point. with a circadian effect at the 6-hour post-dose time point. Sleep-wakefulness should be considered as a translational biomarker for the D1PAM mechanism.

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**Significance Statement:** This is the first translational study describing the effects of a selective DIPAM on sleep wakefulness in the hD1 mouse and in sleep deprived healthy male volunteers. In both the human and mouse, drug exposure was correlated to sleep latency supporting the use of sleep-wake activity as a translational central biomarker for the mechanism. Overall, the wake-promoting effects of DIPAMs including mevidalen may offer therapeutic opportunities in several conditions including sleep disorders and excessive daytime sleepiness related to neurodegenerative disorders.

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## Introduction

Dopamine (DA) plays a key role in several central functions including cognition, motor activity, wakefulness, mood, and reward. Several CNS therapeutics work through the dopaminergic system. For example, DA agonists for Parkinson's disease and DA releasers (amphetamine like compounds) for attention deficit hyperactivity disorders, narcolepsy, and excessive daytime sleepiness (Banerjee et al., 2004). The importance of the DA D1 receptor subtype for higher cognitive functions such as working memory, attention, and executive function has triggered interest in developing novel therapeutics including D1 receptor agonists (Arnsten et al., 2017; Gao et al., 2003; Hall et al., 2019).

Development of novel D1 receptor agonists has been challenging due to poor drug-like properties including inverted U-shaped dose-response, tachypylaxis, poor metabolic stability, and tolerability issues. As an alternative to the D1 receptor agonist approach, we recently discovered a series of D1 positive allosteric modulators (DIPAMs) that could address some of the issues associated with D1 receptor agonists and offer a more physiological approach to activation of D1 receptors with temporal and spatial resolution related to endogenous DA release (Bruns et al., 2018; Hall et al., 2019; Svensson et al., 2019; Svensson et al., 2017). Preclinical studies in transgenic mice expressing the human D1 receptor (hD1 mice) suggest that selective DIPAMs, while sharing many pharmacological properties with D1 receptor agonists, fail to induce inverted U-shaped dose response or rapid tolerance on various behavioral endpoints (Hao et al., 2019; Meltzer et al., 2019; Svensson et al., 2017). Recently discovered DIPAM are represented by the close structural analogs DETQ and DPTQ, as well as mevidalen (LY3154207), which also goes under the generic name mevidalen (Hao et al., 2019; Svensson et al., 2019). When dosed to hD1 mice, DIPAM produce a dose-related increase in forward

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locomotion and rearing behavior over a wide dose range without induction of stereotyped behaviors at high doses that impair locomotion and other functions (Hao et al., 2019; Svensson et al., 2017). A sleep electroencephalogram (EEG) study in the hD1 mouse with DETQ showed dose-related increases in wakefulness and latency to fall asleep (Bruns et al., 2018). In another study the D1 receptor agonist SKF82958 was also wake-promoting, in agreement with preclinical literature (Isaac and Berridge, 2003). The D1 antagonist SCH39166 was found to be sleep promoting in the hD1 mouse, which is in line with sleep inducing effects of D1 antagonism in humans (Eder et al., 2003). The wake-promoting effects of D1 receptor activation is likely linked to central arousal effects including enhanced locomotion and general cortical activation through enhanced release of both acetylcholine and histamine (Bruns et al., 2018).

Single and multiple ascending dose studies in healthy male volunteers with mevidalen were recently reported (Wilbraham et al., 2020) where it demonstrated an acceptable safety and tolerability profile with dose-dependent increase in activating Adverse Events (AE), e.g. insomnia.

Herein we present data for mevidalen on sleep-wake activity in the hD1 mouse and in sleep deprived healthy male volunteers. The preclinical studies with DETQ demonstrated an hD1 mediated wake-promoting effect that could serve as a translatable biomarker to demonstrate central penetration and confirm target engagement in humans. Wake-promoting effects of d-amphetamine, modafinil and the H3 antagonist MK-0249 have been demonstrated in single dose administration studies with sleep deprivation used to induce increased sleep pressure (Chapotot et al., 2003; James et al., 2011), and these compounds have also been shown to have wake-promoting effects in rodent studies (Stocking and Letavic, 2008). In further support of the translatability of sleep signals, basic phenomenological aspects of rodent and human sleep such

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as REM versus NREM states, and homeostatic versus circadian influences have similar underlying physiological mechanisms, often with a homologous chemo-neuroanatomy (Brown et al., 2012; Leiser et al., 2011). However, there are important differences between rodent and human sleep. First, rodents are night active (nocturnal) and they are awake during about 2/3 of the dark period and 1/3 of the light period while humans in general are day active. Also, rodents sleep in short naps throughout the circadian cycle, while humans generally consolidate their sleep into 1 or 2 periods per day. Thus, although the underlying sleep physiology may be similar, these timing differences require care in selecting the timing of drug administration and the background sleep pressure when comparing rats and mice with humans. In this work we present the preclinical work to demonstrate mevidalen's wake-promoting properties in rodents and a clinical trial in healthy male volunteers that demonstrates for this mechanism that this biomarker translates to humans, and thereby confirms central target engagement at tolerable doses.



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## Materials and Methods

### Nonclinical

All experimental procedures were pre-approved by the local animal care and use ethical committee according to NIH guidelines and the Declaration of Helsinki. In the UK all procedures were carried out in accordance with the UK Animals Scientific Procedures Act (1986) and associated guidelines, as well as the European Communities Council Directive of 24 November 1986 (86/609/EEC). All procedures passed the Lilly UK ethical review. The Eli Lilly and Company animal care and use program is fully accredited by AAALAC.

*Animals, housing and care:* Transgenic male mice in which the mouse D1 receptor was replaced with the human D1 were derived as previously described (Svensson et al., 2017). The strain was maintained in the homozygous state. The hD1 mice were bred at Charles River (Margate, UK) and animals were 27-52 weeks of age. Age matched male wild-type (WT) C57Bl/6 NTac mice were also purchased from Charles River (Margate, UK) for preliminary pilot studies. Genotyping was routinely performed by the breeder.

*Surgery:* EEG recordings were made using tethered mice. Surgery was performed by in-house surgeons. Subjects were anaesthetised and sedated (2% isoflurane in 100% oxygen, 0.1 mg/kg medetomidine HCl subcutaneous (SC) injection) and fitted with a cranial implant affixed to the skull by dental acrylic and cyanoacrylate. The cranial implant consisted of bilateral stainless steel screws (2 frontal [+2mm AP from bregma,  $\pm$  2mm ML] and 2 occipital [-3mm AP,  $\pm$  3mm ML]) for EEG recordings. For EMG recordings, two Teflon-coated stainless steel wires were positioned under the nuchal trapezoid muscles. Atipamezole (0.5 mg/kg, SC) was administered to reverse the medetomidine. Carprofen (5 mg/kg, SC) was administered pre-operatively, post-surgery and on the morning of the first post-operative day. Cefovecin (8 mg/kg, SC) was

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administered post-surgery. Body temperature and locomotor activity were monitored via a miniature transmitter (Minimitter G2Emitter, Philips Respironics, Bend, OR, USA) placed in the abdomen during the same surgical procedure. In addition, a non-steroidal anti-inflammatory drug (meloxicam 0.15 mg/kg) was administered orally twice daily for 6 days post-surgery.

Prophylactic antibiotic treatment (cefalexin 20 mg/kg) consisted of an oral dose 24 hours prior to and immediately before surgery, and twice daily for 7 days after surgery (Loomis et al., 2015).

At least 2 weeks were allowed for recovery from surgery.

In the first sleep study, the home cage is the recording cage (Hanley et al., 2019). Each animal was housed individually within a specially modified Ancare™ microisolator cage having a custom polycarbonate filter-top riser and a custom ultra-low-torque slip-ring commutator (Hypnion, Inc.) on a 12-hour light/dark cycle with free access to standard animal laboratory food and water. Each cage was located within separate, ventilated compartments of a stainless steel sleep-wake recording chamber, and had an infrared light source and digital video cameras to allow a minimum of twice-daily remote visual monitoring. Animals were undisturbed for 48 hours before and after each treatment. All treatments were administered at zeitgeber 5 (ZT-5, 5 hours after the start of the light cycle). This dosing time resulted from an empirically optimized assessment of the evaluation of wake-promoting compounds (Edgar and Seidel, 1997; Olive et al., 1998). A general outline of the preclinical testing scheme is shown in [Figure 1a](#).

*Sleep restriction:* To examine the effect of mevidalen preclinically in a paradigm more closely resembling a healthy volunteer human pharmacology study, in a second study we increased sleep pressure over the first protocol by employing sleep deprivation prior to the dosing of compound. To employ sleep restriction (SR) mice were housed in individually custom-designed SR chambers during the course of the sleep study. Each chamber consisted of a cylinder constructed

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of plexiglass rods positioned horizontally inside a plexiglass frame. During periods of SR, following the real-time detection of a NREM sleep epoch, a motor rotated the cylindrical SR chamber around its axis in a pseudo-random direction for 10 seconds. This movement caused a mild vestibular stimulus sufficient to initiate immediate awakening. Our method of inducing physiological SR did not reduce time asleep to zero, rather the amount of functionally effective sleep was zero since no sleep bout was longer than 10 seconds; a reliable, reproducible amount of sleep loss was achieved (Hanley et al., 2019). The sleep restriction period, between ZT-0 and ZT-5 is shown in [Figure 1a](#). Overall, mice undergoing the sleep restriction lost 79.3% of their sleep relative to baseline (7.5 minutes of sleep per hour vs 36.3 minutes per hour during the baseline) and the remaining sleep was limited to periods no longer than 10 seconds of NREM sleep before the activation of the wheel. REM sleep was completely abolished using this protocol. On average the animals attempted to sleep 45 times per hour, causing activation of the wheel.

*Test compound:* Mevidalen (Synonyms: mevidalen, LY3154207 hydroxybenzoate) chemically known as 2-(2,6-dichlorophenyl)-1-[(1S,3R)-3,4-dihydro-3-(hydroxymethyl)-5-(3-hydroxy-3-methylbutyl)-1-methyl-2(1H)-isoquinolinyl]ethanone (Hao et al., 2019) was administered as parent compound or the hydroxybenzoate co-crystal form (as indicated in the result section). For animal studies, drug formulations occurred immediately before each treatment. Test drug was weighed using a Mettler Toledo AB104-S analytical balance (d=0.1 mg), then mixed with vehicle (20% hydroxypropyl betacyclodextrin) using a sterile ground glass mortar and closely fitting pestle (i.e., tissue homogenizer) of appropriate volume until finely suspended, and transferred using multiple washes to a clean screw top glass vial. Suspensions were agitated immediately before being drawn into a syringe for oral administration by gavage. Mice were

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dosed orally (PO) by gavage using a dose volume of 10 mL/kg. To administer the treatment, each mouse was removed from its cage for about 60 to 90 seconds to be weighed and treated.

*Dose-response and sleep-deprivation studies in hD1 mice:* In the first experiment vehicle and doses from 3-100 mg/kg PO of mevidalen were evaluated in a 4-period incomplete crossover study design. In general, animals were not naïve to drug treatments. At least 6 days washout preceded and followed any treatment. In the second study vehicle, 20 and 60 mg/kg PO of mevidalen were evaluated in the sleep deprivation protocol. Where possible, animals from the dose-response study were reused in the sleep deprivation study.

*Measurement of sleep and wakefulness in hD1 mice:* Sleep and wakefulness were determined using SCORE-2000™ - a microcomputer-based sleep-wake and physiological monitoring system (Van Gelder et al., 1991). In the present study, the system monitored amplified EEG (X10,000, bandpass 0.1-30 Hz [Grass Corp., Quincy, MA]; initial digitization rate 400 Hz), integrated EMG (bandpass 10-100 Hz, RMS integration), and telemetered body temperature and non-specific LMA from all mice simultaneously in each experiment. Vigilance states were classified on-line as NREM sleep, REM sleep, wake, or theta-dominated wake every 10 seconds using EEG period and amplitude feature extraction and ranked membership algorithms.

Individually taught EEG-arousal-state templates and EMG criteria differentiated states of arousal. Spectral frequency bands were delta (0.5 to 4 Hz), theta (5.1 to 9 Hz), alpha (9.1 to 12 Hz) and beta (12 to 20 Hz) based on internal and external data (Holton et al., 2020). Total power was selected as 0.1 to 30 Hz.

*Pharmacokinetics:* Mevidalen was given to a satellite group of fed male CD-1 mice (N=6 per dose group with 3/timepoint) as a single oral dose of 3, 10, 30, 60 and 100 mg/kg. The dosing formulation used mevidalen mixed with vehicle (20% hydroxypropyl betacyclodextrin). Blood

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samples were collected at 0.25, 0.5, 1, 2, 4, 7, 12, and 24 hours post dose from orbital sinus bleeding and a terminal cardiac puncture. The plasma samples were analyzed for mevidalen using a non-GLP LC/MS-MS bioanalytical method (Hao et al., 2019).

*Statistical Analysis:*

All statistical analyses were performed using version 9.4 of the SAS System for Windows, Cary, NC, USA and plots created using GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA, [www.graphpad.com](http://www.graphpad.com). Sleep was evaluated over the 60-hour recording period shown in [Figure 1a](#). To quantify the compound effects on sleep/wake parameters, the 12-hour light (0-12 hours) and dark (12-24 hours) periods were used to characterize baseline sleep. On the day of drug administration, the period of 5–12 hours of lights after drug administration was used to estimate sleep latency. Sleep latency was defined as the time to the first minute of continuous sleep following drug administration, whilst REM sleep latency was defined as the duration from the onset of sleep to the first 20 seconds of REM sleep. Other sleep/wake parameters were also evaluated in this period. Finally, the recovery phase was evaluated as two separate light and dark periods 7 to 19 hours and 19 to 31 hours after drug administration. To represent the homeostatic book-keeper, the accumulated minutes of wakefulness were calculated as the accumulated sum difference to baseline at each hour following the treatment.

All outcomes were analyzed by a mixed-model repeated measures analysis of variance using treatment (drug dose), and treatment date as factors, and a compound-symmetry covariance structure to model multiple drug treatments on the same animal. Contrasts were used to test for statistical significance of the change from vehicle for each dose. Sleep latency and other outcomes analyzed on the log scale were back-transformed to report geometric means and mean

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ratio-to-vehicle results, denoted in the table of results as n-fold differences from vehicle. Post-hoc analyses were performed using Tukey's honestly significant differences test.

### Clinical

Protocols for both the studies were reviewed and approved by Independent Institutional Review Board, before study start. Written informed consent was obtained from all subjects before study participation. Both studies were conducted in accordance with the International Conference on Harmonization guideline for Good Clinical Practice and the original principles embodied by the Declaration of Helsinki. The studies are registered at ClinicalTrials.gov as NCT02603861.

*Subjects:* The study enrolled overtly healthy males who are at least 18 years old, and who had a body mass index (BMI) of 18 to 35 kg/m<sup>2</sup> and a regular sleep pattern with routine time spent in bed between 6.5 hours and 9 hours each night. Subjects were excluded if they had a significant abnormality in the 12-lead electrocardiogram (ECG), hypertension, or medical history capable of significantly altering the absorption, metabolism, or elimination of drugs, believed to increase the risk associated with taking the study medication, or of interfering with the interpretation of data. Subjects with serious or active medical or ongoing psychiatric disorders, those who had used or intended to use over-the-counter or prescription medication including herbal medications within 14 days prior to dosing were excluded. Subjects with symptoms consistent with a sleep disorder. were also excluded.

*Study design and treatments:* We designed a clinical study modelled after the Merck H3 antagonist MK-0249 to confirm the wake promotion effect in humans (Iannone et al., 2010), with the principal difference being the selection of the multiple sleep latency test (MSLT) as the primary endpoint instead of the maintenance of wakefulness test (MWT) used in the previous study. The MSLT was selected over the MWT to ensure sufficient window to identify effects for

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a compound with strong wake-promoting effects. Both protocols are similar with the key difference being the command to try to go to sleep in the MSLT vs the command to try to remain awake during the MWT. This inpatient Phase 1 study was a single-dose, placebo-controlled, single-blind (subject- and investigator-blinded) for mevidalen and placebo, open-label for assay control with modafinil, randomized, 4-period, incomplete crossover study design in sleep-deprived healthy male subjects. The design was unbalanced with all subjects randomized to receive placebo and 3 of the 4 remaining treatments (mevidalen dose levels: 15 mg, 30 mg, and 75 mg and modafinil 200 mg). The washout period between dosing in each period was at least 7 days. Subjects were blindfolded during dosing procedures to maintain a level of blinding such that they were swallowing the same total number of capsules or capsules and tablets each period.

#### *Sleep restriction*

Five days prior to each admission, subjects maintained a normal day-awake/night-sleep schedule. Subjects were admitted to the clinical research unit (CRU) on the day prior to receiving study drug (Day -1) for the sleep deprivation night. Sleep was not allowed within 24 hours, before the start of the study. Subjects were to remain within the sleep research laboratory and asked to remain awake throughout the trial. Subjects had access to media including phones, TV and books but were not allowed to leave or perform vigorous exercise. They were continuously monitored by trained staff at the sleep facility to ensure compliance with the protocol.

Study drug was dosed approximately 8 am. Throughout each study period, subjects were provided standardized meals that were devoid of caffeine and alcohol. Each study period lasted for approximately 3 days, with discharge on Day 2 (after the completion of 24-hour procedures), at the discretion of the investigator. All subjects were required to attend a post study follow-up

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assessment at least 7 days after the final dose of study drug prior to discharge from the study. A schematic overview of the clinical study design is shown in [Figure 1b](#).

### *Pharmacodynamics*

*Multiple Sleep Latency Test, MSLT:* Twenty-minute MSLT evaluations were conducted as per the schedule shown in [Figure 1b](#). Subjects laid supine and were instructed to close their eyes and try to sleep. The room lights were turned off to signal the start of the MSLT. Sleep Latency (SL) for the MSLT was defined as the time from lights out to the first epoch of any sleep stage, or first epoch of greater than 15 seconds of cumulative sleep in a 30-second epoch. To prevent accumulation of sleep during the evaluation day, lights were turned on after the occurrence of sleep, or after 20 minutes of wakefulness. SL was compared across treatment groups by time (4 post-dose time points of 2, 4, 6, and 8 hours) and the overall effect was the average across the 4 post-dose times.

*Statistical Analysis and Sample Size Considerations:* All statistical analyses were performed using version 9.4 of the SAS System for Windows, Cary, NC, USA and plots created using GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA, [www.graphpad.com](http://www.graphpad.com). SL was analysed across time using a mixed-effects model repeated measures (MMRM), fitting treatment, period, time, and the treatment-by-time interaction as fixed effects. Time was accounted for as a repeated effect within subject and period using an unstructured covariance model. Subject and subject-by-period effects were fitted as a random effect in the model.

The overall sleep latency effect was also analysed using an MMRM fitting treatment and period as fixed effects. Subject and subject-by-period effects were fitted as a random effect in the model. Change from placebo was estimated for each dose group by a contrast. Confidence



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intervals for the difference not containing zero indicated statistical significance. Post-hoc analyses were performed using Tukey's honestly significant differences test.

The plan was to enroll up to 18 subjects to ensure that approximately 12 completed all 4 study periods. The target sample size of 12 subjects was estimated to have a 90% confidence interval half width of 1.51 minutes in the 4-period average MSLT.

*Karolinska Sleepiness Scale (KSS):* The KSS is a single-question, 9-point self-rating scale used to measure levels of sleepiness during the last 5 minutes (Kaida et al., 2006; Miley et al., 2016). The scale ranges from 1=very alert to 9=very sleepy, great effort to stay awake or fighting sleep. A score of 7 or more indicates excessive sleepiness. KSS was measured before dosing on Day 1 as well as 5 minutes before and after each MSLT assessment performed at the post-dose time points of 2, 4, 6, and 8 hours. Baseline was defined as the pre-dose value on Day 1. Change from baseline in KSS was calculated and summarized by treatment group and time point.

*Pharmacokinetics:* At doses of 15 mg, 30 mg and 75 mg blood samples were collected at pre-dose and at 1, 3, 5, 7.5 and 9 hours post-dose on Day 1 and 14 of the study design to measure plasma concentrations of mevidalen. Plasma concentrations of mevidalen were assayed using a validated liquid chromatography/tandem mass spectrometry method at Covance Laboratories Inc. (Madison, Wisconsin, USA). The lower limit of quantification (LLOQ) of mevidalen plasma was 0.500 ng/mL, and the upper limit of quantification was 2500 ng/mL (Wilbraham et al., 2020). From the mevidalen plasma concentration – time data, standard noncompartmental methods of analysis were used to calculate the time to maximal concentration ( $t_{max}$ ), the maximum plasma concentration ( $C_{max}$ ), the area under the plasma concentration - time curve from time 0 to infinity ( $AUC_{0-\infty}$ ) and the AUC from 0 to 24 hours ( $AUC_{0-24}$ ).

*Exposure – Response Analysis*

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The objective of the exposure-response (ER) analysis was to assess the relationship between mevidalen plasma concentrations and sleep effects in human and mouse. In human, MSLT evaluations were performed at 2, 4, 6, and 8 hours post-dose, and PK blood samples were collected at 1, 3, 5, and 7.5 hours during the MSLT time frame. In order to time-match the MSLT observations with mevidalen plasma concentrations for the ER analysis, a population PK model (not reported herein) that provided an adequate fit to the observed mevidalen PK was developed. Subsequently, a model-based PK simulation was conducted to predict the mevidalen plasma concentration for each individual at each MSLT time based on their PK model parameter estimates. In the mice, individual PK parameter estimates were not possible due to the nature of the study and therefore only treatment group PK levels were estimated from at the average latency to sleep. A log-linear fit to the data was performed using GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA, [www.graphpad.com](http://www.graphpad.com).

#### *Safety Assessments and Analyses*

Safety data included documentation of adverse events (AEs), safety laboratory parameters, ECG parameters and vital signs. Suspected unexpected serious adverse reactions (SUSARs) were defined as serious events that were not listed in the Investigator's Brochure and that investigator identified as related to investigational product or procedure. Clinical and laboratory AEs were coded according to the Medical Dictionary for Regulatory Activities (version 18.0).

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## Results

### Nonclinical

#### *Effect of mevidalén on sleep and wakefulness in human D1 knock-in mice*

Preliminary studies of mevidalén in hD1 and WT mice confirmed on-target hypothesis of mevidalén's wake-promoting properties (not shown). In the dose-response study in the hD1 mouse with mevidalén dosed at ZT-5, mevidalén showed a rapid onset of the wake-promoting effects in a dose dependent fashion with a delay to the onset of sleep (Figure 2a-c and Figure 3a). Relative to the vehicle control animals, which required on average  $34 \pm 3$  minutes to fall asleep, the dose-related increase in sleep latency ranged from 1.4-fold to 9.7-fold longer (Figure 3a), a statistically significant result at all doses (3 to 100 mg/kg, PO). NREM sleep reductions during the first 7 hours post treatment was also dose-related and statistically significant starting at 20 mg/kg (Supplementary Table 3). In all treatment groups, wake-promoting efficacy subsided within the first 7 hours with little evidence of a rebound-like increase in sleep. Overall, accumulated wakefulness was lost in small increments sleep during the subsequent dark period at the 30 and 60 mg/kg doses. (Figure 2c).

#### *Effects of mevidalén on sleep latency in sleep deprived hD1 mice*

Mevidalén maintained equivalent wake-promoting effects in mice subjected to 5 hours of prior sleep restriction using the custom-designed SR chambers. Following 20 mg/kg PO of mevidalén, mice fell asleep on average, in  $107 \pm 7$  minutes, or 5.5-fold longer than the vehicle controls, which took on average on average  $19.5 \pm 2$  minutes ( $p < .0001$ , Figure 3b), which was not significantly different to the  $125 \pm 9$  minutes, or 4-fold longer than their vehicle controls ( $p < .0001$ ) seen without prior sleep deprivation. At the 60 mg/kg, PO dose level of mevidalén, mice subjected to 5 hours of sleep restriction fell asleep, on average, in  $297 \pm 20$  minutes, 15.2-

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fold longer than their vehicle controls ( $p < 0.0001$ , [Figure 3b](#)), which was once again not significantly greater than the  $250 \pm 19$  minutes, 8.1-fold longer than their vehicle controls ( $p < 0.0001$ , [Figure 3b](#)) seen without prior sleep deprivation.

### *Pharmacokinetics*

The pharmacokinetics of mevidalen at doses of 3 – 100 mg/kg in the satellite group of mice are presented in [Supplementary Figure 1](#). Across doses, the mean  $t_{\max}$  ranged from 0.25 – 0.5 hours and the mean  $t_{1/2}$  ranged from approximately 2 – 3 hours. Mevidalen demonstrated an approximately dose proportional increase in  $C_{\max}$  and  $AUC_{0-24}$  ([Supplementary Table 1](#)).

### Clinical

#### *Effects of mevidalen on sleep latency in sleep-deprived healthy subjects using MSLT:*

Mevidalen significantly increased sleep latency at all doses in the first sleep latency test at all doses ([Figure 4a](#)). However, at later time points, as sleep pressure continues to build and the quantity drug reduces, the treatment effect disappears. Consistent with the results from the analysis by time point, in the overall MSLT, mevidalen demonstrated dose dependent increases in sleep latency within the MSLT ([Figure 5](#)). Based on the LS mean differences compared to placebo (where subjects remained awake for only 40.2 seconds, 95% CI 10.1, 121.8), the mevidalen 75mg dose group had a 5.2-minute increase compared to placebo (95% CI: 3.31, 7.10) and at the 30mg dose had a 3.2-minute difference (95% CI: 1.35, 5.06). On average, mevidalen did not significantly increase sleep latency at the 15 mg dose (estimate=1.95 min, 95%CI: 0.40, 3.51), but the time series plots indicate efficacy at the 2-hour post treatment time point ([Figure 4a](#)).

In a covariate analysis investigating the effects of machine and technician used for the MSLT, no notable differences were seen for either of these factors. In addition, no significant period effect

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was observed, although it appears that subjects, on average, took longer to fall asleep during their first MSLT assessment in Period 1. However, this was already accounted for by the planned inclusion of period as a fixed effect in the MMRM analysis.

*Effects of mevidalen on sleepiness in sleep deprived healthy subjects using KSS:*

A line plot of mean KSS scores pre-dose and at each post-dose time point (pre- and post-MSLT) is presented by treatment group in [Figure 4b](#). Mean scores indicated a clear dose response with the mevidalen 75mg dose group having the largest increase in levels of alertness compared to the other mevidalen dose groups, and placebo. This is consistent with the same dose response observed for sleep latency from the MSLT. However, the circadian effect observed in the mevidalen 75mg and 30mg dose groups at the 6-h post-dose time point for sleep latency (that is, subjects fell asleep quicker compared to the earlier 4-h post-dose time point) was not observed in the KSS score.

*Pharmacokinetics*

The plasma concentration – time profile of mevidalen at doses of 15, 30 and 75 mg are shown in [Supplementary Figure 2](#) and the PK parameters are summarized in [Supplementary Table 2](#).

Across all dose levels,  $t_{max}$  was achieved at about 3 hours and  $C_{max}$  and AUC increased in a dose proportional manner. The PK profile of mevidalen in this study was consistent with what has been reported previously (Wilbraham et al 2020).

*Exposure – Response Relationship for Human and Mouse*

[Figure 6](#) illustrates the ER relationship between mevidalen plasma concentrations (log scale) and increase in sleep latency for human and mouse in the non-SD experiment. The ER was dose proportional within the ranges tested and for each species the data was characterized using a linear regression model. The slope and y-intercept was 6.16 and -8.04 for human, and 5.92 and -

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11.1 for mouse, respectively, indicating an increasing wake-promoting ER in both species. The similarity of the slopes suggests similar fold-increases in wake-promotion for equivalent exposure increases.

### *Safety and Tolerability*

No deaths, serious AEs, or discontinuations due to AE occurred. All AEs were mild in severity. A total of 12 TEAEs were reported by 9 subjects, the most common of which was contact dermatitis (4 subjects). No other TEAEs occurred in more than 1 subject. Of the 17 subjects who received 1 more dose of study treatment, 2 subjects (11.8%) reported 2 AEs that were considered related to treatment. One subject experienced mild somnolence after receiving mevidalen 15mg. There were no apparent drug- or dose-related trends in the clinical laboratory data and 12-lead ECG parameters (with the exception of dose-related increases in pulse rate and blood pressure) following doses of mevidalen consistent with previous trials of mevidalen in healthy volunteers (Wilbraham et al., 2020).

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## **Discussion:**

This is the first translational study describing the effects of a selective and brain penetrant D1PAM on sleep wakefulness in the hD1 mouse and in sleep deprived healthy volunteers. In human and mouse, exposure was correlated to sleep latency, supporting the use of sleep-wake activity as a central biomarker for the D1PAM mechanism. In addition, these data support several therapeutic opportunities including excessive daytime sleepiness, narcolepsy, and other disorders related to impaired circadian rhythms.

Dopamine releasers such as d-amphetamine are used clinically to increase wakefulness, and D1 receptor agonists have been shown to cause behavioral activation with increased wakefulness and reduced sleep in animals (Isaac and Berridge, 2003). Conversely, D1 receptor antagonists increase slow wave and REM sleep with reduced time spent awake both in animals (Monti et al., 1990) and in humans (Eder et al., 2003). We recently reported that the selective D1PAMs DETQ and mevidalen produce dose-dependent increases in locomotion in hD1 mice, similar to D1 receptor agonists, but importantly without inducing inverted U-shaped dose responses (Hao et al., 2019; Svensson et al., 2017). In addition, DETQ showed dose-related increases in wakefulness and reduced sleep in hD1 mice and these effects were similar to a D1 receptor agonist but opposite to a D1 antagonist in this model (Bruns et al., 2018). The mechanism by which D1PAMs increase wakefulness is likely via enhanced receptor activation from the naturally present dopamine and subsequently enhanced release of acetylcholine and histamine in cortical and subcortical areas (Bruns et al., 2018; Hao et al., 2019). Both neurotransmitters are well known to have arousal and wake-promoting effects (Watson et al., 2010).

In this report we found that mevidalen enhanced wakefulness (latency to fall asleep) in the hD1 mouse in a dose dependent fashion when measured during the light (ZT-5) and predominantly

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inactive phase. This is in agreement with previous studies (Hao et al., 2019; Svensson et al., 2017), where increased wakefulness and motor stimulation were observed at doses that produced unbound brain drug levels in the hD1 mouse around the EC50 value in the in vitro hD1 cAMP assay (3 nM). Following the wake-promoting effect of mevidalen there were no significant rebound-like increases in NREM sleep. However, there was some gradual recovery of lost sleep throughout the dark period at the highest doses. The lack of a rebound increase in sleep after extended waking is similar to what is reported for modafinil (Edgar and Seidel, 1997) but different from what is observed with traditional stimulants like d-amphetamine (Hanley et al., 2019). The duration of the wake-promoting effects also paralleled increases in locomotor activity (data not shown).

Treatments for disorders of excessive sleepiness have to remain effective in situations of high sleep pressure. In the current study in hD1 mice mevidalen or vehicle was administered at ZT-5 either with or without a prior sleep restriction of 5 hours. Mevidalen was clearly still wake-promoting following prior sleep deprivation with 5.5 and 15.2-fold longer times to fall asleep when compared to the vehicle treated animals, after the 20 and 60 mg/kg PO doses respectively. Wake time was similar to that without sleep restriction suggesting that the additional sleep pressure did not reduce the efficacy.

In the human study, 24-hour sleep-deprived subjects were dosed and 2 hours later allowed to fall asleep within a 20-minute interval. Subjects were awakened and asked to leave the test room as soon as sleep onset has occurred. In this way sleep propensity should have minimally affected the repeated testing. The test was repeated every 2 hours throughout the day (Mitler et al., 1998; Seidel et al., 1984).



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Consistent with the preclinical findings in hD1 mice, mevidalen demonstrated a dose-dependent increase in SL and all doses showed significant effects separated from placebo at the first 2-hour post-dose time point (Figure 4a). Based on the LS mean differences compared to placebo, the 75 mg dose had a 5.2-minute difference compared to placebo. A delay in sleep latency of this magnitude represents a clinically meaningful effect based on available literature for modafinil (Minzenberg and Carter, 2008), which is marketed as a wakefulness-promoting agent. For example, a Phase 3 study of armodafinil 150 mg versus placebo for jetlag showed a total increase of sleep latency of 6.9 minutes (Rosenberg et al., 2010). However, in the present study, Modafinil did not show a statistically significant effect relative to placebo on SL as measured in the MSLT overall or at any time point. Pharmacokinetic analyses of modafinil in plasma samples revealed levels in agreement with published data (Darwish et al., 2009; Wong et al., 1999) for the 200 mg dose of modafinil (data not shown). This contrasts with previous reports that modafinil increases SL in the MWT (Chapotot et al., 2003; Dinges et al., 2006; Iannone et al., 2010; James et al., 2011), and we did not expect the variation in primary endpoints to affect the outcome of modafinil. However, the use of MWT remains the principal difference in methodology between those studies and ours. In the MSLT the subject is told to try to sleep whereas in the MWT they are told to remain awake. These findings suggest that the motivational instruction given to the subject affects modafinil performance, but this hypothesis would have to be confirmed in future studies.

Further support of the clinical meaningfulness of the changes in MSLT with mevidalen, a dose-dependent decrease in subjective sleepiness as measured by the KSS was seen, with the greatest increases in alertness occurring at the 75mg dose. The wake-promoting effects of mevidalen observed in this study are also consistent with previous Phase 1 safety studies where

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higher doses of mevidalen resulted in CNS activating adverse events including insomnia (Wilbraham et al., 2020). Safety and tolerability were overall consistent with our previous studies (Wilbraham et al., 2020). Only a very small number of treatment emergent adverse events (TEAEs) were reported, regardless of causality (data not shown). The most common TEAE was contact dermatitis. No other TEAE occurred in more than 1 subject. As a result, we believe mevidalen to be safe for further evaluation in an efficacy study in disorders of excessive sleepiness.

Analyses were conducted to assess the relationship between mevidalen plasma concentrations and sleep latency in humans and mice. Our findings show a parallel relationship between species (similar slope) across the concentration range examined, but sleep effects in human can be achieved at lower mevidalen plasma concentrations than mouse (Figure 6). Based on the linear regression analysis results, a 5-fold increase in sleep latency compared to vehicle/placebo would require a mevidalen plasma concentration of about 524 ng/mL in mouse and 130 ng/mL in human with a potency ratio (human/mouse) to be about 4.

Possible explanations may be that the design and methods of the studies in mice and human necessarily have differences and there may be biological differences between species resulting in different sleep patterns across species. SL in rodents is measured immediately after dosing versus 2 hours post dose in humans. No instructions can be given to rodents. Pharmacologically mevidalen shows about 30-fold higher potency for the human vs the mouse D1 receptor (Hao et al., 2019). Although we used the fully humanized D1 mouse for current studies, these animals show about 50% lower D1 receptor expression in the brain as compared to their corresponding wild types (Svensson et al., 2017). This, together with potential differences in signal transduction mechanisms such as G-protein receptor coupling, could possibly contribute to the human/mouse

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potency ratio described above. We have estimated previously that the human  $K_{pu,u}$  (CSF concentration/unbound plasma ratio) is about 0.3 (Wilbraham et al., 2020) and similar to that of mouse (Hao et al., 2019), so it does not appear that the more potent sleep effects in human are driven by enhanced mevidalen penetration into the CNS. These data are important in understanding the translatability of mouse to human sleep latency data for a DIPAM and potentially supports the use of wake promotion as a translational biomarker for this mechanism. Furthermore, complete translatability (herein defined as a potency ratio of 1) does not preclude use of a translational biomarker; what is useful to know is the relationship across species, which can then be used to make inferences and insights from preclinical to clinical experiments.

In this study, only male animals were used and compared to male human subjects. This was for practical reasons, such as ensuring animals reached a certain size to accommodate the EEG implant and reducing variability. As a result this limits the direct interpretation of these findings to males. However, indirect evidence suggests these findings will be independent of sex, since female mice were briefly characterized, they were found to respond in a similar fashion to DIPAM treatment, with behavioral activation but with slightly higher baseline spontaneous locomotor activity (unpublished data). Later clinical studies in phase 1b and phase 2 studies in Parkinson's disease and Lewy Body Dementia patients (Wilbraham et al., 2021b and Biglan et al., 2021, respectively) did include female subjects. Based on these findings, there is no evidence to suggest that we have a gender difference in the overall pharmacodynamic effects of mevidalen.

Although the recovery sleep assessment was not a formal sleep evaluation (Supplementary Table 4) with fixed times for lights off (start of sleep) and lights on (morning waking), the data generated suggest that there were some residual effects from the 75mg mevidalen dose (slightly longer sleep latency and larger percentage of time in the WASO state) but, importantly, the sleep

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architecture was not significantly disrupted. This is important information for a molecule with wake-promoting activity that is not being developed for that indication.

In conclusion, these results suggest that increased wakefulness is a translatable central biomarker for the D1PAM mevidalen in humans, and that the wake-promoting efficacy of mevidalen might not be markedly diminished by sleep depriving humans or rodents prior to quantitative EEG assessment of wakefulness. Finally, the wake-promoting effects of selective D1PAMs including mevidalen may offer therapeutic opportunities in several conditions including sleep disorders and excessive daytime sleepiness related to neurodegenerative disorders. Ongoing Phase 2 studies in patients with Lewy Body Dementia, including Parkinson Disease Dementia and Dementia with Lewy Body, may also inform whether this new mechanism has wake-promoting benefits in this population.

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**Acknowledgments:** The authors wish to acknowledge the subjects, investigators, and clinical staff involved in the study, as well as Sumit Arora for providing medical writing services on behalf of Covance, Inc.

### **Authorship Contributions**

All authors wrote or contributed to the writing of the manuscript

Andrew McCarthy, Brian Eastwood and Claire Brittain performed data analysis

Kjell Svensson, William Kielbasa, Kevin M Biglan, Keith A. Wafford participated in research design

Elaine Shanks conducted experiments

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## **Footnotes**

### **Declaration of Conflicting Interests:**

All authors are current or former employees of Eli Lilly and Company Inc., and may own stock in this company.

**Funding:** This study was funded by Eli Lilly and Company Inc.

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**Legends for Figures:**

**Figure 1:** Figure 1. Experimental designs for sleep EEG studies in the mouse (a) and humans

(b). Abbreviations: CRU=clinical research unit; KSS=Karolinska sleepiness scale;

MSLT=multiple sleep latency test; ZT= Zeitgeber Time (Circadian Time, CT).

**Figure 2:** Pharmacodynamic effects of mevidalen on wakefulness in hD1 mice for the placebo (n=21), 10 mg/kg (n=10), 30 mg/kg (n=11) and 60 mg/kg (n=12) groups: (a) hourly plot showing baseline, treatment and recovery periods following drug administration at ZT/CT-5 (5 hours after lights on) (b) the first 5 hours following treatment in 5-minute bins, and (c) accumulated minutes of wakefulness over 29 hours post treatment. Dark phases are represented by grey shaded regions and dosing at ZT-5 by a black vertical line.

**Figure 3:** Dose dependent effects of mevidalen on sleep latency in, (a) hD1 mice for placebo (n=21), 3 mg/kg (n=17), 10 mg/kg (n=10), 20 mg/kg (n=13), 30 mg/kg (n=11), 60 mg/kg (n=12) groups and 100 mg/kg (n=18) groups. All treatment groups were compared to vehicle, with p-values <0.0001. Dose dependent effects of mevidalen on sleep latency (b) in hD1 mice following 5 hours of sleep deprivation (SD) and a non-sleep deprived control. Compared to vehicle controls, all treatment groups showed significant increases in sleep latency (p<0.0001). In addition, pairwise comparisons to non-sleep deprived controls were included, showing significant increases in latency.

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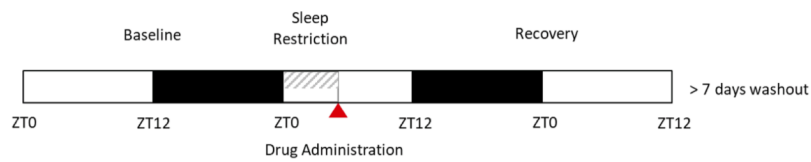
**Figure 4:** Pharmacodynamic effects of mevidalen on wakefulness in healthy human subjects using the MSLT and KSS scales. Time course for (a) MSLT for dose levels of 15mg (n= 12, \* p<0.05), 30mg (n= 13, # p<0.05), 75mg (n= 9, ∅ p<0.05) vs placebo (n= 16); and (b) KSS time series with measures taken prior to and immediately after the MSLT. Sample sizes were the same as MSLT. Modafinil not shown for clarity.

**Figure 5:** Dose dependent effects of mevidalen on sleep latency (MSLT) in humans. Values are presented as fold change in latency averaged from the 4 tests performed (see Figure 1). Pairwise comparisons showing significant differences (p<0.05) are marked with \*, 15mg (n=12) 30mg (n= 13), 75mg (n= 9), modafinil (n= 12).

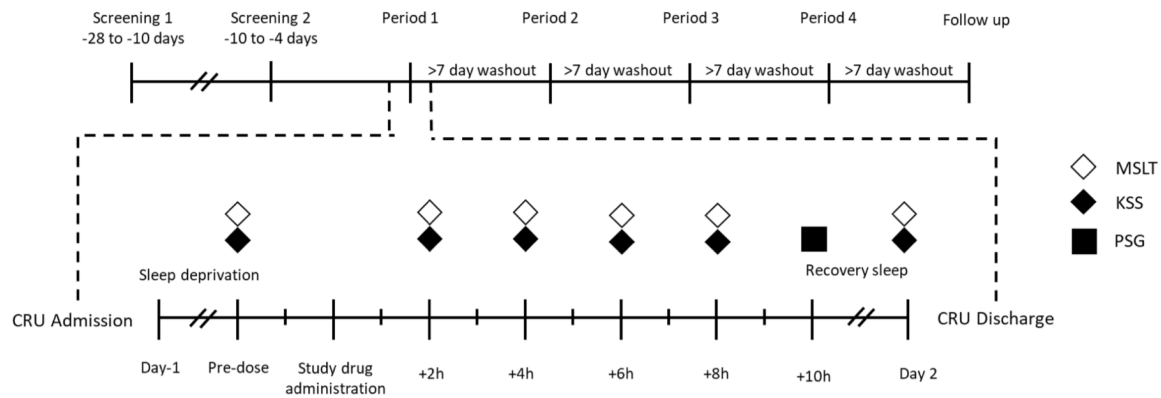
**Figure 6:** Translational PKPD plot of mevidalen in hD1 mice and healthy human volunteers. Shown are the mean and standard error on both axes for each timepoint by dose level. Log linear relationships are shown for human ( $y = -8.04 + 6.16 \cdot \log(x)$ ) and mouse ( $y = -11.1 + 5.92 \cdot \log(x)$ ).

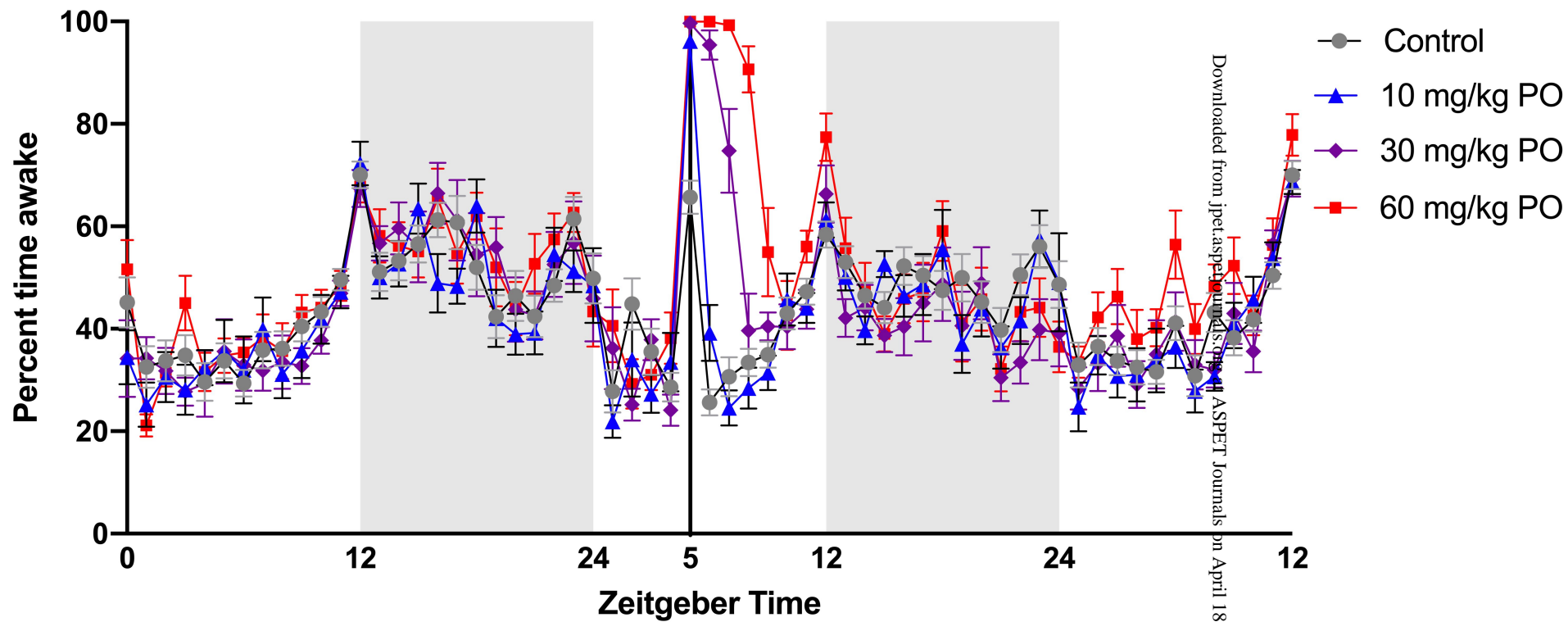
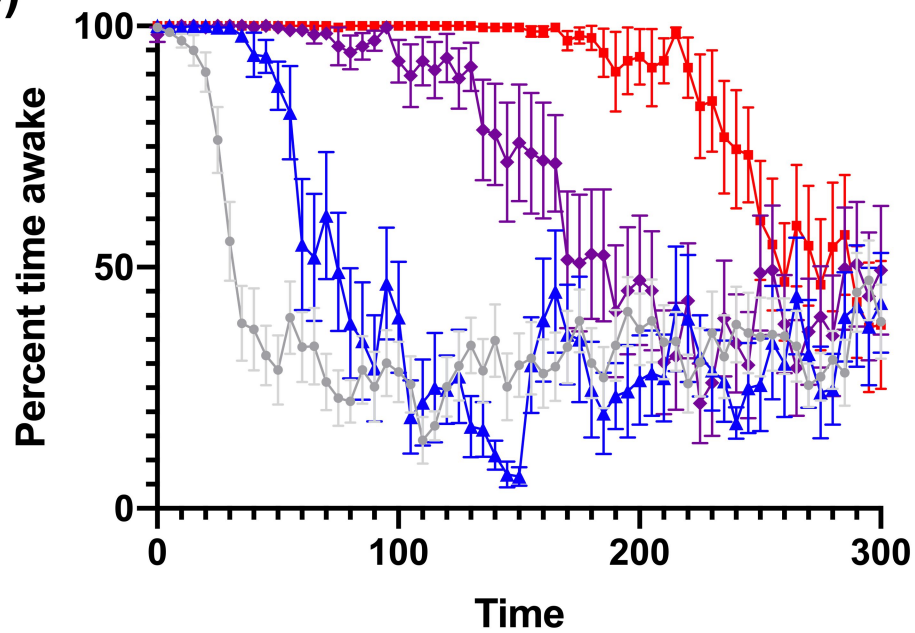
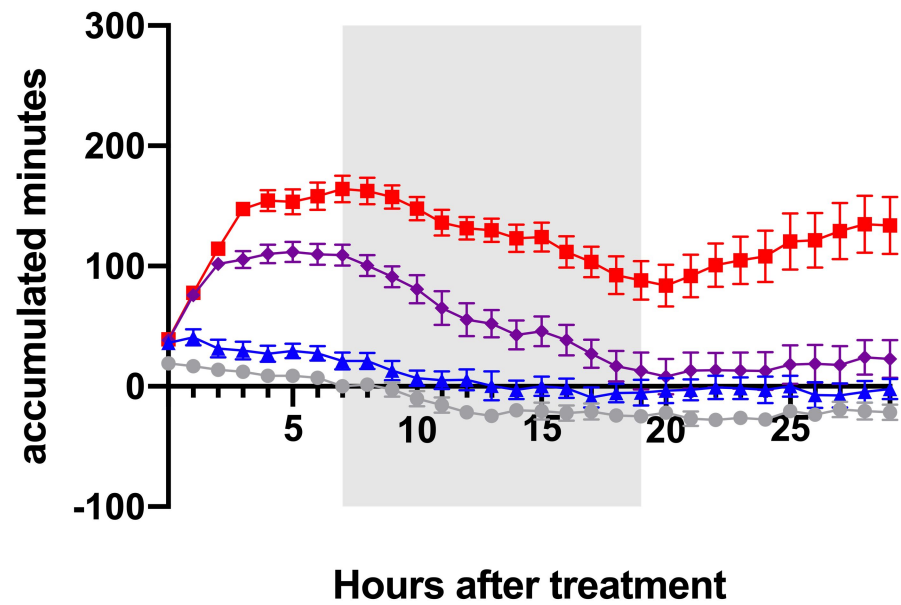
**Figure 1**

(a) Mouse Experimental Design



(b) Human Experimental Design

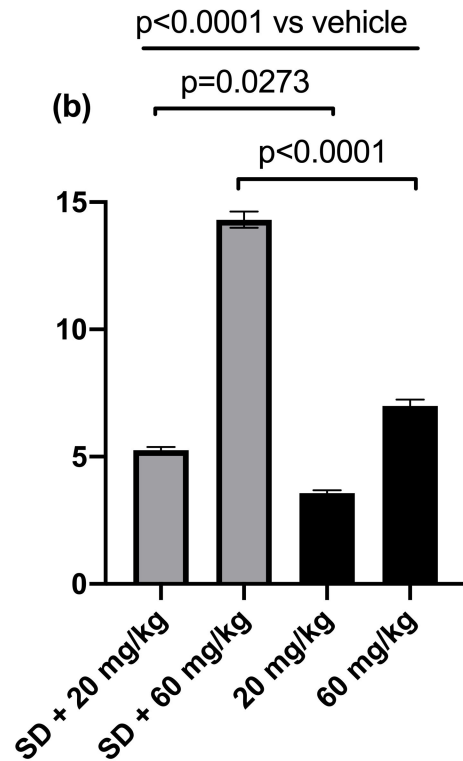
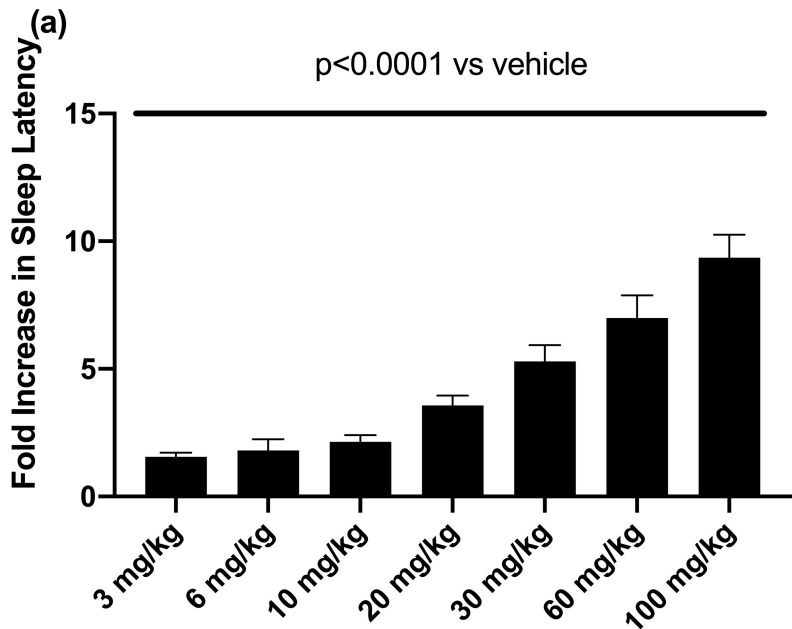


**Figure 2****(a)****(b)****(c)**

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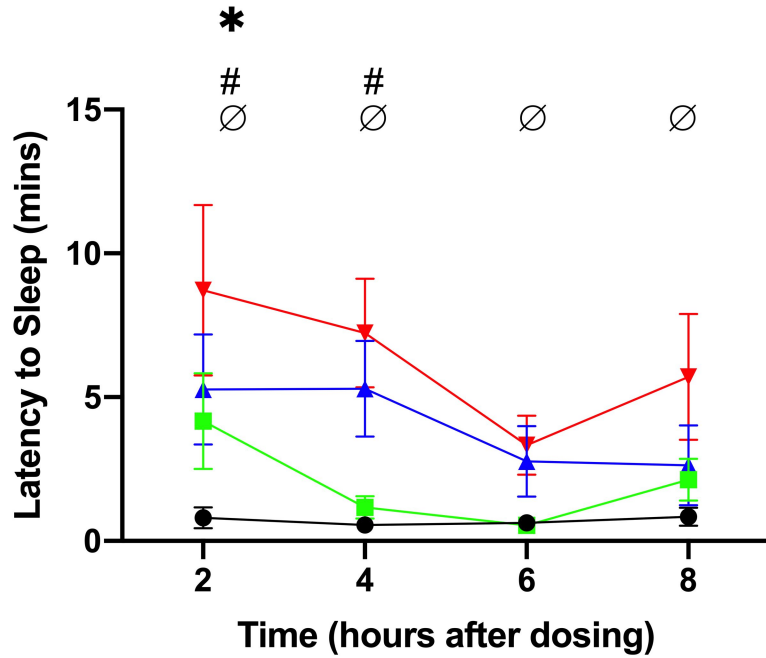
Figure 3



Treatment

# Figure 4

(a)



(b)

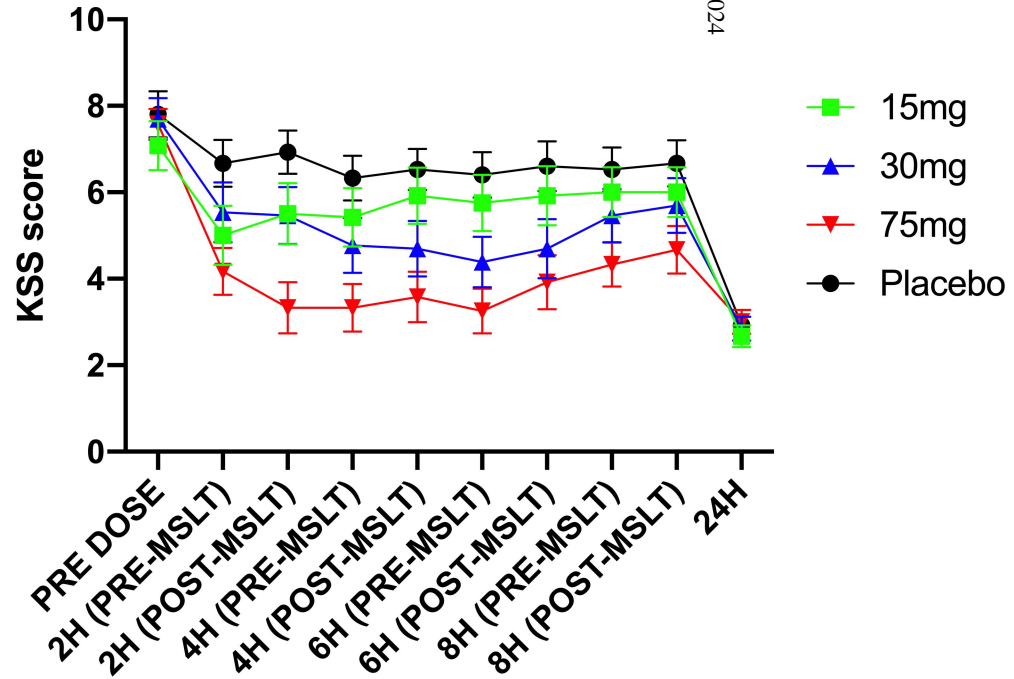
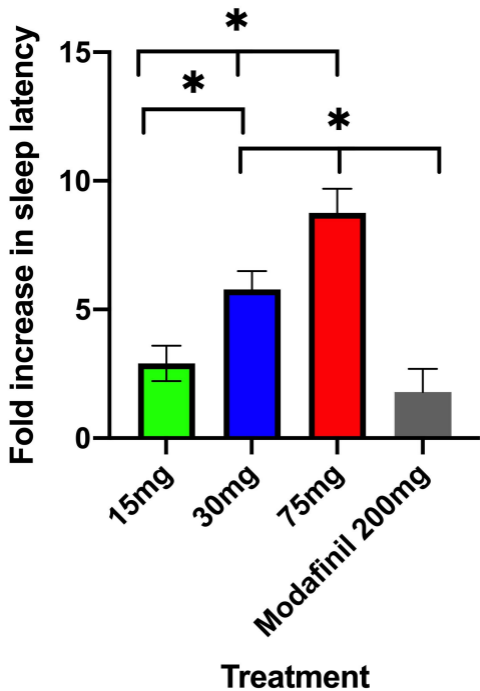


Figure 5



**Figure 6**

