ORX142, an Oral, Highly Potent and Selective Orexin Receptor 2 Agonist, Promotes Wakefulness in Non-Human Primates

Sarah Wurts Black[1], Tod Steinfeld[1], Karl Gibson[1], Gregory R. Ott[1], Emiliangelo Ratti[2], David Grainger[2], Mario Alberto Accardi[2], Deborah S. Hartman[1]

1. Centessa Pharmaceuticals LLC (USA) | 2. Centessa Pharmaceuticals (UK) Limited

Introduction

Excessive daytime sleepiness is associated with multiple neurological indications—some with intact orexin neurotransmission and others with low orexin tone, such as Narcolepsy Type 1 (NT1). We previously demonstrated reduction of NT1 symptoms by orexin receptor-2 (OX2R) agonists in translationally predictive mouse models[1,2]. Non-human primates provide a direct translational model due to their monophasic, diurnal sleep pattern, similar to humans. Here, healthy marmosets with functional orexin neurotransmission were evaluated for wake promotion during their normal rest phase at night after ORX142, an investigational, orally administered, highly potent, and selective OX2R agonist.

Materials & Methods

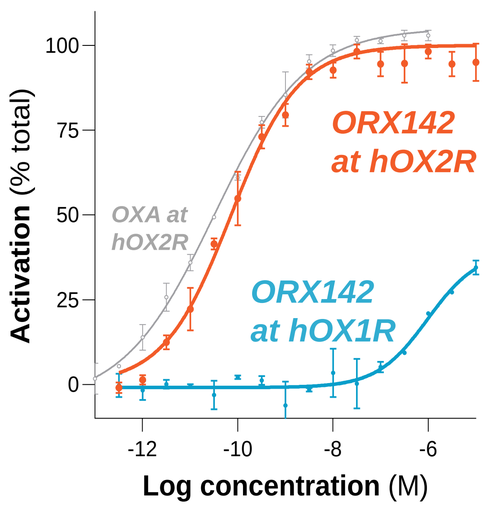
***In Vitro* Pharmacology. i)** Calcium mobilization (FLIPR) assays were performed in Chinese hamster ovary (CHO) cells stably expressing human recombinant OX2R or orexin receptor 1 (OX1R). Cells were loaded with a calcium-sensitive fluorescent dye and then monitored for changes in fluorescence that were agonist concentration-dependent. **ii)** Pathhunter β-arrestin recruitment assays were performed in CHO cells co-expressing the ProLink™ (PK)-tagged OX2R and Enzyme Acceptor (EA)-tagged β-Arrestin (Eurofins). Agonist-stimulated activation of the tagged OX2R resulted in β-arrestin recruitment and complementation of β-galactosidase enzyme fragments, PK and EA. The resulting functional enzyme hydrolyzed a substrate generating a chemiluminescent signal that was agonist concentration-dependent. **iii)** Activity at over 400 additional targets was assessed using *in vitro* assays at test concentrations up to 10 micromolar in Eurofins' SAFETYscan47, GPCRMax, and Drug Abuse Potential SafetyScreen panels and up to 30 micromolar in Metrion's Comprehensive *in vitro* Proarrythmia Assay (CiPA) panel.

Animals and recordings. Common marmosets (*Callithrix jacchus,* 350-450 g) were surgically prepared with telemeters (HD-S02) for electroencephalogram (EEG), electromyogram (EMG) monitoring with concurrent video recording; arousal states were manually scored in 10 sec epochs using NeuroScore (Data Sciences Inc., St. Paul, MN, USA). All stages of NREM sleep were combined into the single classification of NREM sleep to simplify analysis. Marmosets were recorded from home cages in LD12:12, with *ad libitum* water and environmental enrichment, and housed with a non-implanted marmoset of the opposite sex. Each marmoset per pair received the same treatment per dosing day, but only one animal was recorded per cage throughout the study (n=4 males, 3 females per analysis condition). All experimental procedures were approved by the Italian Ministry of Health (Project Code No. 37101 at Evotec).

Formulation, dosing, and efficacy study design. ORX142 was formulated for acute oral dosing. Each dosing day, ORX142 was serial diluted and administered 15 min prior to lights-off in a counterbalanced design. Data presented were the mean ± S.E.M. during the 8 h post dose, unless otherwise noted. Significant differences from vehicle (indicated by * on graphs) were determined by Holm-Sidak contrasts following repeated-measures analysis of variance.

Results

1 ORX142 showed high in vitro potency at OX2R and strong selectivity vs. OX1R

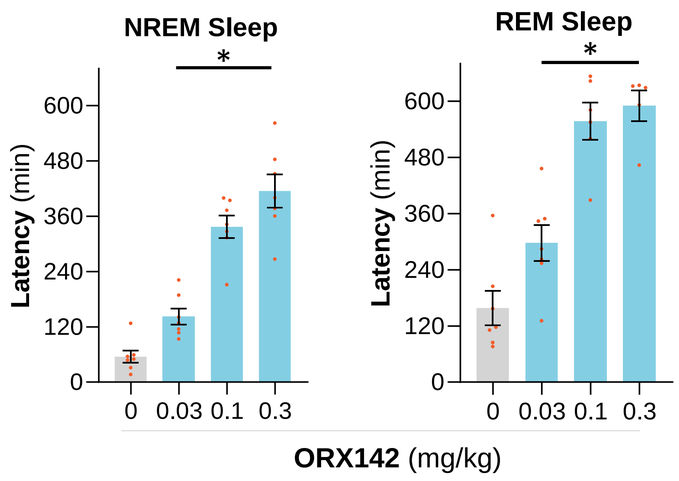


EC_{50} 0.069 nM at hOX2R

> 13,000-fold selective vs. hOX1R

- ORX142 hOX2R agonist profile (geometric mean EC_{50} ± SD = 0.069 ± 0.060 nM; n=24) was comparable to OXA (geometric mean EC_{50} ± SD = 0.036 ± 0.016 nM; n=199) and no biased agonism (not shown)
- No significant differences in OX2R potency were observed across human, mouse, rat, dog, monkey recombinant receptors *in vitro*
- No significant pharmacological activity was observed in GPCR selectivity and *in vitro* safety panels

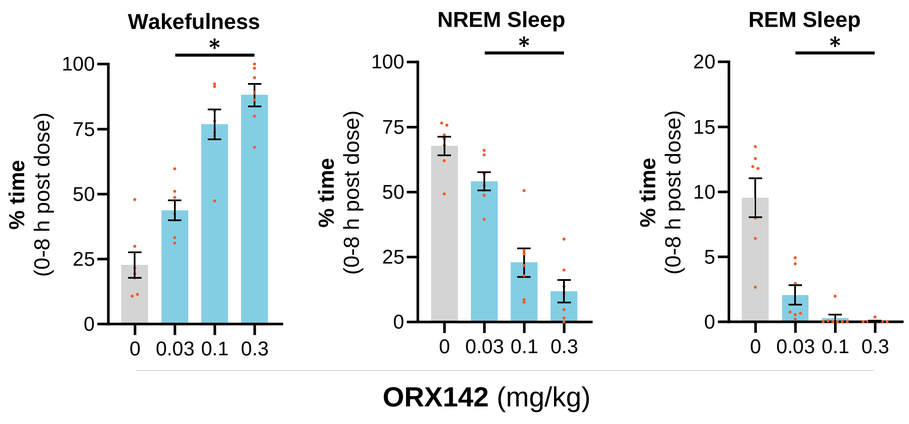
2 ORX142 promoted wakefulness with increased latencies to NREM and REM sleep in a dose related manner



- The latencies to REM sleep were longer than for NREM sleep, as expected (1.7-2.9 h longer)
- NREM sleep resumed by 2.4-6.9 h and REM sleep resumed by 5.0-9.8 h post dose

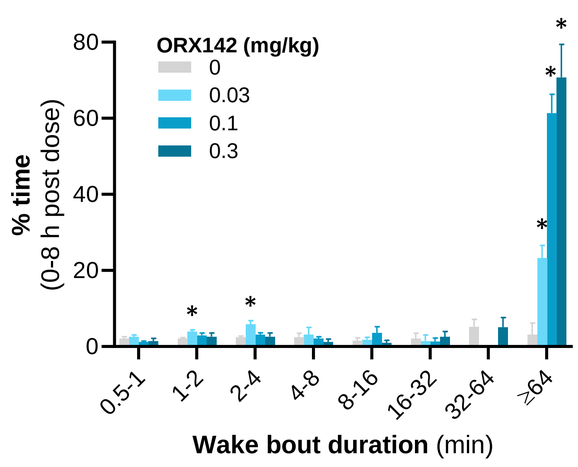
Latency to sleep increased at 0.03 mg/kg, the lowest dose tested in marmosets

3 ORX142 increased time awake and decreased NREM and REM sleep in a dose related manner



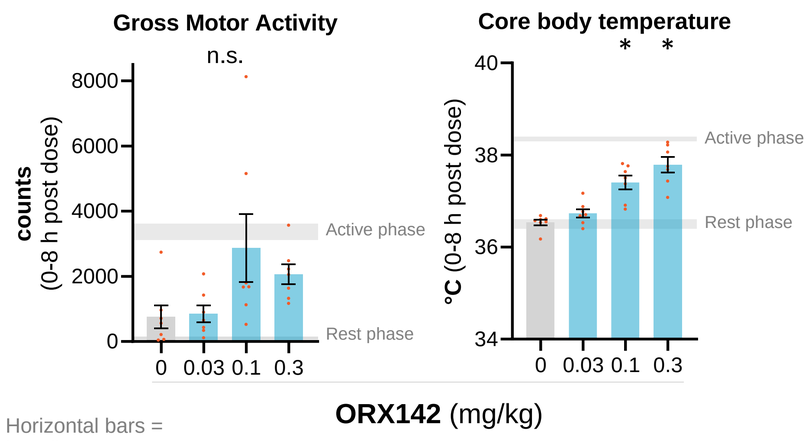
Wake time and consolidation increased at 0.03 mg/kg, the lowest dose tested in marmosets

4 ORX142 induced long, consolidated bouts of wakefulness



- During the 8 h after ORX142 (0.3 mg/kg), 70% of the time was spent in wake bouts that were longer than 1 h

5 Motor activity and body temperature were within normal diurnal variation after ORX142

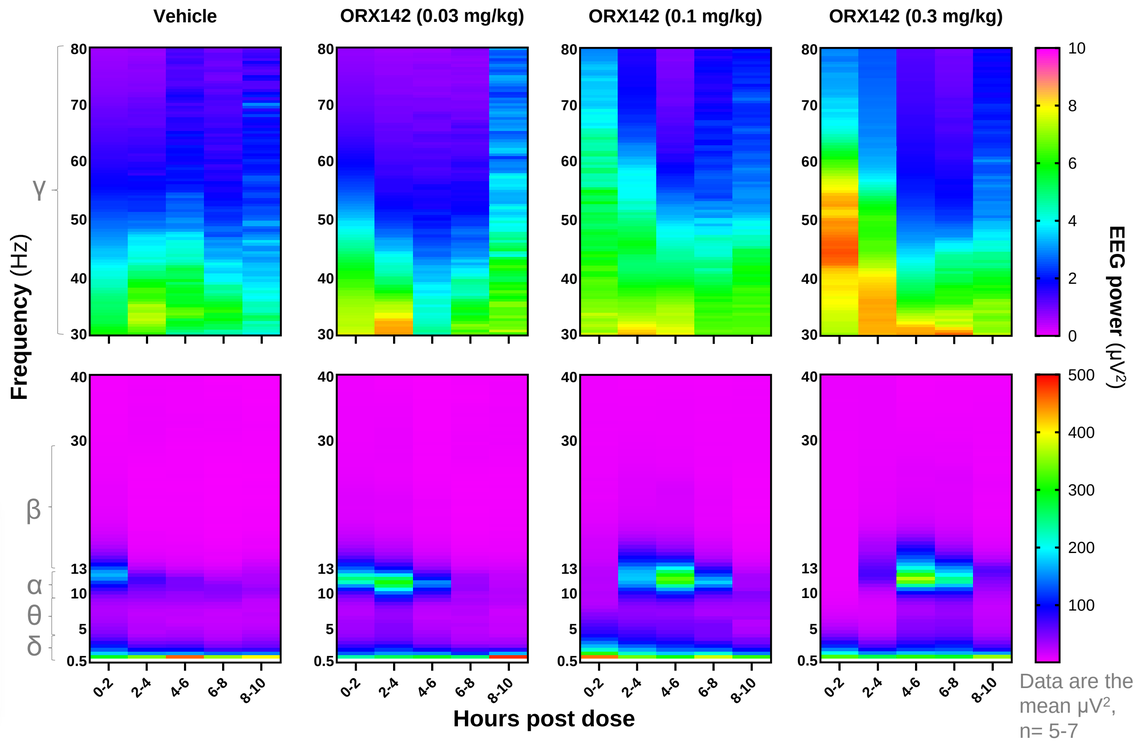


Horizontal bars = baseline mean ± SEM

- No significant increase was seen in gross motor activity
- Increases in core body temperature (< 1°C) were observed

ORX142 wakefulness is associated with normal arousal

6 ORX142 shifted EEG spectral density in wakefulness toward higher frequencies



Data are the mean μV², n= 5-7

- **EEG power increased in the gamma band (>30 Hz) during wakefulness** in a dose related manner after ORX142 (top panels)
- As EEG power increased in the higher frequencies of the gamma band during the first few hours post dose (top panels), power in the alpha (9.5-12.5 Hz) and theta (4.5-9.5) bands decreased (lower panels)
- Moderate levels of gamma activity at lower frequencies were accompanied by increased power in the alpha band

Conclusions

- **ORX142 is an investigational, orally administered, highly potent, and selective OX2R agonist**
- ORX142 potently activated the OX2R with an EC_{50} that was within 2-fold of the EC_{50} for the native ligand OXA
- In marmosets, ORX142 induced sustained increases in wakefulness that suppressed NREM and REM sleep at the lowest dose tested, 0.03 mg/kg
- Wakefulness induced by ORX142 was associated with normal physiological arousal and EEG power spectra signatures of enhanced alertness and attention[3,4]

We believe ORX142 has the potential to alleviate excessive sleepiness in neurological, neurodegenerative, and psychiatric conditions with intact orexin neurotransmission

References

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Acknowledgements

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