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ORIGINAL ARTICLE

Central nervous system effects of the histamine-3 receptor antagonist CEP-26401, in comparison with modafinil and donepezil, after a single dose in a cross-over study in healthy volunteers

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Aims: In previous studies, the histamine-3 receptor antagonist CEP-26401 had a subtle effect on spatial working memory, with the best effect seen at the lowest dose tested (20 μg), and a dose-dependent disruption of sleep. In the current study, 3 low-dose levels of CEP-26401 were compared with modafinil and donepezil.

Methods: In this double-blind, placebo- and positive-controlled, randomized, partial 6-way cross-over study, 40 healthy subjects received single doses of placebo, CEP-26401 (5, 25 or 125 μg) or modafinil 200 mg or donepezil 10 mg. Pharmacokinetic and pharmacodynamic measurements were performed predose and at designated time points postdose.

Results: The main endpoint between-errors of the spatial working memory-10-boxes task only improved for the 125 μg dose of CEP-26401 with a difference of 2.92 (confidence interval [CI] −1.21 to 7.05), 3.24 (CI −1.57 to 8.04) and 7.45 (CI 2.72 to 12.19) for respectively the 5, 25 and 125 μg dose of CEP-26401, −1.65 (CI −0.572 to 1.96) for modafinil and − 3.55 (CI −7.13 to 0.03) for donepezil. CEP-26401 induced an improvement of adaptive tracking, saccadic peak velocity and reaction time during N-back, but a dose-related inhibition of sleep and slight worsening of several cognitive parameters at the highest dose. CEP-26401 significantly changed several subjective visual analogue scales, which was strongest at 25 μg, causing the same energizing and happy feeling as modafinil, but with a more relaxed undertone.

Discussion: Of the doses tested, the 25 μg dose of CEP-26401 had the most optimal balance between favourable subjective effects and sleep inhibition. Whether CEP-26401 can have beneficial effects in clinical practice remains to be studied.

KEYWORDS

drug metabolism, neurology, pharmacodynamics, pharmacokinetics, psychopharmacology

The authors confirm that the PI for this paper is Prof. Dr J.M.A. van Gerven and that he had direct clinical responsibility for patients.

1 | INTRODUCTION

Histamine-3 receptors (H_3Rs) have been suggested as a drug discovery target for many different indications, because of their influence on several neurotransmitter systems.[1,2] The highest levels of this receptor are found in the thalamus, caudate nucleus and cortex.[3,4] High levels of expression are also found in the hypothalamus, hippocampus and olfactory tubercle. H_3Rs are located presynaptically and act as inhibitory auto- and hetero-receptors, decreasing the release of histamine and of several important neurotransmitters, such as acetylcholine (ACh), dopamine (DA), γ-aminobutyric acid (GABA), norepinephrine and serotonin.[5-7] Like all histamine receptors, the H_3R is a Gi-protein coupled receptor which leads to inhibition of the formation of cyclic adenosine monophosphate.[8] Also, the β- and γ- subunits interact with N-type voltage-gated calcium channels, to reduce action potential mediated influx of calcium and hence reduce neurotransmitter release.[9,10] H_3R antagonists are expected to increase the release of neurotransmitters, including acetylcholine, dopamine and norepinephrine, and are therefore suggested as possible enhancers of cognitive functions in central nervous system (CNS) diseases with cognitive impairments, such as Alzheimer disease, schizophrenia and attention deficit hyperactivity disorder (ADHD).[7]

In preclinical studies, mainly in mice and rats, positive effects of H_3R antagonists were found on working memory, memory consolidation, social memory, spatial orientation and attention and impulsivity.[11,12] These positive effects were also seen in models for negative symptoms of schizophrenia, but not in Alzheimer disease models.[11,12]

Human studies have mainly focused on treatment of ADHD and excessive daytime sleepiness. Pitolisant, an H_3R inverse agonist, has been shown to improve ADHD symptoms and reduce excessive daytime sleepiness in patients with narcolepsy and obstructive sleep apnoea syndrome.[13,14] The effects of H_3R antagonists on cognitive disturbances in Alzheimer disease and schizophrenia were not consistent.[15-17]

CEP-26401 ([6-[4-[3-[(2R)-2-methyl-1-pyrrolidinyl]propoxy]phenyl]-3-(2H)-pyridazinone hydrochloride]) is an H_3R antagonist/inverse agonist that displays high-affinity H_3R binding and potent functional antagonism in both rat and human recombinant cell and native rat brain cortical systems.[18-22] Two clinical studies with orally administered single and multiple doses of CEP-26401 in healthy volunteers have been performed prior to this study with interesting results on the spatial working memory (SWM) task.[23] In this task, several boxes are presented on the screen, in 1 of which a token is to be found. The token never appears in the same box more than once and the test continues until a token had been found in all of the boxes once. Each click on an empty box is counted as an error. Applying a population pharmacokinetic/pharmacodynamic (PK/PD) model, an effect on SWM was found with a maximal decrease of 10.8 errors (clinically relevant improvement of cognitive function) at plasma levels ≤0.01 ng/mL (dose ≤20 μg), but with a maximal increase of 17.6 errors (worsening of cognitive function) at plasma levels ≥0.1 ng/mL (dose ≥80 μg). Sleep was affected in a dose-related fashion with an increase in time awake after sleep onset to about a 2.4-fold increase at plasma levels ≥16 ng h/mL (dose ≥50 μg) after single dose. Although data were derived from 2 different studies with parallel-group designs, where differences between groups and study design may have played a role (i.e. studies were not powered nor specifically designed to detect differences in cognition enhancement), the PK/PD-model based on these studies consistently indicated the largest cognitive effects at the lowest dose.[23] It was therefore of interest to investigate the dose–response relationship of CEP-26401 on cognition at a dose range below as well as above 20 μg.

The primary objective of this study was to evaluate the dose–response relationships of single doses of CEP-26401 5, 25 and 125 μg on SWM and a range of other CNS functions in healthy subjects. Secondary objectives were the assessment of the effects of CEP-26401 on sleep; comparison of the effects of CEP-26401 with those of positive controls, modafinil and donepezil; and assessment of pharmacokinetics (PK), safety and tolerability of a low dose range of CEP-26401.

What is already known about this subject

- Histamine-3 receptors (H_3R) influence several neurotransmitter systems, including acetylcholine, dopamine and norepinephrine.
- In preclinical studies, H_3R antagonists induced an improvement on several cognitive domains.
- The H_3R antagonist CEP-26401 positively influenced spatial working memory with the best effect at the lowest dose.

What this study adds

- CEP-26401 did not have any positive effects on cognitive tests.
- CEP-26401 induced energizing, relaxed and happy feelings in healthy volunteers and a dose-related improvement of tests measuring attention, reaction time and alertness.
- Of the doses tested, the 25 μg dose of CEP-26401 had the most optimal balance between favourable subjective effects and sleep inhibition.

2 | METHODS

2.1 | Study design

This was a single centre, double-blind, placebo- and positive-controlled, randomized, partial 6-way cross-over study to investigate the pharmacodynamics and pharmacokinetics of CEP-26401 (5, 25 and 125 μg) following single-dose administration to healthy male and female subjects. All subjects were informed about study procedures and signed the informed consent form before any study activity took place. All subjects had a screening visit within 4 weeks prior to their 1st study day, followed by 4 treatment periods and a follow up visit. Each study treatment period was separated by a 14-day wash out.

Within 4 weeks of their 1st check-in day (day −1), subjects had a training session to familiarize them with the pharmacodynamic tests. After the training session, subjects performed the SWM test (the primary outcome parameter) and test scores were compared with reference values to ensure normal cognitive performance which was an inclusion criterion. Subjects also underwent polysomnography (PSG) during a single habituation night, to get accustomed to this procedure before the effects of study treatment were investigated.

Eligible subjects were admitted to the study centre on study day −1 and their eligibility to participate in the study was confirmed. On the morning of day 1, after a light meal, subjects were randomized to cohorts and underwent baseline assessments. After completion of baseline assessments, subjects received a single oral dose of their randomized treatment after which pharmacodynamic and safety assessments and pharmacokinetic sampling were performed at specified time points. Subjects remained in the study centre during day 1 and until the morning of day 2. Subjects were then released and requested to return to the study centre for check-in procedures for the next treatment period after a washout period of at least 2 weeks.

All dosed subjects had final procedures and assessments performed approximately 1 week after the last administered dose.

The study was approved by the medical ethical committee (Stichting Bebo, Assen, The Netherlands) and the competent authority (CCMO, The Hague, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice and the Declaration of Helsinki. The trial was registered in the European Union Clinical Trials Register (2013–001883-51) and on www.clinicaltrials.gov (NCT01903824). All pharmacological nomenclature nomenclature conforms to BJP's Concise Guide to PHARMACOLOGY 2015/16.[24]

2.2 | Subjects

A total of 40 healthy male and female subjects in an approximate 1:1 ratio, aged 18–50 years (inclusive) with a body mass index of 18.0–30.0 kg/m^2 (inclusive), were recruited for this study. Main exclusion criteria were smoking or use of nicotinic products within 3 months before inclusion, alcohol or drug abuse, excessive daily use of caffeine (>800 mg per day), use of medication with CNS effects or PK interactions and irregular diurnal rhythm. Because CEP-26401 could bind to melanin containing tissues (data on file, Teva Pharmaceuticals), subjects with a dark skin (Fitzpatrick scale 5 or 6[25]) were excluded. Also, subjects had to have a performance score on the spatial working memory test within normal range in order to reduce ceiling effects on cognitive testing.

2.3 | Randomization

In this partial 4-period, 6-treatment cross-over study, subjects were 1st randomized to 1 of the 3 cohorts, each with a different combination of treatments (Table 1). Within each cohort, subjects were randomly assigned to a treatment sequence using a Williams design. Each of the cohorts was comprised of 13 or 14 subjects. A total of 40 subjects were enrolled, with the intention of at least 36 subjects completing the entire study, 12 from each cohort. All treatments were administered as a single dose, with 14 days separating each treatment administration. Each subject underwent 4 study periods and received placebo in 1 of these periods. As this was a double dummy design, each subject received on each occasion CEP-26401 or placebo, modafinil or placebo and donepezil or placebo. Modafinil and donepezil and their matching placebos were over-encapsulated.

2.4 | Study medication and dosing rationale

2.4.1 | CEP-26401

CEP-26401 dose levels were determined based on clinical findings from the 2 completed clinical studies with CEP-26401 and PK/PD modelling.[23] A dose of 20 μg was anticipated to have the largest cognition-enhancing effect in a subset of Cambridge Neuropsychological Test Automated Battery (CANTAB) tests. Because 20 μg was the lowest dose tested in previously completed clinical studies, a dose of 5 μg was chosen to test the possibility of further improvement at lower concentrations. The 25-μg dose was close to the most active previously tested dose of 20 μg. The high dose of 125 μg would assist in assessing a possible inverted U-shaped dose–response relationship for cognitive enhancement and in confirming awakening effects during sleep periods. CEP-26401 and its placebo were administered as an aqueous solution.

TABLE 1 Treatments per cohort

	Placebo	CEP-26401 5 μg	CEP-26401 25 μg	CEP-26401 125 μg	Donepezil HCl 10 mg	Modafinil 200 mg
Cohort 1	+	+	+	+	–	–
Cohort 2	+	+	+	–	+	–
Cohort 3	+	+	–	+	–	+

2.4.2 | Modafinil

Modafinil was selected as a positive control, because it is a CNS-stimulant compound whose effects include noradrenergic and dopaminergic enhancement, which (among others) are also indirectly produced by H_3R antagonists such as CEP-26401.[1,26] Modafinil is used for the treatment of patients with excessive sleepiness associated with certain disorders and has been studied in ADHD, which may be potential therapeutic areas for H_3R antagonists.[1,2,7,11] A 200-mg dose of modafinil was chosen because it has repeatedly demonstrated effects on the SWM task in the CANTAB battery of tests.[27,28] Modafinil also demonstrated statistically significant improvements of other working memory tasks (memory span) that were not studied with CEP-26401, but were improved in studies with donepezil in healthy subjects.[28]

2.4.3 | Donepezil

A 10-mg dose of donepezil HCl was selected as an additional positive control. This cholinergic cognitive enhancer is registered for cognitive impairment in patients with mild-to-moderate AD.[29,30] As CEP-26401 also has indirect cholinergic effects, memory disorders are a potential therapeutic indication for this compound.[7] If an effect of donepezil can be measured in healthy volunteers, this could provide a benchmark for CEP-26401 activity related to a registered memory enhancer. Although most studies in healthy subjects have used repeat dose designs or cognitive impairment models, single donepezil HCl doses of 5-mg have caused small improvements of various aspects of memory and attention.[31] None of the tests used were previously employed in CEP-26401 studies. Therefore, the current study incorporated tests that have shown effects of donepezil HCl, including the n-back (data on file, CHDR1104, Centre for Human Drug Research Leiden, The Netherlands) and maze learning tasks.[32] A dose of 10 mg was chosen in view of the limited effects of the 5 mg dose in previous research, while adverse reactions were still expected to be minimal.[31]

2.5 | Pharmacodynamic methods

Pharmacodynamic tests were performed using 2 different computerized testing platforms. The NeuroCart is a battery of drug-sensitive tests, developed by the CHDR, for a wide range of CNS domains, including neuropsychological, neurophysiological and subjective measurements, to examine different kinds of CNS-active drugs. CANTAB is a specific neuropsychological test battery, developed by Cambridge Cognition, UK. Tests were performed predose and at selected time points after drug administration (Figure 1). Measurements were performed in a quiet room with ambient illumination with only 1 subject per session in the same room to minimize distraction. A short test description is given below. More details, including primary and secondary outcome parameters per test, can be found in the supplementary material. The primary parameters are chosen based on their known sensitivity to drug effects.

2.6 | Cognitive tests

2.6.1 | SWM

Several boxes were presented on the screen, in 1 of which a token was to be found. The token never appeared in the same box more than once and the test continued until a token had been found in all of the boxes once. The primary outcome parameter on this test was the total of between errors on 10 and 12 box trials. Between errors is the number of times a subject touches a box already found to contain a token.[33]

2.6.2 | Rapid visual information processing

Numbers were presented consecutively on the screen with a speed of 100 numbers per minute. The subject had to press a button if a predefined sequence of even or uneven numbers was seen.[34]

2.6.3 | Stop signal task

The stop signal task (SST) is a classic stop signal response inhibition test. An arrow pointing either to the left or to the right is displayed on the computer screen. Subjects had to indicate in which direction the arrow on the screen pointed, but when an audio tone was presented at the same time, they had to inhibit the response.[33]

2.6.4 | Paired associate learning

Several boxes were presented and automatically opened in a random order. In some of the boxes a pattern was shown. Then patterns were shown and the subject had to indicate which box contained the pattern.[33]

2.6.5 | Visual verbal learning task

The visual verbal learning test contains 3 different subtests that cover basically the whole scope of learning behaviour (i.e. acquisition, consolidation, storage and retrieval). Volunteers performing the visual verbal learning test were presented 30 words in 3 consecutive word trials. Each trial ended with a free recall of the presented words (Immediate Recall). Approximately 30 minutes after start of the 1st trial, the volunteers were asked to recall as many words as possible (delayed recall). Immediately thereafter, the volunteers underwent memory recognition test, which consisted of 15 presented words and 15 new *distractors* (recognition).[35]

2.6.6 | Maze learning

Subjects had to complete a maze by using trial and error learning to locate a 28-step pathway (from upper-left to bottom-right) that was hidden beneath a 10 × 10 grid of tiles. Individuals had to find the same pathway on five successive trials. Approximately 30 minutes after the start of the 1st trial, the volunteers were asked to

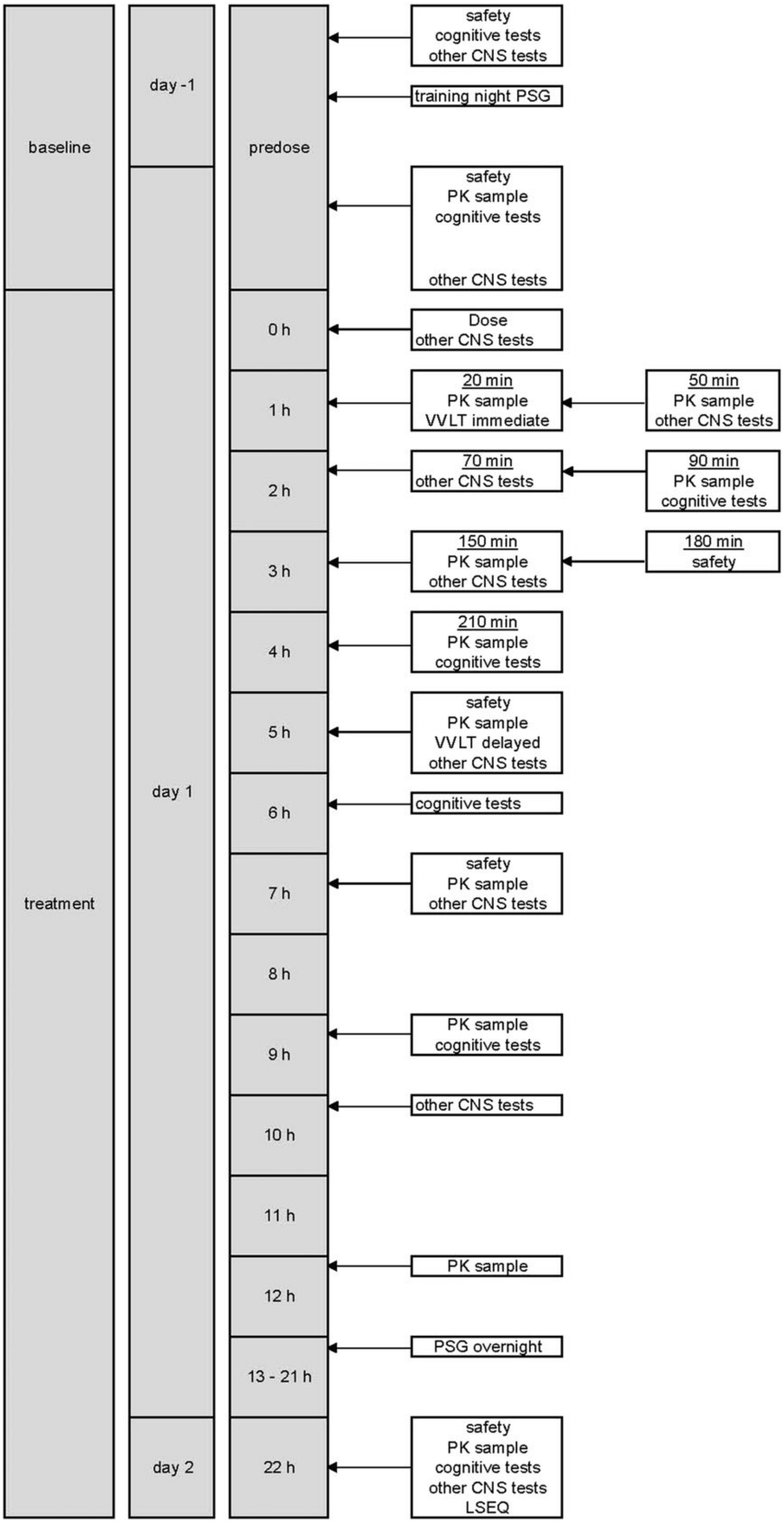

FIGURE 1 Schedule of assessments. CNS, central nervous system; LSEQ, leeds sleep evaluation questionnaire; PSG, polysomnography; PK, pharmacokinetic; VVLT, visual verbal learning test

identify the same maze again (delayed test, 1 trial). Immediately thereafter, the volunteers underwent the reversed test, which consists of 1 trial of the same maze backwards (from bottom-right to upper-left).[36]

2.6.7 | N-back

This test evaluates working memory and requires buffering and updating consonants, matching, encoding and responding. The N-back test consists of 3 conditions, with increasing working memory load. Letters were presented consecutively on the screen with a speed of 30 letters per minute. In the 1st condition subjects had to indicate whether the letter on the screen was an X. In the 2nd condition, subjects indicated whether the letter seen was identical to the previous letter. In the 3rd condition, subjects were asked to indicate whether the letter was identical to 2 letters before the letter seen.[37-39]

2.6.8 | Stroop choice reaction time

The distraction task is a parametric version colour-word response conflict task.[40] The words Left and Right were displayed either at the left or the right side of a computer screen. Response instructions are to respond quickly (by pressing a corresponding button) to the meaning of the word irrespective of its location.

2.7 | Subjective measurements: visual analogue scale (VAS) Bowdle, VAS Bond & Lader, VAS Task Enjoyment

Subjective feelings were assessed using classical VASs according to Bowdle and Bond & Lader.[41,42] From these questionnaires, composite scores were derived for *internal perception* and *external perception*, originating from the VAS Bowdle. The VAS score for task enjoyment was evaluated by means of a classical VAS (0–10 cm) device, with cut-off points as follows: 0–1 (no enjoyment), 2–4 (mild enjoyment), 5–7 (moderate enjoyment) and 8–10 (high enjoyment).

2.8 | Other CNS tests

2.8.1 | Adaptive tracking

Adaptive tracking is a pursuit-tracking task, measuring attention and eye–hand coordination.[43-48] A circle moves pseudo-randomly about a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. Each test was preceded by 3 training sessions and included 2 baseline measurements.

2.8.2 | Eye movements

Both saccadic and smooth pursuit eye movements were measured using a computerized test system to generate a moving dot on the screen, which had to be followed with the eyes by the subject, while the head was stabilized.[47,49,50]

2.8.3 | Body sway

The body sway was measured with an apparatus similar to the Wright ataxia meter.[51] The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. During sway measurements, subjects are instructed to keep their eyes closed for 2 minutes.

2.8.4 | Pharmaco-electroencephalography

Pharmaco-electroencephalography (EEG) was used to monitor any drug effects, which can be interpreted as evidence of penetration and activity in the brain.[52-54] EEG recordings were made at Fz, Cz, Pz and Oz. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes (power) in the δ-1 (0.5–2 Hz), δ-2 (2–4 Hz), θ (4–7.5 Hz), α- (7.5–13.5 Hz), β- (13.5–35 Hz) and γ- (35–48.9 Hz) frequency ranges. The duration of EEG measurements was 64 seconds per session.[52-54]

2.9 | Measurement of sleep

2.9.1 | Polysomnography

The PSG consisted of EEG, electrooculography, electromyography and electrocardiography (ECG) and cardiorespiratory measurements. In PSG, the electromyography is typically recorded from under the chin; since muscles in this area show very dramatic changes associated with the sleep stages. ECG is used for artefact removal.[55] PSG data were analysed by The Siesta Group Schlafanalyse GmbH (Vienna, Austria).

2.9.2 | Leeds sleep evaluation questionnaire

The Leeds sleep evaluation questionnaire (LSEQ) has 10 questions, the answers for which are captured on a VAS scale. This clinical tool allows test persons to qualitatively assess their sleep. Composite scores were computed for *getting to sleep*, *quality of sleep*, *awakening following sleep* and *behaviour following wakening*.[56,57]

2.10 | Assessment of safety

All subjects underwent medical screening before study entry, including medical history, physical examination, ocular pressure measurement, vital signs measurement, 12-lead ECG, urinalysis, drug screen and safety chemistry and haematology blood sampling. During study periods, safety was monitored based on adverse events (AEs), ocular pressure measurement, vital signs, ECG, safety chemistry and haematology blood sampling, urinalysis, physical examination and concomitant medication usage. In previous studies, CEP-26401 has been administered to healthy volunteers in doses up to 5 mg.[23] In these studies, intraocular pressure emerged as a safety finding of possible concern. In the current study, subjects with intraocular pressure > 22 mmHg were excluded at screening, and pressure was measured repeatedly using an ICare TA01 tonometer (ICare, Finland).

2.11 | Pharmacokinetic methods

Venous blood samples were collected via an indwelling catheter before drug administration, and at preselected time points after drug administration (Figure 1). Samples were collected in lithium heparin tubes, centrifuged to obtain plasma and frozen.

CEP-26401 concentrations in plasma samples were determined by Pharmaceutical Product Development (PPD) (Richmond, Virginia) using ultraperformance liquid chromatography (UPLC) with tandem mass spectrometric detection method that had been validated as per Food and Drug Administration guidelines. The final extracts were injected onto an Acquity UPLC system with chromatographic separation achieved via an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm; Waters, Milford, MA, USA). Detection was performed using a Xevo TQ-S mass spectrometer (Waters) in positive ion-mode. The assay range is 0.500–250 pg/mL. At the minimum, the method was required to have intra- and interday precision (coefficients of variation) for pooled plasma quality control samples of ≤15% except at the lower limit of quantitation, where ≤20% was acceptable. The calculated concentrations (both inter- and intraday) were required to be within 15% of nominal at all concentrations except the lower limit of quantitation, where up to 20% deviation from nominal was acceptable. The precision and accuracy of the method exceeded these minimum requirements for assay validation. In addition, stability of the analyte in frozen lithium heparinized human plasma was demonstrated for periods exceeding the storage periods of the samples prior to analysis, as well as under all conditions to which study samples or working solutions were subjected.

The following PK parameters were calculated for CEP-26401 by noncompartmental methods using WinNonlin software (Enterprise version 5.1.1; Pharsight Corporation, Mountain View, CA, USA): area under the plasma concentration–time curve from time zero to the time of the last measurable concentration ($AUC_{0\text{-}t}$), maximum observed plasma concentration (C_{max}) and time to C_{max} (t_{max}).

2.12 | Statistical analysis

For statistical analysis of PD parameters, mixed-model analyses of covariance (using SAS PROC MIXED) were performed with treatment, treatment period, time and treatment by time as fixed effects, and with subject, subject by treatment and subject by time as random effects, and with the average baseline value per period as covariate, where baseline is defined as the average of the available values obtained prior to dosing. Treatment effects were reported as contrasts where the average of the measurements was calculated within the statistical model up to last time point. Effect sizes for all treatments compared to placebo were calculated as change from baseline. Data were presented with a 95% confidence interval (thus a critical α of 0.05). As this was an exploratory study, no correction for multiple testing was employed.[58,59]

2.13 | Power calculation

Prestudy power calculations were based on the effects of CEP-26401 on between errors of the SWM task with 10 boxes, in previous studies with CEP-26401 in healthy volunteers and PK/PD-modelling of this data.[23] In the study reported in this manuscript, 24 subjects were planned to have a cross-over comparison between CEP-26401 5, 25 or 125 μg and placebo. A sample size of 24 would have 80% power to detect a difference in means of 6.6 assuming a standard deviation of differences of 11.0, using a paired *t* test with a 0.05 2-sided significance level. Thirty-six subjects were planned to have a cross-over comparison between CEP-26401 5 μg and placebo, which would have 80% power to detect a difference in means of 5.3 under the same assumptions. Modafinil and donepezil were included as active comparator compounds for the effect profile of CEP-26401 and were each administered to 12 subjects. This sample size would have at least 80% power to detect a difference in means of 12.7 in between errors of the SWM task with 10 boxes, assuming a standard deviation of differences of 11.0, using a paired *t*-test with a 0.05 2-sided significance level. A recent parallel design study showed an average improvement of 7.2 errors on this test, after a single 200-mg dose of modafinil in adults with ADHD.[27] The effects of donepezil on the tests used in this study were unknown at the time this study was planned. Therefore, no formal power calculations could be made to determine sample sizes for the effects of this compound. However, 12 subjects had previously been sufficient to obtain statistically significant effects of donepezil 5 mg in various study designs on working and visual memory, digit span backward, and maze learning in healthy elderly.[31,32] These functional domains were also covered in this study.

2.14 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[60] and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18.[61]

3 | RESULTS

3.1 | Demographics and disposition

A total of 80 subjects were screened for enrolment into this study. Of these, 40 subjects met inclusion criteria and were considered eligible for enrolment into the study. Of the 40 subjects who were not enrolled, 29 were excluded based on enrolment criteria and 11 subjects did not participate for other reasons. Of the 40 subjects enrolled, all received at least 1 dose of study drug and were evaluated for safety. Four subjects withdrew from the study for personal reasons (10%). Of these subjects, 1 subject completed 3 occasions, 1 subject completed 2 occasions and 2 subjects completed 1 occasion. All 4 missed their placebo occasion. Consequently, 36 subjects completed a placebo occasion. The cohorts were similar with regard to age, weight and body mass index (Table 2).

TABLE 2 Demographics

	Cohort 1 (*n* = 13)	Cohort 2 (*n* = 14)	Cohort 3 (*n* = 13)	Total (*n* = 40)
Age (years)	29.0 (18–48)	25.4 (19–48)	26.2 (18–48)	26.8 (18–48)
Sex (*n* male)	5 (38%)	10 (71%)	7 (54%)	22 (55%)
Weight (kg)	70.8 (55.5–92.3)	76.1 (53.8–95.2)	71.9 (47.6–86.3)	73.0 (47.6–95.2)
BMI (kg/m^2)	23.4 (19.3–29.4)	23.7 (18.3–28.2)	23.2 (18.2–28.9)	23.4 (18.2–29.4)

For age, weight and body mass index (BMI): mean (range). For sex: number of male subjects (%).

3.2 | Pharmacodynamics

3.2.1 | Cognitive effects

The most relevant parameters of the cognitive tests are presented in Table 3. A complete overview of summary data of all tests and parameters is provided as supplementary material. After administration of CEP-26401 in all doses tested, no improvement on any of the cognitive tests could be observed. Of particular interest was the SWM task that showed some evidence of positive effect, which was also observed in the previous phase-1 studies.[23] The number of errors in this task was not different from placebo, after 5 and 25 μg. Similar to the previous findings, performance in the SWM task was statistically significantly worse at the high dose of 125 μg. A slight worsening effect on the paired associate learning (PAL) task was also seen with the 25 and 125 μg doses of CEP-26401. Accuracy on the 2-back condition of the N-back test deteriorated at 125 μg. After administration of modafinil 200 mg, an improvement was observed on rapid visual information processing (RVIP), but no significant effects were observed on other cognitive tests. There were no significant improvements on cognitive tests after administration of donepezil. Detailed results of all parameters are presented in the supplementary material.

3.2.2 | Subjective effects

The 2 lowest doses of CEP-26401 induced significant improvements on several subscales of the VAS Bond & Lader, which were strongest at the 25 μg dose (Figure 2). Administration of CEP-26401 5 μg led to feelings of alertness, energy, contentedness, quick-wittedness, attention, happiness and gregariousness ($p < .05$). Administration of CEP-26401 25 μg induced feelings of strength, clear-headedness, coordination, contentedness, quick-wittedness, attention, proficiency, happiness, interest and gregariousness. The increase in alertness and energy almost reached significance at this dose of CEP-26401. Administration of CEP-26401 125 μg did not lead to any statistically significant changes on the VAS Bond & Lader. All doses of CEP-26401 induced a significant improvement on the VAS score for task enjoyment. Task enjoyment was also improved by modafinil, which additionally only increased VAS Bond & Lader scores for energy and happiness. Administration of donepezil did not lead to any changes on the VAS Bond & Lader, or task enjoyment, but there was an increase on VAS Bowdle scores of feeling high, change in surroundings and feeling of unreality. These effects in VAS Bowdle were not seen with CEP-26401 or modafinil.

3.2.3 | Other CNS performance effects

Dose related improvements of CNS performance were observed after administration of CEP-26401 on adaptive tracking, saccadic peak velocity and reaction time (during the 2-back condition of the N-back task, but not the 0-back condition; Table 3). There was an increase in frontal γ frequency on the EEG, which was statistically significant for the 2 lowest doses of CEP-26401. No statistically significant differences were found for other frequency bands of the EEG, finger tapping or body sway after administration of CEP-26401. Administration of modafinil led to an improvement on adaptive tracking, body sway and saccadic peak velocity and an increase in frontal γ frequency on the EEG, although the latter might be influenced by muscle artefacts. Reaction time and finger tapping were not affected by modafinil. No statistically significant effects of donepezil were seen on any of the parameters (detailed data in supplementary material).

3.2.4 | Effects on sleep

CEP-26401 had an inhibitory, dose dependent effect on all sleep parameters measured during PSG, not with 5 μg but starting at 25 μg and increasing at 125 μg (Table 4). On the subjective assessment of sleep, a similar effect was seen, except for the questions related to awakening following sleep. Modafinil had a significantly inhibitory effect on sleep for sleep efficiency, sleep latency, total sleep time and wake after sleep onset. The subjective scales showed a decrease in the ease of getting to sleep and awakening following sleep. Administration of donepezil led to a slight reduction of frequency of stage shifts; no effects were seen on other parameters of the PSG or on the subjective sleep assessment.

3.3 | Pharmacokinetics

CEP-26401 was absorbed with median t_{max} values of approximately 3.5–5.0 hours (Figure 3, Table 5). After reaching peak plasma levels, CEP-26401 slowly declined with mean concentrations at the 22-hour time point representing approximately 60% of C_{max}. Systemic

TABLE 3 Cognitive, subjective and general central nervous system effects compared to placebo, using a mixed-model analysis of covariance

	CEP-26401 5 μg (*n* = 38)	CEP-26401 25 μg (*n* = 26)	CEP-26401 125 μg (*n* = 25)	Modafinil 200 mg (*n* = 13)	Donepezil 10 mg (*n* = 13)
Spatial working memory—between errors 10 boxes	2.92 (−1.21 to 7.05) *P* = .1630	3.24 (−1.57 to 8.04) *P* = .1837	***7.45 (2.72 to 12.19)*** ***P* = .0024**	2.30 (−3.84 to 8.45) *P* = .4583	−0.71 (−7.12 to 5.71) *P* = .8276
Rapid visual information processing—a prime	0.00 (−0.00 to 0.01)	0.00 (−0.00 to 0.01)	0.00 (−0.00 to 0.01)	*0.01 (0.00 to 0.02)*	***−0.01 (−0.02 to -0.00)***
Stop signal task—reaction time	−11.57 (−26.28 to 3.15)	−8.79 (−25.46 to 7.89)	−11.39 (−28.07 to 5.28)	−1.79 (−23.12 to 19.55)	19.77 (−2.32 to 41.86)
Paired associate learning—total errors adjusted	1.78 (−0.47 to 4.02)	***2.69 (0.12 to 5.26)***	***2.97 (0.42 to 5.53)***	−2.41 (−5.47 to 0.91)	4.97 (1.51 to 8.42)
N—back—0-back reaction time (ms)	−3.37 (−16.43 to 9.68)	10.52 (−4.78 to 25.82)	2.58 (−12.52 to 17.67)	3.51 (−15.76 to 22.78)	0.48 (−19.81 to 20.76)
N-back—2-back accuracy	0.0 (−0.03 to 0.03)	−0.01 (−0.04 to 0.03)	***−0.04 (−0.08 to -0.00)***	−0.02 (−0.07 to 0.03)	0.00 (−0.05 to 0.05)
N-back—2-back reaction time (ms)	−17.65 (−36.66 to 1.36)	***−25.04 (−47.34 to −2.73)***	***−39.25 (−61.10 to −17.40)***	−5.97 (−34.32 to 22.38)	−27.72 (−57.26 to 1.82)
Vas task enjoyment	***3.21 (0.86 to 5.55)***	***3.87 (1.16 to 6.58)***	***3.19 (0.49 to 5.89)***	***4.64 (1.17 to 8.12)***	0.52 (−3.10 to 4.13)
Adaptive tracking (%)	***0.74 (0.06 to 1.43)***	***1.08 (0.28 to 1.88)***	***1.20 (0.42 to 1.98)***	***1.80 (0.80 to 2.81)***	0.49 (−0.57 to 1.54)
Saccadic peak velocity (degree/sec)	4.00 (−2.29 to 10.28)	6.75 (−0.50 to 13.99)	***16.99 (9.73 to 24.24)***	***24.62 (15.32 to 33.92)***	3.06 (−6.92 to 13.04)
Body sway (mm)	−16.89 (−46.08 to 12.30)	3.43 (−30.08 to 36.93)	−28.72 (−61.94 to 4.51)	***−54.44 (−97.29 to −11.60)***	24.22 (−20.54 to 68.88)
EEG frontal γ frequency	***0.06 (0.01 to 0.12)***	***0.07 (0.01 to 0.14)***	0.05 (−0.01 to 0.12)	***0.10 (0.01 to 0.18)***	0.06 (−0.03 to 0.14)

Mean (confidence interval). Statistically significant differences in bold.

EEG, electroencephalography.

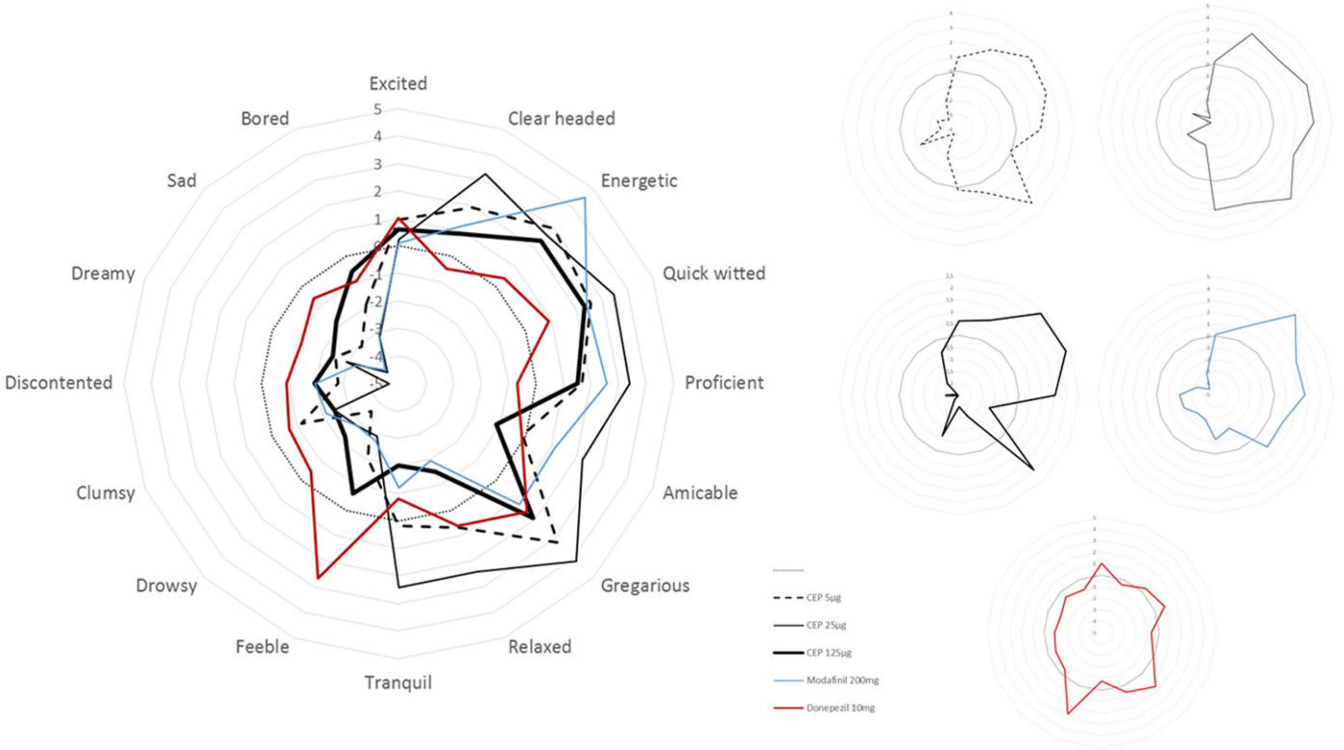

FIGURE 2 Effect on visual analogue scale Bond & Lader compared to placebo. The order of items corresponds with the order of the questionnaire items

exposure to CEP-26401, (C_{max}, $AUC_{0\text{-}t}$) increased in an approximately dose-proportional manner across the dose range evaluated. The mean C_{max} values for the 5-, 25- and 125-μg doses were 9.1, 45.4 and 245.4 pg/mL, respectively, and the corresponding mean $AUC_{0\text{-}t}$ values were 152, 743 and 3925 pg h/mL. The coefficient of variation associated with these parameters was between 15 and 20%. Despite 14-day washout periods, low but quantifiable levels of CEP-26401 were observed in some of the predose samples from all treatments. This finding was not completely unexpected given the long terminal elimination half-life observed for CEP-26401 in previous PK studies[23] and in consideration of the sensitivity of the bioanalytical method.

3.4 | Safety

During the 4 double-blind treatment periods, 27 of 36 (75%) subjects on placebo, 25 of 38 (66%) on CEP-26401 5 μg, 16 of 26 (62%) on CEP-26401 25 μg, 16 of 25(64%) on CEP-26401 125 μg, all 13 (100%) on donepezil 10 mg and 10 of 13 subjects (77%) on modafinil 200 mg reported at least 1 AE (Table 6).

The most important adverse effects, which occurred in at least 10% of subjects and more often with active treatment than after placebo, were as follows: headache at all 3 doses; nausea was more common with CEP-26401 125 μg and 25 μg than with placebo; CEP-26401 125 μg was also associated with more dizziness and hyperhidrosis than placebo. CEP-26401 5 μg was associated with more fatigue than placebo. Somnolence was less frequent with all CEP-26401 doses. In the modafinil group, headache, hypervigilance, nasopharyngitis and oropharyngeal pain were reported in at least 10% of subjects and more frequently than after placebo. For donepezil: nausea and vomiting; headache; abdominal pain; dizziness and procedural dizziness; feeling hot, hot flush and hyperhidrosis; and tremor all occurred at least in at least 10% of subjects and more frequently than after placebo. In contrast, somnolence and fatigue were reported less often after donepezil than under placebo.

All AEs were mild or moderate, except for 1 subject with severe headache and vomiting after administration of CEP-26401 5 μg. One subject had an asymptomatic increased intraocular pressure of 23 mmHg on the right eye at 5 hours after administration of CEP-26401 125 μg, which was normalized at the next measurement at 22 hours after drug administration. Three subjects had an increase in eosinophils during the study. One experienced a progressive rise throughout the study. Two others had eosinophilia at baseline and experienced fluctuations during the study with 1 reaching 23.41% eosinophils (absolute eosinophil count of 1730 × 10^6/L) before returning to near baseline. A relationship between the eosinophilia and the study drug could not be excluded, but the AEs for these subjects did not seem to point to clinical significance for the eosinophil elevation. There were no clinically significant changes in other laboratory values, vital signs, ECG or physical examination.

No deaths or other serious AEs were observed during this study. During the study, no subjects were withdrawn due to AEs.

TABLE 4 Effects on sleep compared to placebo, using a mixed-model analysis of covariance

	CEP-26401 5 μg (*n* = 38)	CEP-26401 25 μg (*n* = 26)	CEP-26401 125 μg (*n* = 25)	Modafinil 200 mg (*n* = 13)	Donepezil 10 mg (*n* = 13)
Number of awakenings per night	−1.98 (−4.39 to 0.43)	**−3.66 (−6.48 to −0.84)**	**−3.20 (−5.92 to −0.47)**	−1.65 (−.572 to 1.96)	−3.55 (−7.13 to 0.03)
Frequency of stage shifts per night	−11.34 (−24.37 to 1.69)	**−24.75 (−39.78 to −9.36)**	**−39.24 (−53.94 to −24.53)**	−14.35 (−33.82 to 5.13)	**−19.32 (−35.58 to −0.05)**
REM latency (min)	−3.58 (−19.64 to 12.48)	15.04 (−3.67 to 33.74)	**44.70 (26.33 to 63.07)**	22.21 (−1.74 to 46.17)	7.61 (−16.04 to 31.25)
Sleep efficiency (%)	−1.48 (−5.58 to 2.62)	**−9.04 (−13.58 to −4.24)**	**−16.01 (−20.65 to −11.38)**	**−12.13 (−18.28 to −5.98)**	−2.61 (−8.71 to 3.48)
Sleep latency (minutes)	3.90 (−8.49 to 16.29)	7.76 (−6.63 to 22.16)	**20.72 (6.67 to 34.68)**	**30.32 (11.89 to 48.76)**	11.44 (−6.74 to 29.63)
Total sleep time (minutes)	−13.40 (−38.85 to 12.05)	**−44.79 (−74.32 to −15.72)**	**−70.82 (−99.47 to −42.17)**	**−59.86 (−97.66 to −22.06)**	−35.24 (−72.50 to 2.02)
Wake after sleep onset (minutes)	1.09 (−13.91 to 16.09)	**35.36 (17.68 to 53.05)**	**57.56 (40.57 to 74.55)**	**29.35 (6.73 to 51.98)**	0.91 (−21.60 to 23.41)
LSEQ–getting to sleep (average mm change)	−2.34 (−6.62 to 1.95)	**−6.39 (−11.13 to −1.65)**	**−12.37 (−17.10 to −7.64)**	**−13.40 (−19.47 to −7.34)**	−1.71 (−7.89 to 4.47)
LSEQ–quality of sleep (average mm change)	−4.05 (−9.67 to 1.56)	**−6.93 (−13.13 to −0.73)**	**−20.88 (−27.08 to −14.68)**	−6.78 (−14.71 to 1.16)	−3.46 (−11.49 to 4.57)
LSEQ–awake following sleep (average mm change)	2.76 (−1.97 to 7.50)	−0.07 (−5.29 to 5.14)	2.92 (−2.29 to 8.14)	**10.94 (4.16 to 17.71)**	−1.32 (−8.09 to 5.46)
LSEQ–behaviour following wakening (average mm change)	−0.95 (−5.01 to 3.11)	−3.71 (−8.19 to 0.77)	**−5.34 (−9.82 to −0.87)**	0.35 (−5.40 to 6.10)	−4.94 (−10.82 to 0.93)

Mean (confidence interval). Statistically significant differences in bold. Sleep efficiency is the percentage of time in bed while the subject is asleep. LSEQ, Leeds sleep evaluation questionnaire; REM, rapid eye movement.

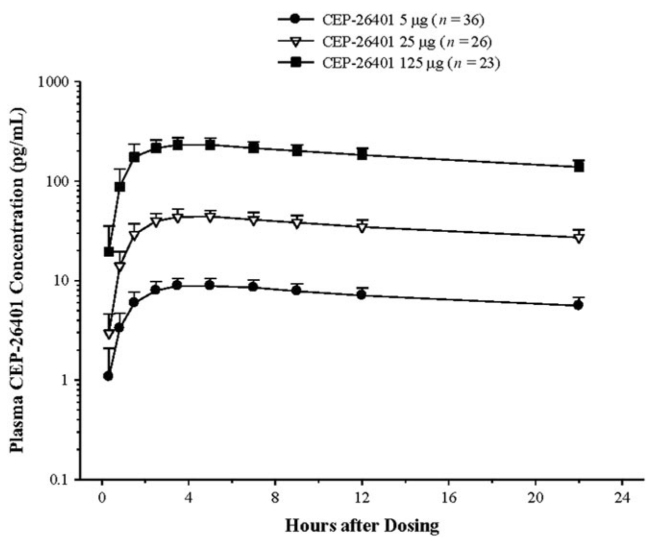

FIGURE 3 Mean (+standard deviation) plasma concentration–time profiles of CEP-26401 in subjects administered single oral doses of CEP-26401 at 5, 25 and 125 μg

TABLE 5 Pharmacokinetic parameters of CEP-26401 in healthy subjects administered Single Oral Doses of CEP-26401 at 5, 25, and 125 μg

Parameter	CEP-26401 5 μg(*n* = 36)	CEP-26401 25 μg(*n* = 26)	CEP-26401 125 μg(*n* = 23)
C_{max}, pg/mL	8.97 (1.770)	44.83 (7.344)	242.56 (38.818)
t_{max}, h[a)]	4.205 (1,300)	4.453 (1,243)	3.685 (1,756)
$AUC_{0\text{-}t}$, pg h/mL	149.63 (29.168)	732.93 (126.472)	3882.36 (612.045)

Geometric mean (standard deviation).

4 | DISCUSSION

In this study, CEP-26401 caused significant excitatory effects on a range of drug-sensitive CNS-tests including adaptive tracking, saccadic peak velocity, reaction time (during the most demanding 2-back paradigm of the N-back task), and frontal EEG γ frequency. As reaction time of the N-back task did not decrease during the zero-back condition, this is probably an effect on working memory processing speed, not on sensorimotor speed. The effect on EEG γ frequency might be an artefact as, in awake subjects it is almost impossible to distinguish EEG γ frequency from muscle artefacts. Some of the other effects already reached statistical significance at the 5 μg dose of CEP-26401, and most were significant with the 125 μg dose. This demonstrates the high potency and stimulatory effects at very low doses of this H_3R antagonist.

Despite the significant CNS-stimulating effects that were demonstrated with the NeuroCart, CEP-26401 did not have any beneficial effect on cognitive testing, even though this was expected based on previous studies with CEP-26401 in healthy volunteers.[23] At the

TABLE 6 Adverse events occurring in at least 10% of subjects

MedDRA system organ class MedDRA preferred term	Number (%) of subjects[a] Placebo (*n* = 36)	CEP-26401 5 μg (*n* = 38)	CEP-26401 25 μg (*n* = 26)	CEP-26401 125 μg (*n* = 25)	Modafinil 200 mg (*n* = 13)	Donepezil 10 mg (*n* = 13)
Number of subjects with at least 1 AE	**27 (75)**	**25 (66)**	**16 (62)**	**16 (64)**	**10 (77)**	**13 (100)**
Gastrointestinal disorders						
Abdominal pain	1 (3)	2 (5)	1 (4)	2 (8)	1 (8)	2 (15)
Nausea	1 (3)	2 (5)	3 (12)	5 (20)	1 (8)	12 (92)
Vomiting	0	1 (3)	0	0	0	7 (54)
General disorders and administration site conditions						
Fatigue	7 (19)	9 (24)	4 (15)	3 (12)	1 (8)	2 (15)
Malaise	1 (3)	1 (3)	0	0	1 (8)	
Feeling hot	0	0	0	1 (4)	1 (8)	4 (31)
Infections and infestations						
Nasopharyngitis	3 (8)	2 (5)	2 (8)	2 (8)	2 (15)	0
Injury, poisoning and procedural complications						
Procedural dizziness	0	0	0	0	1 (8)	2 (15)
Nervous system disorders						
Headache	6 (17)	7 (18)	6 (23)	6 (24)	5 (38)	3 (23)
Somnolence	10 (28)	7 (18)	3 (12)	1 (4)	0	2 (15)
Dizziness	3 (8)	3 (8)	2 (8)	3 (12)	0	6 (46)
Tremor	0	0	0	0	0	2 (15)
Psychiatric disorders						
Hypervigilance	1 (3)	0	0	1 (4)	3 (23)	0
Respiratory, thoracic and mediastinal disorders						
Oropharyngeal pain	0	3 (8)	0	0	2 (15)	0
Skin and subcutaneous tissue disorders						
Hyperhidrosis	0	1 (3)	1 (4)	3 (12)	0	3 (23)
Vascular disorders						
Hot flush	0	0	0	0	0	2 (15)

Number of subjects (%).

MedDRA, Medical Dictionary for Regulatory Activities.

highest dose of 125 μg there was even some decline at the accuracy of the N-back task and an increase in total errors on SWM and PAL. As administration of modafinil led to an improvement on RVIP, it is unlikely that the lack of effect of CEP-26401 on this test is due to inadequate study design or test conditions. As the cholinesterase inhibitor donepezil did not induce any measurable effects on SWM, PAL or SST, it is also possible that this was precluded by ceiling effects in this healthy population or that the tests used were not sensitive enough. The improvement in RVIP after administration of modafinil argues against this explanation, although it is possible that SWM, PAL and SST have a ceiling effect, while RVIP has not. Another possibility is that cognitive enhancement was obscured by AEs of the 10 mg dose in these young subjects. Previous studies at CHDR have shown positive effects of donepezil 10 mg on N-back and adaptive tracking in healthy elderly volunteers.[62] It is possible that a slight, age related cholinergic deficiency in elderly subjects has contributed to the measurability of these effects, and that they tolerate the drug better.[63] The current study, however, provides no indication that CEP-26401 might have cognitive enhancing effects, and does not provide reasons to assume efficacy in cognitive disorders such as AD. This is in contrast with results from several preclinical studies with other H_3 antagonists, which demonstrated an effect on working memory, memory consolidation, spatial orientation and attention.[11] Also, 2 clinical trials in patients with mild to moderate Alzheimer's disease reported small improvements in attention and memory with the H_3R antagonist GSK239512.[15,64] By contrast, it is consistent with a large phase 2 trial with 2 doses of an H_3 antagonist in patients with AD, which was aborted prematurely, because futility criteria were met.[17] Other trials aimed to improve cognitive impairment associated with schizophrenia with an H_3R antagonist, also failed to demonstrate efficacy.[16,65] This study seems to add to the evidence against beneficial cognitive effects of H_3R antagonists.

Although not immediately expected, CEP-26401 had extensive positive effects on several subjective VAS scales, which were significant in 8/16 scales at 5 μg, in 12/16 scales at the 25 μg dose, but in none of the scales at the 125 μg dose. The positive effects were not limited to feelings of energy, happiness and task enjoyment, as was observed after administration of modafinil, but also included feelings of contentedness, proficiency, interest and gregariousness. It is of interest that the 2 lowest doses of CEP-26401 also produced the lowest number of cognitive AE reports (31–32%)—lower even than placebo (56%) and much lower than modafinil (62%) or donepezil (100%). The subjective energetic and alert feeling is also reflected in the dose dependent improvements on adaptive tracking and saccadic peak velocity, as these indicate an increase in vigilance and motivation. Thus, CEP-26401 seems to induce the same, subjectively and objectively measured, energizing and happy feeling as modafinil, but with a more relaxed undertone—at least in the low doses used in this study. It is known that modafinil acts on dopaminergic neurons.[28] Since CEP-26401 affects basically the same parameters as modafinil, it could be suggested that it has—at least—indirect influence on this neurotransmitter system. However, since CEP-26401 has more extensive effects than modafinil, other neurotransmitter systems are probably also involved. This would be consistent with a microdialysis study in rats, where administration of CEP-26401 led to an increase of both dopamine and acetylcholine.[66]

As CEP-26401 is a highly selective H_3R antagonist, it inevitably increases the release of histamine via the inhibitory autoreceptors.[3,4] H_3R antagonists are also expected to increase the release of noradrenaline via heteroreceptors. The combination of increased levels of both histamine and noradrenaline could very well influence alertness and sleep. This is evident in the effects of CEP-26401 on sleep. In this study, CEP-26401 had an inhibitory, dose-dependent effect on sleep, which was significant for many PSG parameters at the 25- and 125-μg doses of CEP-26401. Subjective experience of sleep quality, as measured by LSEQ, also decreased in a dose dependent manner, further suggesting a dose-related disruption of sleep, as was also reported in the previous studies with CEP-26401[23] and also with pitolisant, another H_3R antagonist.[13] Sleep impairment was also observed for modafinil, although this compound had a more prominent effect on falling asleep and on waking up compared to CEP-26401.

Although CEP-26401 did not have the expected positive effect on cognition and cannot be typified as a cognitive enhancer, it may be a useful drug for certain indications that are characterized by a lack of internal drive and energy. The stimulant effects of CEP-26401 on objective CNS tests (PSG, adaptive tracking, saccadic peak velocity) were generally dose-dependent, whereas subjective effects were most favourable at a dose of 25 μg, but virtually disappeared after 125 μg. Except for a dose-dependent inhibitory effect on sleep, CEP-26401 was well-tolerated by most study subjects with only 1 patient experiencing severe AEs (an episode of headache and nausea). Based on these observations, the 25-μg dose of CEP-26401 has the optimal balance between favourable subjective and stimulatory effects, and inhibitory effects on sleep. The more strong, clear-headed, well-coordinated, interested and quick-witted feeling in combination with a more contented, attentive, proficient, happy and gregarious feeling might give benefit to patients suffering from certain types of mood disorders, such as major depression or dysthymia, negative symptoms of schizophrenia or anxiety disorders, especially social anxiety. The energizing aspects of CEP-26401 might give extra benefit to elderly patients with mood disorders, because they usually have more apathy, compared to younger patients.[67]

However, there are also possible challenges with the use of CEP-26401 in a clinical setting. There appears to be a bell-shaped response curve which implies a relatively narrow therapeutic window. It remains to be established whether the pleasurable effect might generate abuse potential, especially in already vulnerable, psychiatric patient populations. Stimulant effects may also be undesirable in (unrecognized) bipolar disorder, and the effects may differ in elderly subjects, particularly with cognitive impairment. Also, the effects on sleep cannot be ignored and might constitute a clinically relevant adverse reaction.

This study has several limitations. Despite the performance of many different tests, a correction for multiple testing was not performed. By contrast, both time profile and response pattern on tests expected to be related to each other are consistent, suggesting that the data are trustworthy. The time courses for the repeated tests were also in agreement with the pharmacokinetic time profile. This suggests that the improvements were driven by pharmacological effects, although no PK/PD-analysis was performed. In general, these consistent observations support the theory that a correction for multiple testing is only necessary in confirmatory studies, studying 1 specific hypothesis without any exploratory objectives.[58,59] The large number of tests on 1 day could induce fatigue or decreased motivation in the subjects. Therefore, drug effects were not compared with baseline, but with the placebo occasion, where fatigue and motivation are expected to play an equal role. The properties of the drug, however, may have helped subjects remain motivated throughout the very intensive study days. Randomization averted decreased motivation over consecutive treatment periods. Although 1 of the objectives of the study was to compare the effects of CEP-26401 with those of donepezil, this objective could not be met, because donepezil did not have any measurable effects in this study. Therefore, it is impossible to deduce whether the lack of pro-cognitive effects of CEP-26401 is caused by a lack of effect on cholinergic neurons or by a lack of sensitivity of the tests used for pro-cholinergic effects in young, healthy volunteers.

In conclusion, CEP-26401 had several simulating CNS effects and induced energizing and positive feelings, with a relaxed undertone at the 5 and 25 μg doses, which disappeared at 125 μg. CEP-26401 caused a dose-dependent inhibition of sleep, which became symptomatic at the highest dose. It is likely that at least dopaminergic and histaminergic neurons are involved in its effects. It remains to be studied whether CEP-26401 can have beneficial effects in clinical practice.

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COMPETING INTERESTS

Co-authors N.G., R.Y., M.F., E.H. and O.S. are employees of Research and Development Teva Pharmaceuticals. Y.G.-S. and A.G. are a former employees of Research and Development Teva Pharmaceuticals. The other authors have no competing interests to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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