Original Research

Pharmacodynamics, Pharmacokinetics, Safety, and Tolerability of Encenicline, a Selective α_7 Nicotinic Receptor Partial Agonist, in Single Ascending-dose and Bioavailability Studies

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ABSTRACT

Purpose: Encenicline (EVP-6124) is a selective α_7 nicotinic acetylcholine receptor partial agonist being developed for cognitive impairment in Alzheimer's disease and schizophrenia. We report on 2 single-dose studies to assess the relative bioavailability, pharmacokinetic profile, tolerability, and cognitive effects of encenicline in healthy volunteers.

Methods: A single ascending-dose study assessed the safety, tolerability, pharmacokinetic, and pharmacodynamic profiles of encenicline in healthy male volunteers. Subjects received a single 1-, 3.5-, 7-, 20-, 60-, or 180-mg oral solution dose of encenicline or placebo. A second single-dose, randomized, open-label, 3-period, crossover study in healthy male and female subjects compared the relative bioavailability of a 1-mg oral capsule versus a 1-mg oral solution dose of encenicline and evaluated the effects of food and sex on encenicline pharmacokinetic profile.

Findings: In the first study, encenicline was well tolerated and dose-proportional increases in C_{max} (mean range 0.59−100 ng/mL) and $AUC_{0-\infty}$ (mean range 45.6−8890 ng · h/mL) were observed over a 1- to 180-mg dose range. Procognitive effects on the Digit Symbol Substitution Test were maximal at the 20-mg dose. In the second study, encenicline 1-mg oral capsules and oral solution were bioequivalent and there was no observed food effect on encenicline pharmacokinetic profile with the 90% confidence intervals of the treatment ratios for both comparisons (ie, capsule to solution and fed to fasted) for C_{max} and AUC being within 80% to 125%. A 30% to 40% higher encenicline exposure in female subjects than respective values in male subjects was consistent with a 33% higher weight of the male subjects. No clinically relevant safety profile or tolerability effects of encenicline were observed.

Implications: Encenicline was well tolerated at single doses up to 180 mg, and doses as low as 1 mg had dose- and time-dependent pharmacodynamic effects on the central nervous system. Oral capsule and solution were bioequivalent and were not affected by food. Although a sex effect on pharmacokinetic profile was observed, it was attributable to weight differences. Clinical Trial Registration at EudraCT: 2006-005623-42 and EudracT: 2008-000029-20. (*Clin Ther*. 2015;37:311–324) © 2015 The Authors. Published by Elsevier HS Journals, Inc.

Key words: α_7 nicotinic acetylcholine receptor agonist, bioavailability, encenicline cognition, pharmacodynamics, pharmacokinetic profile.

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INTRODUCTION

Studies have shown that nicotine can induce significant improvements in measures of attention and psychomotor processing in healthy volunteers[1] and patients with schizophrenia,[2] attention-deficit hyperactivity disorder,[3] and Alzheimer's disease.[4] However, nicotine interacts with multiple nicotine receptors, induces receptor desensitization,[5] is rapidly cleared, has a significant liability for addiction, and is associated with toxicities such as induction of apoptosis, DNA damage leading to oxidative stress, increases in reactive oxygen species, and lipid peroxidation.[6]

Pharmacologic characterization of nicotine suggests the α_7 and $\alpha_4\beta_2$ nicotinic cholinergic receptors (nAcChR) as possible mediators of its procognitive effects, although the affinity of nicotine for the α_7 receptor is substantially lower (approximately 2 log units) than for the $\alpha_4\beta_2$ receptor.[7–9] Among these nicotinic receptor targets, evidence suggests that activation of α_7 nAcChR can be particularly relevant for several aspects of cognition enhancement, including higher cognitive functions,[10] and such disease-specific effects as the normalization of P50 auditory evoked potential gating deficits in schizophrenia[11] and, potentially, reduction of amyloid plaque accumulation in Alzheimer's dementia.[12] The α_7 nAcChR, which is the target of encenicline, is composed of 5 α_7 subunits[13,14] forming a Ca^{2+}-permeable ion channel. The α_7 nAcChR is located in several brain areas involved in cognition and memory, such as the cerebral cortex and the hippocampus.[15] It is predominantly located presynaptically and influences the release of neurotransmitters, such as γ-aminobutyric acid, glutamate, acetylcholine, and dopamine.[16] Activation of the α_7 nAcChR by an agonist can be expected to increase cholinergic neurotransmission.[17] Depending on the brain region studied, α_7 nAcChR agonists also will increase the release of dopamine and glutamate.[18,19] The α_7 nAcChR subunit has an additional advantage over the β_2 nAcChR subunit in that it does not appear to be involved in activation of behavioral reward pathways and, therefore, may not be associated with the development of addiction. It is hypothesized that α_7 nAcChR agonists can provide a cognitive benefit without risk for addiction.[19,20] In the past few years, several selective α_7 nAcChR agonists[16] have shown procognitive effects in animals,[21–23] healthy volunteers,[24] and patients with schizophrenia.[25,26]

Encenicline is an α_7 nAcChR partial agonist that demonstrates procognitive effects at low nanomolar concentrations.[27] This articles describes the safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) profiles of single oral doses of encenicline in humans, as well as the results of a single oral dose relative bioavailability study in fasted and fed adult men and women.

METHODS

For each study, the clinical study protocol, protocol amendments, and informed consent forms were reviewed and approved by an Independent Ethics Committee (Stichting Beoordeling Ethiek Bio-Medisch Onderzoek, Assen, the Netherlands). Both studies were performed at PRA International, Zuidlaren, the Netherlands in accordance with the principles of the Declaration of Helsinki and in compliance with the International Conference on Harmonisation Guidelines for Good Clinical Practice. All participants provided written informed consent before participation.

Single Ascending-Dose Study

Design

This was a randomized, double-blind, placebo-controlled, single-center study. The study consisted of 6 dose groups of 8 subjects each, 6 of whom were randomly assigned to encenicline (1, 3.5, 7, 20, 60, or 180 mg encenicline HCl) and 2 to placebo. Enrollment in any specific dose level occurred only after tolerability was established at the previous dose level. Subjects were confined to the facility the night before dosing and remained there until at least 72 hours post dose.

Subject Selection

Healthy male subjects aged 18 to 45 years and with a body mass index of 18 to 28 kg/m^2 were eligible if they had a medical history with no relevant pathology. Subjects were excluded for an abnormal EEG, use of concomitant medications other than acetaminophen, use of tobacco products within 60 days before drug administration, positive drug screen, positive hepatitis B surface antigen, anti–hepatitis C virus, or anti–HIV1/2 test, intake of >8 cups of coffee or tea per day or intake of >24 units of alcohol per week.

PK Sampling and Analysis

Blood samples (4 mL) were obtained either via an indwelling intravenous catheter or by direct venipuncture into sodium heparin glass tubes at pre dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, 24, 36, 48, and 72 hours post dose for group 1 (1 mg). Additional ambulatory blood-collection time points were added due to the unexpected long plasma half-life (>50 hours) of encenicline (96 and 120 hours in group 2 [3.5 mg], and 168 and 240 hours post dose for subsequent cohorts). Plasma encenicline concentrations were measured using a validated liquid chromatography method that employed tandem mass spectrometry detection. In brief, 1500 μL acetonitrile was added to 400 μL plasma (sodium heparin anticoagulant) and 50 μL internal standard ($^{13}C_2$,D_2-encenicline) and samples were vortexed for 60 seconds. After centrifugation at 3500*g* for 5 minutes, 1 mL supernatant was transferred to a glass tube and 100 μL 10% 1,2-propanediol was added. The sample was reduced to dryness under a stream of nitrogen at 40°C. Reconstitution solvent (100 μL; 40% acetonitrile in water) was added and the sample was transferred to injection vials. A 40-μL aliquot was injected on the high-performance liquid chromatography tandem mass spectrometry system with detection based on monitoring of mass transitions of 321.00 to 110.00 for encenicline and 325.00 to 114.00 for internal standard using a Perkin Elmer API 4000 MS/MS detector (MDS Sciex, Concord, Canada). The assay was validated over a concentration range of 0.01 to 10 ng/mL, with an allowance for up to 400 ng/mL with dilution. Adequate benchtop, autosampler, freeze and thaw, whole blood, and frozen sample stability was demonstrated. Encenicline PK parameters were determined using standard noncompartmental methods using WinNonlin Professional Version 5.01. C_{max} and time of C_{max} values were recorded as observed. The terminal elimination rate constant (λ_z) for encenicline was determined by regression of the terminal log-linear portion of the plasma concentration-time profile. Terminal elimination half-life was calculated as $0.693/\lambda_z$. AUC from time zero to the time of the last sample with quantifiable concentrations (AUC_{0-last}) was determined using linear trapezoidal and logarithmic trapezoidal methods when plasma concentration was increasing or decreasing, respectively. AUC from time zero to infinite time ($AUC_{0-\infty}$) was determined by summing AUC_{0-last} and C_{last}/λ_z, where C_{last} was the concentration of the last sample with quantifiable concentrations.

PD Assessments

PD assessments consisted of the Addiction Research Center Inventory 49-item questionnaire (ARCI49), Bond and Lader Visual Analogue Scale (VAS), and the Digit Symbol Substitution Test (DSST), which were performed pre dose and at 1, 2, 4, 8, and 24 hours post dose. The ARCI49 is a self-rating scale to test subjective effects of drugs.[28] It is composed of 5 subscales: a Morphine Benzedrine (MB) group scale to measure euphoria, A Lysergic Acid Diethylamide group scale to estimate dysphoric and psychotomimetic changes, a Pentobarbital Chlorpromazine Alcohol (PCA) group scale to measure sedation, and a Benzedrine group scale and an Amphetamine Scale to assess stimulant effects. Responses reported as "true" were scored as plus or minus 1 on each relevant subscale. The Bond and Lader VAS questionnaire assessed (1) self-rated alertness, (2) self-rated calmness, and (3) self-rated contentment.[29] The DSST is a neuropsychological test that explores attention, psychomotor speed, and speed of processing[30] and is sensitive for a variety of neuropsychological impairments, including early Alzheimer's disease, with test performance correlating to disease progression.[31]

Quantitative electroencephalogram (qEEG) was performed at 1, 2, 3, 4, 6, 8, and 24 hours post dose. For qEEG, silver/silver chloride disc electrodes were attached to the scalp by means of quick-drying collodion, according to the international standards for EEG practice. The electrodes were placed over T3, T4, F3, F4, O1, and O2. Linked earlobes were used as common reference. The ground electrode was located at the sternum. Electrodes were placed by trained medical research assistants, according to the international 10−20 system. A 24-channel stationary recording system was used (Refa, TMS International, Oldenzaal, the Netherlands). After digital filtering, data were digitized with a sampling frequency of 256 Hz. During the data acquisition, subjects were in the sitting position. The qEEG was recorded during 5-minute resting conditions (subjects were asked to relax with their eyes closed). Data were analyzed off-line using BrainVision Analyzer software.

Safety Profile Assessments

Treatment-emergent adverse events, laboratory evaluations (hematology, blood chemistry, and urinalysis), physical examination, 12-lead ECG, cardiovascular telemetry monitoring, and Holter recordings were monitored throughout the study. A 12-lead ECG and vital signs were performed pre dose and at 1, 2, 3, 4, 5, 6, 8, 24, 48, 72 hours post dose for all dose groups. ECG assessments were also conducted at 96 and 120 hours post dose for all dose groups other than the 1-mg group. Cardiovascular telemetric monitoring was in place from 10 minutes pre dose until 6 hours post dose, and a 3-lead Holter recording was taken from 1.5 hours pre dose to 34.5 hours post dose. Clinical laboratory studies were performed at baseline and at 72 hours post dose in the 1-mg dose group and at 72 and 120 hours post dose in the other dosing groups. Determinations of B- and T-lymphocyte counts were performed pre dose and at 24 hours post dose.

Statistical Analysis

All PK data were summarized using descriptive statistics. Dose proportionality was explored using ANOVA on log-transformed and dose-normalized C_{max} and AUC parameters. For qEEG, a nonparametric ANOVA was used to compare treatment groups for change from baseline for AUC of the frequency bands. Comparison of each active dose to placebo was performed for the different frequency bands, electrode positions, and time points. For the ARCI49 questionnaire, Bond and Lader VAS, and DSST, all data were summarized using descriptive statistics, and change from baseline to end point was summarized. To determine significant differences between active dose and placebo changes from baseline values, the ARCI49 and Bond and Lader VAS data were analyzed using a repeated-measures ANOVA. Least square means were used to estimate the overall differences between active dose and placebo, as well as differences at each time point. The total number of correct responses on the DSST by treatment for each time point, as well as the change from baseline, were summarized using descriptive statistics. A within-subject change using the modified Dunlap's d statistic was calculated according to the following formula: change score = (baseline − post baseline)/WSD (group), where WSD is the within-subject SD of the group from the 2 baseline conditions, baseline is the last observed measurement before dosing, and post baseline is the score on the scheduled post-baseline measurements. Group mean effect sizes (ie, modified Dunlap's d change scores) were then compared between each active dose group and placebo using independent t tests. All p values were 2-sided. Statistical significance was declared at the 0.05 level.

Capsule and Solution Relative Bioavailability and Food and Sex Effect Study

Design

This was a single-dose, open-label, 3-period crossover study in one group of 12 male and female subjects. The 3 treatments were administered in a randomized sequence with at least 14 days' wash out between dosing occasions. Subjects received a single dose of encenicline 1 mg as an oral capsule under fed and fasting conditions, and as an oral solution under fasting conditions.

Drug Administration

For the oral solution, encenicline 1 mg was administered after an overnight fast in 4 mL cranberry juice cocktail (vehicle), followed by an additional 180 mL of vehicle. For all treatment groups, no fluids were allowed from 2 hours before until 2 hours after drug administration (except for 180 mL water used to swallow the capsule or 180 mL cranberry juice taken with the oral solution). On the day of dosing (day 1) for treatment group A (oral capsule under fasting conditions), treatment group B (oral capsule under fed conditions), and treatment group C (solution under fasting conditions), encenicline was administered between 10 AM and 11 AM after having received a light supper the previous evening, followed by a 10-hour overnight fast. For treatment groups A and C, fasting was continued for 4 hours after drug administration until lunch; for treatment group B, a standard breakfast was consumed 30 minutes before dosing and was completed at least 10 minutes before dosing. Lunch on dosing days was given after the 4-hour PK sampling.

Study Assessments

Clinical laboratory, full physical examination (including body weight), vital signs (blood pressure, pulse rate and body temperature), 12-lead ECG, medical history, drug and alcohol screen, hepatitis B surface antigen, anti−hepatitis C virus, anti-HIV1/2, and pregnancy test (females only) were obtained at

Table I. Baseline demographic characteristics.

Parameter/Statistics	Placebo (n = 12)	Encenicline Dose Group 1 mg (n = 6)	3.5 mg (n = 6)	7 mg (n = 6)	20 mg (n = 6)	60 mg (n = 6)	180 mg (n = 6)
Age, y							
Mean (SD)	25 (6)	23 (4)	31 (10)	27 (8)	22 (2)	21 (2)	26 (7)
Range	19–41	18–31	20–44	20–42	20–26	19–26	20–41
Height, cm							
Mean (SD)	181 (5)	187 (6)	180 (5)	181 (8)	186 (3)	184 (5)	179 (6)
Range	171–188	179–195	174–185	168–193	182–189	177–191	170–189
Weight, kg							
Mean (SD)	78.9 (10.3)	81.7 (6.8)	77.8 (7.4)	79.6 (7.4)	82.5 (8.6)	76.8 (11.7)	76.7 (11.4)
Range	58.8–93.9	70.5–91.2	66.2–88.5	67.7–89.5	67.9–92.2	64.2–91.2	63.9–89.6
BMI, kg/m^2							
Mean (SD)	24.0 (2.4)	23.5 (1.7)	23.9 (2.0)	24.3 (2.4)	23.9 (2.0)	22.6 (2.9)	24.0 (3.2)
Range	18.6–26.9	21.7–25.6	20.9–25.9	20.0–27.0	20.3–25.8	19.0–27.2	20.3–28.0

BMI = body mass index.

baseline. Drug and alcohol screen, vital signs (blood pressure, pulse rate, and body temperature), body weight, clinical laboratory, ECG, pregnancy test (females only), concomitant medication, and adverse events were obtained routinely during the study. Blood samples for PK profile of encenicline were obtained pre dose and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 120, 168, and 240 hours post dose. Plasma concentrations of encenicline were determined using a validated liquid chromatography tandem mass spectrometry method, as described. Encenicline PK parameters were determined using noncompartmental methods with WinNonlin Professional Version 5.01.

Statistical Analysis

The bioavailability of encenicline from the oral capsule formulation relative to the oral solution formulation in the fasted state was assessed using an ANOVA on log-transformed encenicline C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ values. Point estimates and 90% confidence intervals (CIs) for the treatment differences on the log scale were exponentiated to obtain estimates and 90% CIs for the geometric mean ratios on the original scale. In addition, the effect of food and sex on the relative bioavailability of encenicline after single oral administration as a capsule was assessed based on determination of 90% CIs for the ratio of the means, using log-transformed data for C_{max}, $AUC_{0.t}$, and $AUC_{0-\infty}$.

RESULTS

Single Ascending-Dose Study

Demographics

Forty-eight subjects were enrolled, received study medication, and completed PD assessments. Demographic characteristics generally were comparable between groups at baseline (Table I).

PK of Encenicline

Across the study sample bioanalytical runs, the accuracy (% bias) of encenicline quality-control samples (0.03 ng/mL to 8.00 ng/mL) ranged from 2.2% to 5.3% and assay precision (%CV) ranged from 3.5% to 21.3%. After oral administration of encenicline at single doses ranging from 1 mg to 180 mg, median T_{max} values ranged from 5.0 to 8.0 hours (Table II and Figure 1). An ANOVA analysis of the extent of systemic exposure, as measured by either C_{max} or $AUC_{0-\infty}$, revealed an increase in a dose-proportional manner over the entire dosing range (1 to 180 mg). Mean terminal elimination half-life values ranged from 52.6 to 63.0 hours and were independent of dose. There was no apparent dose relationship of mean oral clearance values (20.2 to

Table II. Arithmetic mean (%CV) pharmacokinetic parameters for encenicline after administration as an oral solution.

Parameter	Encenicline HCl Dose					
	1 mg	3.5 mg	7 mg	20 mg	60 mg	180 mg
T_{max}, h, median (range)	5.0 (3.0–6.0)	8.0 (5.0–8.0)	5.0 (4.0–8.0)	5.0 (5.0–8.0)	6.0 (2.0–8.0)	5.0 (5.0–8.0)
C_{max}, ng/mL, mean (%CV)	0.588 (23)	2.06 (20)	4.66 (16)	10.4 (10)	35.6 (11)	100 (17)
AUC_{0-last}, ng · h/mL, mean (%CV)	27.4 (24)	116 (30)	306 (9)	780 (18)	2400 (18)	8200 (23)
$AUC_{0-\infty}$, ng · h/mL, mean (%CV)	45.6 (33)	165 (34)	358 (21)	852 (22)	2800 (23)	8890 (24)
λ_z, 1/h, mean (%CV)	0.0145 (30)	0.0118 (20)	0.0122 (24)	0.0127 (23)	0.0113 (17)	0.0117 (17)
$T_{½}$, h, mean (%CV)	52.6 (37)	60.8 (22)	60.3 (30)	57.0 (21)	63.0 (15)	60.6 (17)
CL/F, L/h, mean (%CV)	23.9 (31)	23.1 (30)	20.2 (19)	24.6 (25)	22.4 (22)	21.3 (24)
Vz/F, L, mean (%CV)	1740 (37)	1970 (28)	1680 (10)	1950 (13)	1990 (14)	1810 (16)

CL/F = oral clearance; λ_z = terminal elimination rate constant; Vz/F = volume of distribution of the terminal elimination phase.

24.6 L/h) or apparent volume of distribution of the terminal elimination phase (V_z/F) values (1680 to 1990 L). The degree of variability in PK parameters was low, with most %CV values <25%.

Quantitative EEG

Overall, results from the qEEG analysis suggested that encenicline might have some PD effects. However, a clear and consistent pattern over time or dose relationship was not established. Encenicline had no significant effect on absolute power levels. However, the relative contributions of individual frequency bands in relation to the entire EEG frequency range did show changes with dose and time of measurement. Inspection of the relative power in the EEG signal showed that the contribution of individual frequency bands in relation to the entire EEG frequency range did show changes with dose and time of measurement (dose × band: $p \leq 0.001$; dose × time × band: $p \leq 0.001$). The contribution of α frequencies (8–12.5 Hz) increased compared with power observed in the placebo group, especially for the lower doses of encenicline (1, 3.5, and 7 mg). These effects remained until 8 hours after administration of the active dose. Effects on relative α power in the 1-mg dose group disappeared after 8 hours; however, for the 3.5- and 7-mg doses, the increase of α frequencies in the EEG signal remained visible until 24 hours after administration. In the β (13–32 Hz) and δ (0.5–3.5 Hz) frequency bands, relative power levels decreased in comparison with baseline levels ($p \leq 0.001$). In the 3-hour period after administration of encenicline, the observed decrease in relative δ power was most pronounced with the 3.5-mg dose. Thereafter, the decrease in relative power in the δ band was most apparent with the 60-mg dose. The observed decrease in relative β power was most prominent for the 7-mg and 20-mg doses early after administration of encenicline, and 3 hours after dose administration, effects were observed with the 180-mg dose as well. Relative τ power was not affected by either dose or time of measurement. The relative increase in α power in combination with a decrease of power in the δ frequency band resulted in effects on the α slow-wave index. The change in α slow-wave index was observed most clearly in occipital leads, in the O1 and O2 electrodes. This effect was most pronounced for the 7-, 20-, and 60-mg dose on the left side (O1) and for the 3.5-mg and 7-mg dose on the right side of the brain (dose × channel: $p \leq 0.001$). On the other electrode sites (F3, F4, T3, and T4), the α slow wave index did not show significant changes.

DSST Test

The effects (modified Dunlap's d change score) of procedural learning and repeated practice led to some improvements in performance from baseline during the course of 24 hours in all dose groups (Figure 2). However, there was a clear drug effect above these learning and practice effects. Across all dose groups and time points, the modified Dunlap's d effect sizes

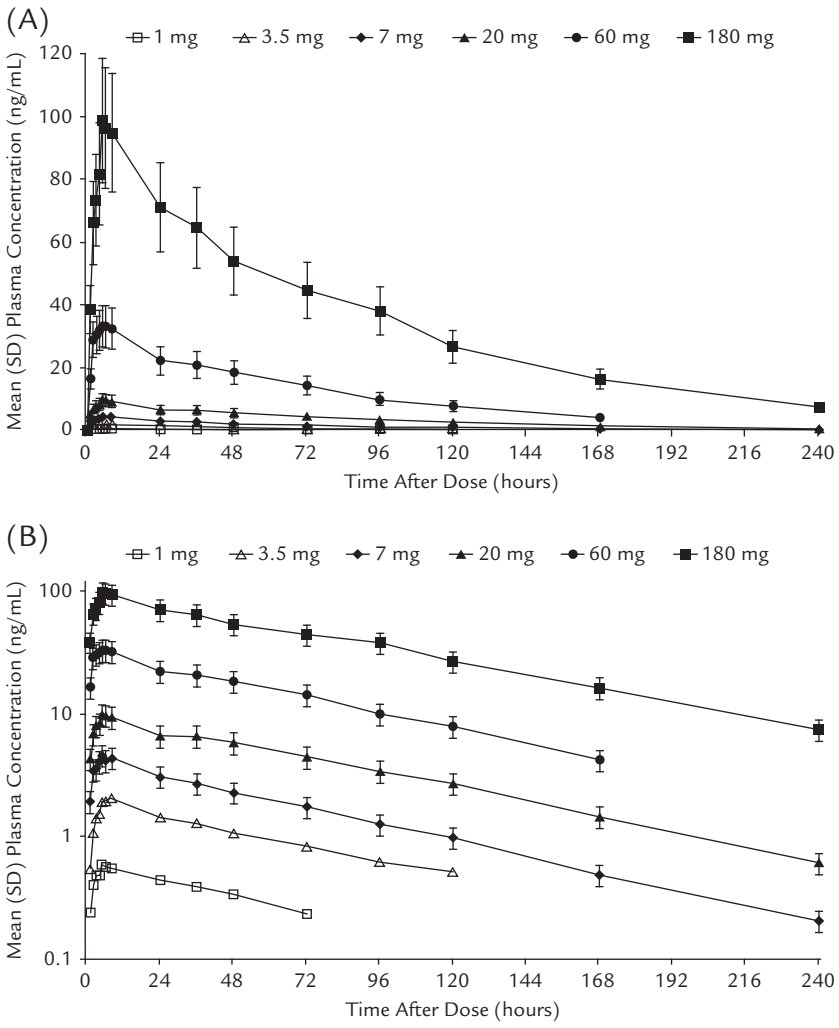

Figure 1. Arithmetic mean (SD) plasma concentration versus time profiles for encenicline at doses of 1 to 180 mg as an oral solution. (A) Linear and (B) semi-log plot.

ranged from −0.1 to 5.7. The improvement in DSST was generally dose dependent, initially increasing with increasing dose, but with an apparent U-shaped dose–response curve and with the effect in the 3.5-mg group being somewhat muted (Figure 2). The maximal effect (d = 3.3−5.7) was observed at the 20-mg dose, with a diminution in peak improvement at the 60-mg and 180-mg doses, Statistically significant differences were observed at the lowest (1 mg) dose group, for which the modified Dunlap's d effect size was 1.7 at 1 hour after dosing, which was more than twice the magnitude of the placebo effect ($p = 0.016$). Significant differences versus placebo ($p \leq 0.050$) were also observed for the 1-mg dose group at 24 hours post dose, the 7-mg dose group at 8 hours and 24 hours post dose, the 20-mg dose group at 4 hours post dose, and the 60-mg dose group at 8 hours post dose.

ARCI49

No statistically significant dose effects were observed for the MB, Lysergic Acid Diethylamide, PCA, Amphetamine, or Benzedrine group scales. Statistically significant differences were observed on comparison of placebo-corrected change from baseline values based on dose or by dose and time point for the following ($p \leq 0.050$): dose, increase in MB group scale at 60 mg and decrease in PCA group scale at 1 mg and 180 mg; dose by time point, increase in Amphetamine scale at 180 mg at 24 hours, increases in MB group scale at 60 mg at 8 hours and 180 mg at

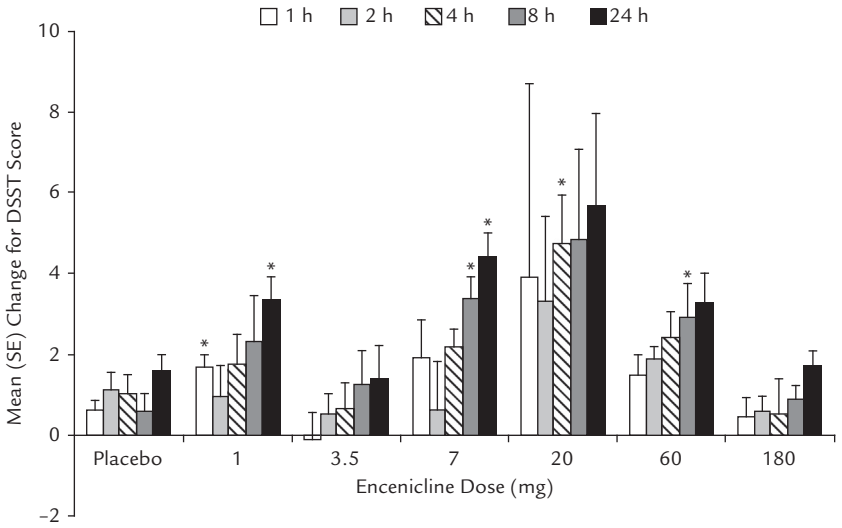

Figure 2. Mean (SE) modified Dunlap's d change scores for Digit Symbol Substitution Test by encenicline dose level and time. $^{*}p \leq 0.050$ versus placebo.

24 hours, decreases in PCA group at 1 mg at 2 hours and 180 mg at 2, 4, and 24 hours. Overall, based on the lack of a dose or time relationship of the statistically significant increases in subscale values the ARCI data suggest encenicline does not have negative effects on endpoints potentially relevant for misuse/abuse.

Bond and Lader VAS

Based on repeated-measures ANOVA, no statistically significant dose effects were observed for the alertness, contentedness, or calmness subscales of the Bond and Lader VAS. On comparison of placebo-corrected change from baseline values, a statistically significant increase in alertness was observed at the 1-, 7-, 60-, and 180-mg encenicline dose levels. Based on a dose by time point analysis of placebo-corrected change from baseline values, statistically significant increases in alertness were observed at 1 and 2 hours post dose at 1 mg, at 24 hours post dose at 60 mg, and at all post-dose time points at 180 mg. Statistically significant decreases in calmness were observed at 2 and 8 hours post dose at 1 mg, and in contentedness at 24 hours post dose at 1 mg. Overall, the Bond and Lader VAS suggest a potential increase in self-rated alertness at the 180-mg encenicline dose level. The data for self-rated calmness and contentedness showed no clear effect of encenicline during the study.

Safety and Tolerability

Thirty-five adverse events (AEs) were reported by 22 of the 48 subjects. Nine AEs were reported before study drug administration, resulting in a total of 26 treatment-emergent AEs (TEAEs) reported by 20 subjects (Table III). The most frequently reported TEAEs were headache (6 events reported by 5 subjects) and rhinitis (3 events reported by 3 subjects). Of the 26 TEAEs, 4 reported by 4 subjects were considered possibly related to treatment: dizziness (2 events; placebo) nausea (1 event; 600 mg dose), and eye irritation (1 event; 20-mg dose). No relationship between TEAEs and increasing doses was observed. No serious AEs, deaths, or discontinuations due to AEs were reported.

Capsule and Solution Relative Bioavailability and Food and Sex Effect Study

Demographics

Twelve subjects were enrolled, 7 female and 5 male, and 11 completed the study. One subject was withdrawn because of influenza-like illness. Mean (SD) age was 51 (16) years, and mean (SD) BMI was 25.0 (3.0) kg/m^2. On average, male subjects were older and had higher mean body weight and BMI. Eleven subjects were white and one female defined her race as white and Asian.

PK Evaluation

Across the study sample bioanalytical runs, the accuracy (% bias) of encenicline quality-control samples

Table III. Treatment emergent adverse events occurring in ≥2 subjects overall.

	Subjects, n (%)						
	Encenicline Dose Group						
Adverse Event	Placebo (n = 12)	1 mg (n = 6)	3.5 mg (n = 6)	7 mg (n = 6)	20 mg (n = 6)	60 mg (n = 6)	180 mg (n = 6)
Any AE	6 (50)	4 (67)	1 (17)	2 (33)	3 (50)	4 (67)	0
Rhinitis	1 (8)	2 (33)	0	0	0	0	0
Dizziness	2 (17)	0	0	0	0	0	0
Headache	1 (8)	1 (17)	0	0	2 (33)	1 (17)	0
Pharyngolaryngeal pain	1 (8)	0	0	0	0	1 (17)	0

AE = adverse event.

(0.03−8.00 ng/mL) ranged from 2.1% to 3.0% and assay precision (%CV) ranged from 1.7% to 5.8%. Mean plasma encenicline concentration-time profiles were similar after the oral solution and capsule formulations and after administration of a capsule under fed or fasted conditions. Encenicline C_{max} was reached at median times of 6 hours for the oral capsule and 7 hours for the oral solution (Figure 3). Thereafter, a gradual decline in mean plasma concentrations was observed with quantifiable concentrations observed up to 240 hours post dose. Encenicline PK parameters were similar after administration as a capsule relative to an oral solution, with point estimates and 90% CIs of the ratio of log-normalized parameters being 104% (98%−111%) for C_{max}, 109% (104%−115%) for AUC_{0-last} and 109% (104%−115%) for $AUC_{0-\infty}$. Therefore, encenicline administered as a capsule formulation was bioequivalent to administration as an oral solution. After encenicline 1-mg capsules under fed and fasting conditions, median T_{max} was similar (6 hours) and mean $t_{½}$ was 67.1 versus 67.4 hours, respectively. Mean C_{max} (0.762 vs 0.717 ng/mL), AUC_{0-t} (65.4 vs 62.3 ng · h/mL), and $AUC_{0-\infty}$ (71.8 vs 68.6 ng · h/mL) were similar for oral capsules under fasting and fed conditions, respectively. The 90% CIs of the treatment ratios (fed over fasted) for C_{max} and AUC parameters were within 80% to 125%, indicating that administration with food did not affect encenicline PK profile. After encenicline 1-mg oral capsules or solution, mean plasma concentrations and exposure parameters were higher in female versus male subjects (Figure 4) with the ratios and 90% CIs of log-normalized parameters being 142% (117%−172%) for C_{max}, 130% (103%−165%) for AUC_{0-last}, and 128% (98%−168%) for $AUC_{0-\infty}$; the sex difference in C_{max} was statistically significant ($p \leq 0.050$) (Figure 5). The approximate 30% to 40% higher C_{max} and AUC parameters observed in female subjects was consistent with the, on average, 33% greater body weight of the male subjects (84.0 kg) enrolled in the study compared with the female subjects (66.4 kg).

Tolerability

Twenty-three TEAEs were reported by 10 of the 12 subjects (83%). Four subjects each reported one or more TEAEs of moderate severity, only one of which (nausea) was considered possibly related to study medication. Five TEAEs were considered to be possibly drug related: headache (3 events, fasted capsule [n = 1] or solution fasted [n = 2] subjects); dizziness (1 event, in fasted capsule subject), and nausea (1 event, solution fasted). No deaths occurred during the study, and no serious AEs or severe TEAEs were reported. No clinically relevant changes in vital signs, 12-lead ECG, telemetric monitoring, Holter monitoring, laboratory parameters (including immunology assessment), and physical examination were observed.

DISCUSSION

This article reports the first clinical experience with encenicline, a selective α_7 nAcChR partial agonist. The results indicate that, after oral administration in healthy male volunteers, single doses of encenicline from 1 to 180 mg exhibited linear kinetics. Encenicline

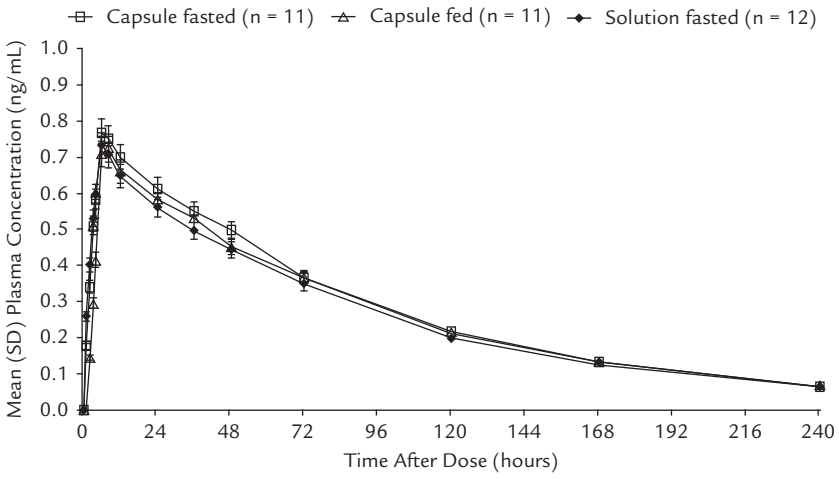

Figure 3. Arithmetic mean (SD) plasma concentration versus time profiles for encenicline after administration of 1-mg oral capsule in fasted and fed states or 1-mg oral solution in fasted state.

was quantifiable in plasma at all doses administered. Absorption was somewhat prolonged, with a T_{max} in the 5- to 7-hour range. Encenicline had a long plasma half-life (estimated 54–62 hours) and a high volume of distribution of the terminal phase (1680–1990 L). It is anticipated that once-daily dosing with encenicline will be appropriate for evaluation of the safety and efficacy profiles for extended periods. The bioavailability of encenicline was similar for oral capsule and oral solution and was not affected by food, but higher C_{max} and AUC values were observed in female versus male subjects, which likely was associated with the higher body weight of male subjects.

Encenicline was very well tolerated after single oral doses of 1 to 180 mg. Most adverse events were of mild or moderate severity, and no serious adverse events occurred. All the adverse events considered related to treatment were mild, and no evidence of a dose-dependency for specific adverse events was noted.

Encenicline had no apparent effect on absolute power levels of the EEG, but the relative contributions of individual frequency bands in relation to the entire

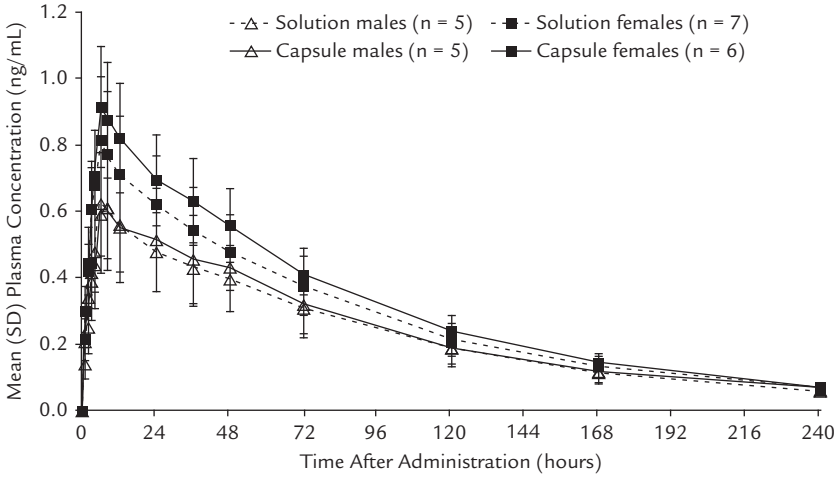

Figure 4. Arithmetic mean (SD) plasma concentration versus time profiles for encenicline after administration of 1-mg oral capsule or solution in fasted state in males and females.

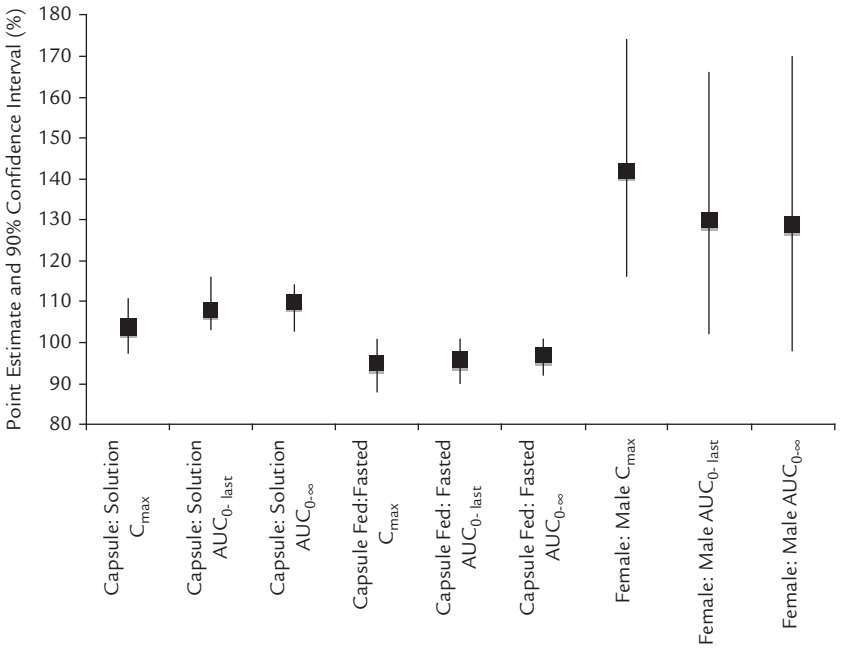

Figure 5. Point estimates and 90% confidence intervals (%) for encenicline AUC and C_{max} parameters for the comparisons of encenicline capsules and solution, the effect of food on encenicline capsules, and the sex effect after administration of encenicline oral capsules.

EEG frequency range did show changes with dose and time of measurement. In general, the contribution of frequencies in the α band increased, especially at the lower doses, while the contribution of the β and δ band attenuated. This latter effect was seen mainly at the higher doses and was delayed in time compared with the effects on the α band. One of the challenges of early drug development targeting central nervous system indications is the difficulty of attaining adequate central nervous system penetration. Various techniques, such as positron emission tomography scans, determinations of compound level in the cerebrospinal fluid, and functional magnetic resonance imaging have been used in attempts to develop an understanding of the PK to PD profile relationship of potential drugs. In the absence of a high-affinity, selective positron emission tomography ligand for neuroimaging studies, we chose qEEG and the DSST test as noninvasive methods indicative of a PD effect of encenicline in humans and these measures did show a response to drug.

No consensus exists about the link between specific brain-wave frequency ranges and cognitive activity or ability. Both τ and α activity have been implicated in memory function.[32] Some groups have found that cognition-enhancing compounds, including acetylcholinesterase inhibitors, increase τ activity.[33] In contrast, cholinesterase inhibitors generally decrease τ activity in patients with Alzheimer's disease.[34,35] This, however, may reflect more of a muscarinic than a nicotinic effect.

At the present time, there is limited knowledge about the effect of nicotinic agonists on EEG, and more specifically of α_7 nAcChR agonists. The nicotinic agonist metanicotine decreased δ and α2 power, while the selective α_7 nAcChR antagonist methyllycaconitine increased power in these ranges.[36] The α_7 nAcChR agonist PNU-282987 did not influence rat hippocampal τ rhythm by itself, but augmented τ activity induced by electrical stimulation of the brain reticular formation[37] and administration of amphetamine.[38] To our knowledge, this is the first report of the effect of a selective α_7 nAcChR agonist on the EEG of the human brain. In healthy male volunteers, TC-1734 (AZD3480, ispronicline), a selective agonist of the $\alpha_4\beta_2$ neuronal AcChR, acceleration of the α centroid and of the α peak was observed, mostly around 1 to 2 hours.[39]

Electroencephalography has been found to be useful in the diagnosis of Alzheimer's disease.[40] Patients suffering from Alzheimer's disease show an

increase of slow EEG activity (δ and τ) at the expense of α power, which might be expected to result in an effect on the α slow-wave index.[41,42] In the present study, the α slow-wave index was found to be sensitive to different doses of encenicline (7, 20, and 60 mg), which might be regarded as preliminary support for a PD effect of encenicline on the central nervous system.

The DSST was included as a measure of cognitive performance. Changes in the DSST with encenicline indicated improvement in a dose-dependent manner and with no adverse effect on cognitive performance in this normal population at all doses tested. The mean modified Dunlap's d scores (DSST) for the groups treated with encenicline appear to increase over time for nearly all doses. At 8 hours, all encenicline treatment groups' mean scores were higher than placebo and at 24 hours, all encenicline treatment groups' mean scores, with the exception of the 3.5-mg dose group, were higher than placebo. This might indicate a positive PD effect occurring after T_{max} at 5 to 8 hours. The maximal effects on DSST were observed at a single dose of 20 mg, but significant effects were observed even at the lowest (1 mg) dose and continued during a 24-hour period. At doses higher than 20 mg, there was an apparent diminution of the positive DSST effects, suggesting that a U-shaped dose response curve might exist, which is compatible with receptor desensitization. The maximal (optimal) procognitive effect of encenicline on the DSST at a dose of 20 mg produces a mean exposure that extrapolates to a steady-state (chronic) dosage per exposure of 2 to 4 mg/d. There were no within-group plasma concentration differences that would explain the lower effect observed in the 3.5-mg dose group. Based on an accumulation ratio of approximately 5, repeat daily administration of 4 mg would be expected to result in plasma concentrations similar to those observed after a single 20-mg dose. Additional studies are needed to evaluate whether long-term therapy in the 2 to 4 mg/d dosage range might be optimal.

CONCLUSIONS

Encenicline appeared to be well tolerated at single doses up to 180 mg in the population studied. Quantitative EEG and the DSST indicated that, even at low doses, encenicline appeared to have PD effects on the central nervous system. Importantly, to our knowledge, this is the first report of the effect of a selective α_7 nAcChR agonist on the EEG of the human brain. No effect of food on the PK profile of encenicline was observed and, although a sex PK effect was observed, it appears to be related to weight differences in the subjects and is not believed to represent sex-specific differences in the disposition and metabolism of encenicline. Additional clinical studies are needed to investigate the anticipated procognitive effects and the safety and tolerability profiles of encenicline after multiple dosing. Dosages in the 2 to 4 mg/d range might provide optimal procognitive effects, but this will require additional studies to confirm.

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A. J. Barbier, A. Van Vliet, M. G. Palfreyman, M. Gawryl, R. Tiessen, W. Timmerman, and D. C. Hilt designed and conducted the study. P. Snyder, N. Dgetluck, and M. Massaro analyzed the data. M. Hilhorst conducted the analytical and PK analysis. A. J. Barbier, D. C. Hilt, M. Gawryl, N. Dgetluck, and M. Massaro were involved in writing and revision of the manuscript. All authors approved submission of the manuscript to the journal.

CONFLICTS OF INTEREST

At the time of this work, Ann J. Barbier, Michael G. Palfreyman, Maria Gawryl, Nancy Dgetluck, Monica Massaro, and Dana C. Hilt were employees of Forum Pharmaceuticals, Boston, MA. Martijn Hilhorst, Andé Van Vliet, Tiessen, and Wia Timmerman were employees of PRA International, Zuidlaren, the Netherlands, which received funding to conduct the studies reported in this article. Peter Snyder is on the faculty of Brown University, Providence, RI and received research support for data analysis for these studies. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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