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Biperiden Challenge Model in Healthy Elderly as Proof-of-Pharmacology Tool: A Randomized, Placebo-Controlled Trial

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Abstract

Selective M₁ muscarinic acetylcholine receptor (mAChR) agonists are being developed as symptomatic treatment for neurodegenerative and neuropsychiatric disorders that lead to cognitive dysfunction. Demonstrating cognition-enhancing effects in early-phase clinical development in healthy subjects is difficult. A challenge with the M₁ mAChR antagonist biperiden could be used to demonstrate procognitive and pharmacological effects of selective M₁ mAChR agonists. The aim of this study was to develop such a model. To this end, 12 healthy elderly subjects participated in a randomized, placebo-controlled, 3-way crossover study investigating tolerability, pharmacokinetic (PK) and pharmacodynamic (PD) effects of 2 and 4 mg biperiden. Repeated PD assessments were performed using neurocognitive tasks and electrophysiological measurements. A population PK-PD model was developed. Four milligrams of biperiden showed significant impairment of sustained attention (−2.1 percentage point in adaptive tracking [95%CI, −3.043 to −1.148], verbal memory (2-3 fewer words recalled [95%CI, −5.9 to −0.2]) and working memory (up to a 50-millisecond increase in the n-back task reaction time [95%CI, 21.854-77.882]) compared with placebo. The PK data were best fitted by a 2-compartment model and showed high interoccasion and intersubject variability. Population PK-PD analysis quantified significant concentration-effect relationships for the n-back reaction time, n-back accuracy, and adaptive tracking. In conclusion, biperiden caused M₁ mAChR-related dose- and concentration-dependent temporary declines in cognitive functioning. Therefore a biperiden pharmacological challenge model can be used for proof-of-pharmacology studies and to demonstrate cognition-enhancing effects of new cholinergic compounds that are being developed.

Keywords

acetylcholine, biperiden, cognition, M₁ receptor, pharmacology, pharmacokinetics

Acetylcholine is a main neurotransmitter of the central nervous system (CNS) and is involved in cognitive processes such as memory and attention.^{1–3} Deficits in the cholinergic system have been found in both neuropsychiatric and neurodegenerative disorders such as Alzheimer's disease and schizophrenia. The current mainly available (ie, registered) therapies for the treatment of cognitive dysfunction in patients with mild to moderate Alzheimer's disease are acetylcholinesterase inhibitors such as donepezil and galantamine. However, these drugs are only effective in a limited number of patients and are associated with significant (gastrointestinal) side effects because the compounds are not selective for the affected parts of the central nervous system. As a consequence, the possibility of reaching effective dose levels is limited.^{4–6} In response to these limitations, selective M₁ muscarinic acetylcholine receptor (mAChR) agonists are under development and entering early-phase clinical trials. These specific muscarinic drugs are expected to cause fewer side effects than the relatively non-specific-acting cholinesterase inhibitors. The M₁ mAChR is a potential target of a

selective muscarinic drug, as this receptor plays a major role in cognitive function.⁷

Several anticholinergic pharmacological challenge models are commonly used to investigate cognition-enhancing effects in early-phase clinical development, the most important of which is the scopolamine model. The idea behind an anticholinergic challenge is that this induces temporarily (reversible) cognitive defects, which involve the same neurobiological mechanisms as

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are targeted by procholinergic drugs. Scopolamine is a competitive mAChR antagonist that is nonselective and thus binds to all 5 subtypes of the mAChRs. This lack of selectivity makes scopolamine a less suitable challenge agent for the investigation of new M₁ mAChR agonists that are currently being developed. In addition, scopolamine has been shown to induce marked sedation, which is difficult to disentangle from its cognition-impairing effects.^{3,8}

Biperiden is a competitive, relatively selective M₁ mAChR antagonist (equilibrium dissociation constant [K_d] for M₁, 0.48 ± 0.02; for M₂, 6.3 ± 0.5; for M₃, 3.9 ± 0.1; for M₄, 2.4 ± 0.03; for M₅, 6.3 ± 0.1).⁹ Administration of biperiden has been shown to lead to impairment in episodic and working memory,^{10–12} attention,¹¹ and posterror control.¹³ Because of the higher M₁ selectivity of biperiden, a biperiden challenge model would be more appropriate to use in early-phase clinical studies of M₁-specific mAChR agonists. Several studies have investigated biperiden as a cognitive challenge model in healthy young,^{12–16} healthy elderly,¹¹ and schizophrenia patients.¹⁰ However, these studies have significant design-related limitations: only 1 session of testing was performed postdose, in most cases around the T_{max} of biperiden (approximately 1 hour postdose); a single dose level was investigated; and it was not always described whether the test battery was also performed before drug administration to serve as baseline measurement. Also, the relation between cognitive pharmacodynamic (PD) effects and the plasma pharmacokinetics (PK) of biperiden was not investigated, as in most cases the biperiden plasma concentrations were not analyzed. A reliable PK-PD model provides an important indication for robust pharmacological activity, and it can be used to optimally design a future study investigating new experimental compounds by calculating the biperiden dose level, sample size, and timing of PK and PD measurements. In addition, biperiden has been studied in only a few elderly subjects. Because M₁ mAChR agonists are under development for the treatment of Alzheimer's disease, it is useful to already know about the behavior of the drug in elderly subjects before moving into the target patient population.

The aim of this study was to develop the biperiden challenge model in healthy elderly, as a tool to prove pharmacology and to provide support for cognition-enhancing effects of new M₁ mAChR agonists that are being developed.

Methods

This study was approved by the ethics committee of the Leiden University Medical Centre (Leiden, The Netherlands). Informed consent was obtained from all

individual participants included in the study. It was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. The trial was registered in the Netherlands Trials Register (NL7146). A randomization code was generated in SAS 9.4 for Windows (SAS Institute Inc., Cary, North Carolina).

Trial Design and Subjects

This was a randomized, double-blind, placebo-controlled, 3-way crossover study in which biperiden 2 and 4 mg and placebo were orally administered to 12 healthy elderly subjects. Akineton 2-mg tablets (Laboratorio Farmaceutico) and placebo tablets were overencapsulated in Swedish orange capsules size 00 at Leiden University Medical Centre Pharmacy in accordance with local regulations. The treatment phase consisted of 3 identical treatment periods separated by a washout period of 1 week between administrations of the medication. The tolerability of a single 4-mg dose was unknown. Therefore, subjects were randomized in such a way that biperiden 4 mg was only administered after the subjects completed the study day with the 2-mg dose. In this way, individual tolerability to 2-mg tablets would be known prior to administration of the 4-mg dose. Before the start of the study day, a light breakfast was allowed, and within 30 minutes prior to dosing, subjects consumed a snack to prevent nausea.

All subjects had to be 65 to 80 years old (inclusive), healthy with no current or past history of any physical, neurological, or psychiatric illness interfering with the study objectives, and a Mini-Mental State Examination score of ≥28. Use of nicotine-containing products was not allowed during the study, and consumption of caffeine was not allowed 24 hours prior to dosing and during the study days.

Safety Assessments

During the study periods, safety was assessed using monitoring of treatment-emergent adverse events, laboratory tests, vital signs, and electrocardiogram.

PK Assessments

To assess the pharmacokinetic characteristics of biperiden, venous blood samples were obtained predose and 0.5, 1, 1.5, 2, 2.5, 4, 7, 10, and 22 hours postdose. Plasma concentrations of biperiden were determined by Ardena Bioanalytical Laboratory (Assen, The Netherlands). Extraction of biperiden from human K₂-ethylenediaminetetraacetic acid plasma samples was performed using liquid-liquid extraction and followed by analysis using a Shimadzu Prominence/Nexera liquid chromatography (LC) system, equipped with a Sciex API 4000 tandem mass spectrometer.

Biperiden-D5 was used as an internal standard. Separation was established on a XBridge Phenyl LC column (4.6 × 100 mm, 3.5 μm) using isocratic elution with 0.025% NH₄OH in 67% acetonitrile at a flow of 1.0 mL/min. The mass spectrometer was equipped with a Turbo Ion Spray probe operated in the positive multiple reaction monitoring mode. The mass transitions were m/z 312 → 143 for biperiden and m/z 317 → 148 for the internal standard. The analytical range of the assay was 0.100.0 ng/mL. The accuracy and precision of the assay were monitored during all analysis runs using quality control samples at the levels low (0.300 ng/mL), medium (1.50 ng/mL), and high (8.00 ng/mL). The overall accuracy was 100.8% for low level, 99.2% for medium level, and 102.1% for high level. The between-day variability, expressed as CV%, was 6.5% for low level, 3.1% for medium level, and 2.1% for high level. Noncompartmental analysis was performed in R, version 2.12.0 for Windows (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria).

PD Assessments

To assess the effects of biperiden on CNS functioning, PD tests were performed repeatedly using the Neuro-Cart, a battery of neuropsychological and neurophysiological tests that can be used to examine the effects of CNS active drugs on a wide range of CNS domains.¹⁷ A customized set of tasks to detect PD effects to be expected of cholinergic drugs was performed twice immediately prior to dosing and 1, 2.5, 4, 7, and 22 hours postdose. The duration of 1 PD testing round was 1 hour. The visual-verbal learning test immediate part was only performed 1.5 hours postdose, and the delayed recall/recognition condition was performed 40 minutes after. Timing of the PD tests was based on the PK characteristics described in the summary of product characteristics: T_{max} between 1 and 1.5 hours after administration and mean half-life of 24 to 37 hours after administration of a single dose of 4 mg in elderly subjects.¹⁸

Adaptive Tracking Test. This is a pursuit-tracking task for the measurement of visuomotor coordination and sustained attention.^{19–22} A circle moved randomly about a screen. The subject was requested to keep a dot inside the moving circle by operating a joystick. If this effort was successful, the speed of the moving circle increased. Conversely, the velocity decreased if the test subject could not maintain the dot inside the circle. In this way, the subject is constantly challenged to perform optimally.²³

N-Back Task. The N-back test was used to evaluate working memory.^{24–26} Per condition, 24 letters were

presented consecutively on the screen with a speed of 30 letters per minute. The target:nontarget rate was 1:3. Subjects were required to press a key for both targets and nontargets. In the 0-back condition, subjects had to indicate whether the letter on the screen was identical to the target letter. In the 1-back condition, subjects indicated whether the letter seen was identical to the previous letter. In the 2-back condition, subjects were asked to indicate whether the letter was identical to 2 letters before the letter seen. The outcome parameters are accuracy measure (correct responses – incorrect responses/total responses) and reaction time.²⁵

Visual-Verbal Learning Test. For the visual-verbal learning test (VVLTL), 30 words were presented. By recalling immediately, acquisition was assessed, by recalling after 30 minutes, recall active retrieval from long-term memory was assessed, and by recognition, memory storage was assessed.^{23,27}

Pupillometry. To determine the pupil diameter, pictures were taken with a digital camera (Canon EOS1100D) and a single flash. The diameters of the pupil and the iris were determined in the number of pixels used horizontally. Pupil size was calculated as the ratio of the pupil diameter over the cornea diameter of each eye.²⁸

Body Sway. The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability.²¹ The total period of body-sway measurement was 2 minutes. All body movements are integrated and expressed as percentage of change.²³

Saccadic and Smooth Pursuit Eye Movements. Saccadic eye movements and smooth pursuit are sensitive parameters for sedation.^{29,30} The use of a computer for the measurements has been described elsewhere.^{23,30,31} The subject was requested to follow a horizontally moving target on a screen at a 58-cm distance. The target moved continuously for measurement of smooth pursuit and jumped from side to side for measurement of saccadic eye movements.

Resting-State Electroencephalography. Resting-state electroencephalography (EEG) is very sensitive to central actions of pharmacological substances. EEG recordings were performed with open and closed eyes for 5 minutes in each eye state.³² Each recording employs alternating periods with eyes open and closed, with a duration of 64-seconds for each period. The EEG was continuously recorded using a 40-channel recording system (Refa-40; TMSi B.V., Oldenzaal, The Netherlands). Twenty-one electrodes were placed

according to the international 10 to 20 system (32-lead cap; TMSi B.V.), but replacing electrodes placed at the earlobes (ie, A1 and A2) with electrodes placed at the mastoids (ie, M1 and M2). Scalp electrode impedance was kept below 5 k Ω . The ground electrode was placed at AFz. In addition, to detect ocular artifacts, vertical and horizontal electrooculogram was also recorded. Two Ag/AgCl electrodes were placed at the outer canthi of both eyes, and 2 Ag/AgCl electrodes were placed approximately 2 cm above and below the right eye. All signals were sampled at a sampling rate of 1024 Hz and were filtered prior to storage using a first-order recursive high-pass filter with a cutoff frequency of 0.1 Hz. Digital markers were recorded by the amplifier, indicating the start and end of each eye state. The electrodes of interest were Fz-Cz, Pz-O1, and Pz-O2. Changes in the amplitude of the following frequency bands were quantified by spectrum analysis (ie, fast Fourier transformation): β -band (12.5-30 Hz), γ -band (30-40 Hz), α -band (8.5-12.5 Hz), and θ -bands (6.0-85 Hz) and δ -bands (1.5-6.0 Hz).

Mismatch Negativity. The mismatch negativity (MMN) auditory event-related potential is a method that is proposed as an index of auditory sensory memory.³³ During an auditory passive oddball task, subjects were watching a silent movie while being presented auditory tones. A total of 750 tones were presented, of which 600 were presented as frequent stimuli and 150 as deviant/infrequent stimuli. The frequent and infrequent tones were 150 milliseconds at a sound pressure level of 75 dB. All tones had a 5-millisecond rise and fall time. Tones were presented at a fixed rate of 2 Hz.

Visual Analog Scales. Visual analog scales (VASs) according to Bond and Lader were used to subjectively assess effects on alertness, mood, and calmness.^{23,34,35} For the VAS nausea, subjects were asked to indicate how nauseous they felt on a 100-mm line.³⁵⁻³⁷

Tapping Test. The finger-tapping test evaluates motor activation and fluency and was adapted from the Halstead Reitan Test Battery.³⁸ The speed of finger tapping was measured for the index finger of the dominant hand while the subject tapped the space bar of a computer as quickly as possible. A session contained 5 performances of 10 seconds. The mean tapping rate and the standard deviations were used for statistical analysis.

Statistics

Usually experimental drugs are investigated in small groups to minimize the exposure of human subjects to a new chemical entity. As this biperiden model might be

used to further investigate new drugs, a small sample size has to be sufficient.

To establish whether significant treatment effects could be detected on the repeatedly measured PD parameters, each parameter was to be analyzed with a mixed-model analysis of covariance with treatment, time, period, and treatment by time as fixed factors and subject, subject by treatment, and subject by time as random factors, and the (average) baseline measurement as covariate.

Single measured PD parameters were analyzed with a mixed-model analysis of variance with treatment and period as fixed factors and subject as a random factor. In these analysis models, all means are estimated. These are called the least-squares means. Biperiden 2 and 4 mg was compared with placebo. Statistical analysis was conducted with SAS 9.4 for Windows (SAS Institute Inc., Cary, North Carolina). Heat plots were generated using the EEG analysis outcomes.

Population PK-PD Analysis

Population PK-PD Model Development. To investigate the relationships between biperiden plasma concentration and PD parameters, a population PK-PD model was developed using nonlinear mixed-effects modeling (NONMEM V7.3).³⁹

For the PK model, 1- 2- and 3-compartmental models, with and without lag time on the absorption of biperiden and transit compartments, were explored. Interindividual variability and between-occasion variability were included in the model parameters following a bottom-up inclusion procedure and were included if significant ($P < .01$) improvement in model fit was obtained. The empirical Bayes estimates were fixed for the development of the PD models. The existence of a learning/placebo effect over time was explored using a linear or Bateman function on data from the placebo occasion only. To capture the concentration-effect relationship, linear, E_{\max} , and sigmoidal E_{\max} relationships were explored.

Age, sex, body weight, and body mass index (BMI) were tested as potential covariates for parameters from which interindividual variability (IIV) could be identified. Covariates were stepwise introduced to the base models (PK and PD), and the covariates that were significant at $P < .01$ were added to the model, followed by a backward exclusion step ($P < .001$).

Model selection was based on the objective function value, the precision of the parameter estimates (relative standard error [%RSE]), and the goodness-of-fit plots consisting of the individual predictions and population predictions of the model versus the observations and the conditional weighted residuals with interactions versus PRED and time.

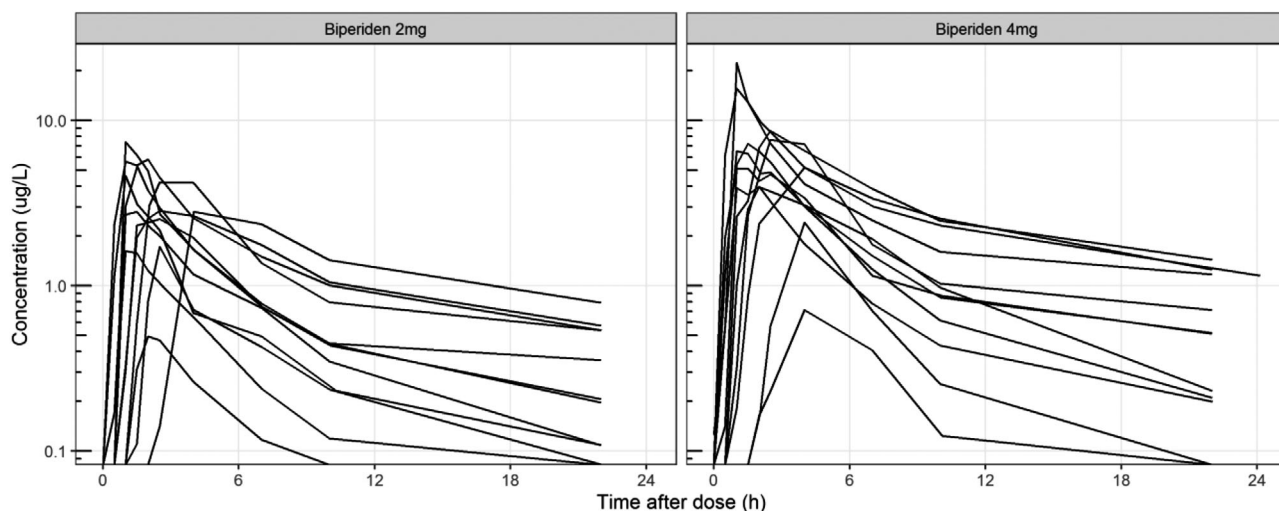


Figure 1. Individual biperiden plasma concentrations after 2 and 4 mg oral biperiden hydrochloride.

Simulation of Statistical Power. The developed population PK-PD model was used for the simulation of different scenarios in which biperiden was used as a challenging compound on the adaptive tracking task. A 4-mg oral dose in parallel and crossover study designs were explored. Hypothetical scenarios in which the investigational drug reduced the response on the adaptive tracking task by 25%, 50%, or 100% were explored.

Each scenario was simulated in 1000 individuals, with 2 baseline measurements and PD measurements 1, 2, 3, 4, and 5 hours postdose. Simulated data were analyzed with linear mixed-effects models with treatment, time, and treatment by time as fixed factors and subject or subject, subject by treatment, and subject by time as random factors for parallel or crossover designs, respectively. The mean of both baseline measurements was included as a covariate. A significance of $P < .05$ was used for the determination of the statistical power.

Results

Subjects

A total of 12 healthy elderly (5 women, 7 men) were enrolled and completed the study. Subjects had a mean age of 71.6 years (range, 69-78 years), weight of 76.2 kg (range, 56.2-88.7 kg), and BMI of 26.2 kg/m² (range, 20.5-31.1 kg/m²).

Pharmacokinetics

The PK of biperiden showed high variability between occasions and high intersubject variability after 2- and 4-mg dosing (Figure 1). The median T_{max} of the plasma concentration was 2 hours postdose (range, 1-4 hours). The mean C_{max} was 3.51 ng/mL (range, 0.50-7.40 ng/mL; CV, 56.7%) after the 2-mg dose and 7.45 ng/mL (range, 0.72-22.30 ng/mL; CV, 80.4%) after the 4-mg dose. The AUC_{0-last} was 18.4 ng·h/mL (range,

1.64-35.16 ng·h/mL) following 2 mg and 39.47 ng·h/mL (range, 3.36-79.7 ng·h/mL) following 4 mg biperiden.

Pharmacodynamic Effects

Adaptive Tracking Test. A significant and dose-related decrease in mean adaptive tracking test performance of 1.36 percentage point was observed after 2 mg biperiden (95%CI, -2.31% to -0.42%; $P = .0075$) and of 2.10 percentage point after 4 mg biperiden (95%CI, -3.04% to -1.15%; $P = .0002$); see Figure 2.

N-Back Task. Visual inspection of n-back graphs indicated a dose-related increase in reaction time in all 3 conditions of the task; however, only the mean reaction time following 4 mg biperiden was significantly different compared with placebo for the 0-back condition (mean difference, 37.2 milliseconds; 95%CI, 6.40-68.0 milliseconds; $P = .0212$) and 1-back condition (mean difference, 49.9 milliseconds; 95%CI, 21.9-77.9 milliseconds; $P = .0016$). The accuracy was slightly but significantly decreased, with 0.06 (95%CI, -0.12 to -0.01; $P = .0209$) after 4 mg biperiden in the 2-back condition compared with placebo (Figure 2). No significant change in reaction time and accuracy was observed following 2 mg biperiden.

Visual Verbal Learning Test. Visual inspection of the VVLT graphs showed a dose-related decrease in performance of all parts of the memory test. Only the effects following 4 mg biperiden were significantly different from placebo on all parameters except for the first immediate recall round. During the second immediate recall round, 2.5 fewer words (95%CI, -4.9 to -0.1 words; $P = .0387$) were recalled. During the third immediate recall round, 2.9 fewer words (95%CI, -5.8 to -0.1 words; $P = .0453$) were recalled; 3.1 fewer

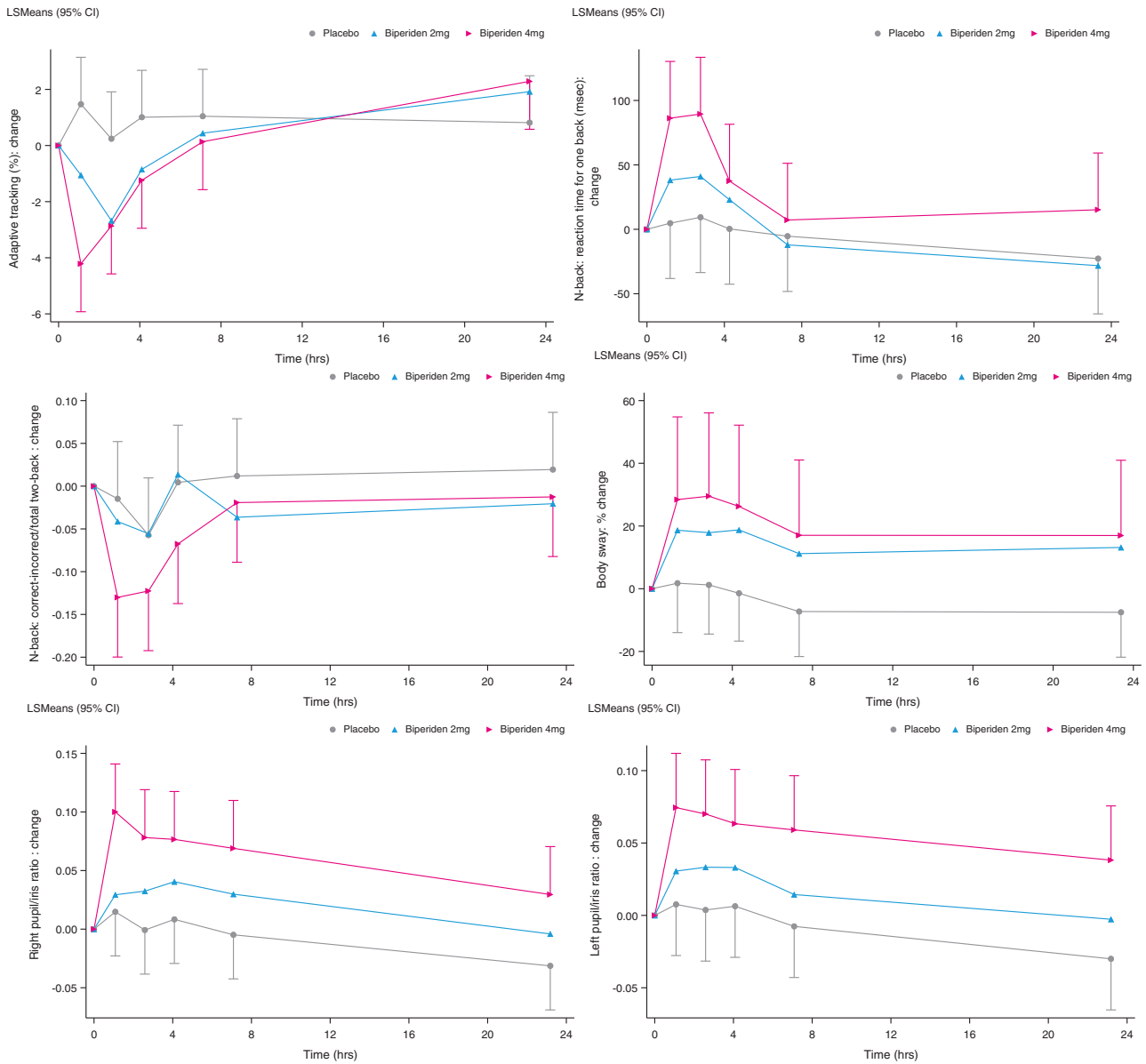


Figure 2. Pharmacodynamic effects on adaptive tracking, n-back test, body sway, and pupil size presented as change from baseline.

words (95%CI, -5.9 to -0.2 words; $P = .0344$) were recalled after a delay of 30 minutes, and 6.5 fewer words (95%CI, -10.8 to -2.2 words; $P = .0053$) were recognized after a delay, whereas the reaction time was 92.2 milliseconds (95%CI, 5.1-179.3 milliseconds; $P = .0390$) longer.

Pupillometry. Inspection of the pupil/iris ratio graphs showed a dose-related increase in pupil size in both eyes, with only the change following 4 mg biperiden significantly different from placebo (right eye: mean difference, 0.07341; 95%CI, 0.02957-0.11725; $P = .0033$; left eye: mean difference, 0.065; 95%CI, 0.02789-0.10211; $P = .0028$). Following the maximum mean change, the

pupil/iris ratio in both eyes decreased; however, it was not normalized 22 hours postdose (Figure 2).

Body Sway. The body sway graphs suggested a dose-related increase postural movements. Only after 4 mg biperiden, body sway increased significantly, 27% (79.7 mm), compared with placebo (95%CI, 3.4%-55.9%; $P = .025$; Figure 2).

Saccadic and Smooth Pursuit Eye Movements. Smooth pursuit decreased with 3.55 percentage point following 4 mg biperiden compared with placebo (95%CI, -5.58% to -1.53%; $P = .0016$). No significant effect was observed after 2 mg biperiden. No significant

Table 1. Population PK Model Parameter Estimates of Oral Biperiden

Parameter	Estimate (CV%)
Lag time, hours	0.54 (BOV = 75%)
Absorption rate constant, /hour	2.73 (BOV = 97.7%)
Volume of distribution—central, L/F	491.40 (IIV = 79.5%)
Volume of distribution—peripheral, L/F	1537.00
Intercompartmental clearance, L/h/F	79.03
Clearance, L/h/F	78.06 (IIV = 172%, BOV = 12%)
Proportional residual error (σ^2)	0.03

BOV, between-occasion variability; IIV, interindividual variability. CV% calculated by $\sqrt{e^{\omega^2} - 1}$.

Biperiden was modeled as biperiden hydrochloride. A relative bioavailability of 1 was assumed. Covariance IIV V_d -central versus clearance was 0.74.

effects were observed on saccadic inaccuracy, peak velocity, or reaction time for both doses compared with placebo.

Resting-State Electroencephalography. All EEG results are summarized in Supplemental Table S1. Most significant changes were observed following 4 mg biperiden. The changes per electrode and per frequency band after 4 mg biperiden compared with placebo are shown in Figure 3A,B. In all cortical areas, alpha and theta power were decreased during the eyes-closed condition after 4 mg biperiden. Beta power was decreased at the central location, and delta power was increased in the frontal cortical area during the eyes-closed condition. The significant changes in gamma power that were observed were not consistent. During the eyes-open condition, there was a decrease in beta power at the central location, and a diffuse increase in delta power.

Mismatch Negativity. MMN latency at Fz increased significantly, with 12.1 milliseconds after 2 mg biperiden (95%CI, 3.004–21.282 milliseconds; $P = .0119$) and with 13.9 milliseconds after 4 mg biperiden (95%CI, 5.071–22.773 milliseconds; $P = .0038$) compared with placebo.

VASs and Tapping Test. No significant changes were observed after both doses on tapping-test performance or on the VAS Bond and Lader subscales of mood, alertness, and calmness or on VAS nausea scores.

Population PK-PD Analysis

Population PK-PD Model Development. The PK data were best fitted by a 2-compartment model with linear elimination. Inclusion of a lag time and transit compartment was required to correctly capture the absorption phase of biperiden. Significant variability was estimated on the absorption parameters, the volume of distribution, and the clearance of biperiden (Table 1). No covariates were

identified. The model-derived terminal half-life was 29.5 hours.

PD results of the adaptive tracking test and n-back test were included in a population PK-PD analysis. No learning or placebo effect was found in any of these PD results. The population PK-PD analysis quantified multiple significant concentration-effect relationships. An inhibitory direct linear concentration-related effect on the adaptive tracking (slope, -0.98 percentage point/ng/mL [RSE, 12.3%; IIV, 32.4%]) was identified. On the reaction time of the n-back 0-back condition, a sigmoid E_{max} drug effect (EC_{50} , 6.72 ng/mL [RSE, 23.2%]; E_{max} , 288.5 milliseconds [RSE, 24.1%; IIV, 37.0%]; Hill coefficient, 2.25 [18.9%]) was best fitted for this purpose. Reaction time in the n-back 1-back condition showed a linear drug effect (slope, 16.18 ms/ng/mL [RSE, 16.5%; no IIV]). Reaction time in the n-back 2-back condition demonstrated a linear drug effect (slope, 11.08 ms/ng/mL [RSE, 28.6%; no IIV]). Regarding the accuracy of the n-back tests, a linear drug effect was quantified for the 1-back accuracy measure (slope: -0.011 /ng/mL [RSE, 46.7%; no IIV]) and for the 2-back accuracy measure (slope: -0.2 /ng/mL [RSE, 31.0%; IIV, 76.4%]). No significant effect was quantified on the 0-back accuracy measure. The typical concentration-effect relationships on the explored PD tests are shown in Figure 4.

Simulation of Statistical Power. The population PK-PD model was used to explore different study designs and the impact on the statistical power on the adaptive tracking task. Simulations presenting the PK after oral dosing and the corresponding power at multiple sample sizes in a study are shown in Figure 5.

Results show that 15 subjects are required in both parallel and crossover study designs to achieve a power of 80% when an M_1 agonist is able to fully reverse the biperiden-induced effects. When a 50% reduction of the concentration-effect relationship was established, fewer subjects ($n = 32$) were required in a crossover design compared with a parallel design ($n = 50+$) to achieve a power of 80%. However, even though the group size was smaller, subjects had to participate in 2 study occasions. Therefore, the number of performed occasions would remain comparable between parallel and crossover study designs. This agreement between crossover and parallel study designs is because of the high between-occasion variability (BOV) present in the model.

A 25% reversal of the biperiden-induced effects by the M_1 agonist has low statistical power that does not increase above 50% at a sample size of 50. This indicates that to identify these small effect sizes using the biperiden challenge model, an increased dose should be given or the sample size should be increased.

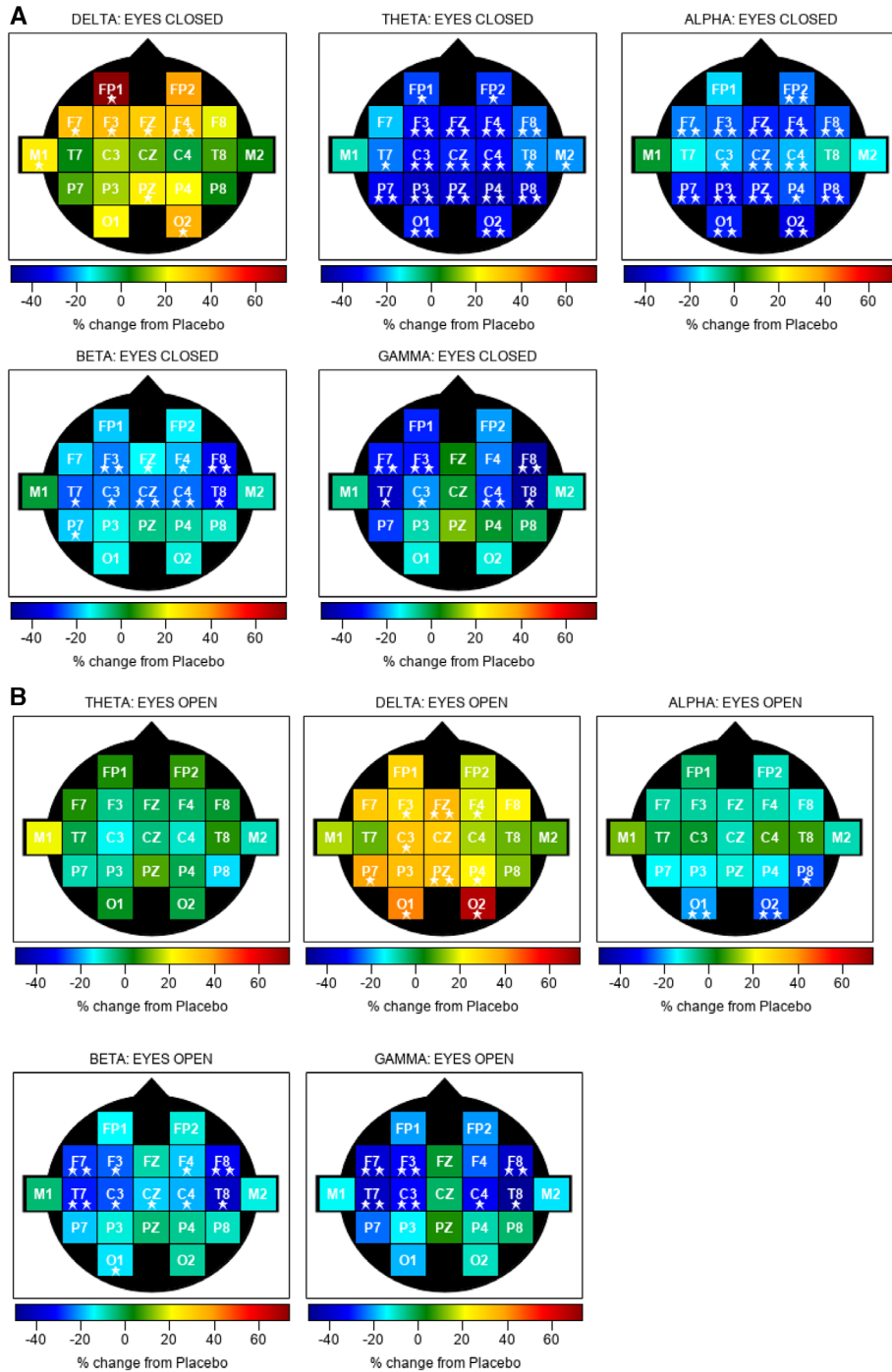


Figure 3. (A) Heat plots showing the effects of 4 mg biperiden on EEG eyes-closed condition. For each frequency band and each electrode (representing a cortical area) the percent change in power compared with placebo is shown. * $P < .05$; ** $P < .01$. (B) Heat plots showing effects of 4 mg biperiden on EEG eyes-open condition. For each frequency band and each electrode (representing a cortical area) the percent change in power compared with placebo is shown. * $P < .05$; ** $P < .01$.

Discussion

This study was performed to develop a biperiden challenge model as a tool to prove pharmacology and to provide support for cognition-enhancing effects of

new M₁ mAChR agonists in future studies. Previous studies investigated the effects of biperiden on cognitive functioning, mainly in young subjects, with only 1 session of testing postdose, in most cases around the expected T_{max} of biperiden, although no PK was

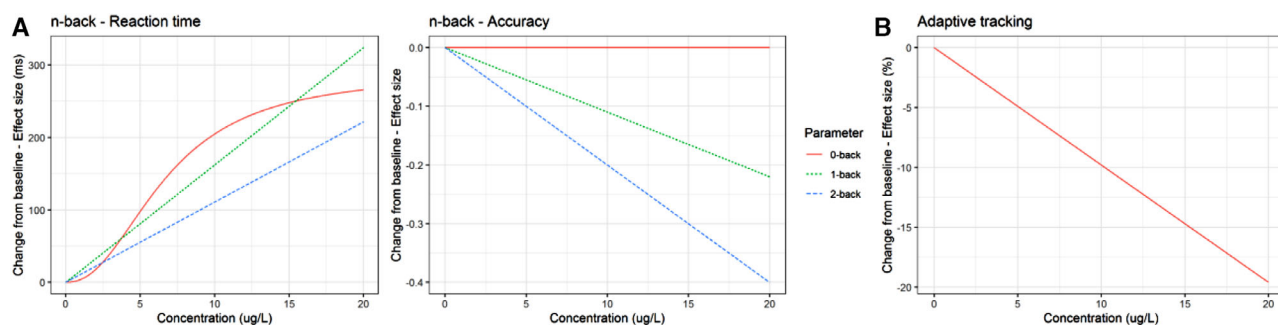


Figure 4. Visualization of the typical concentration-effect relationships for the n-back (A) and the adaptive tracking (B) tasks.

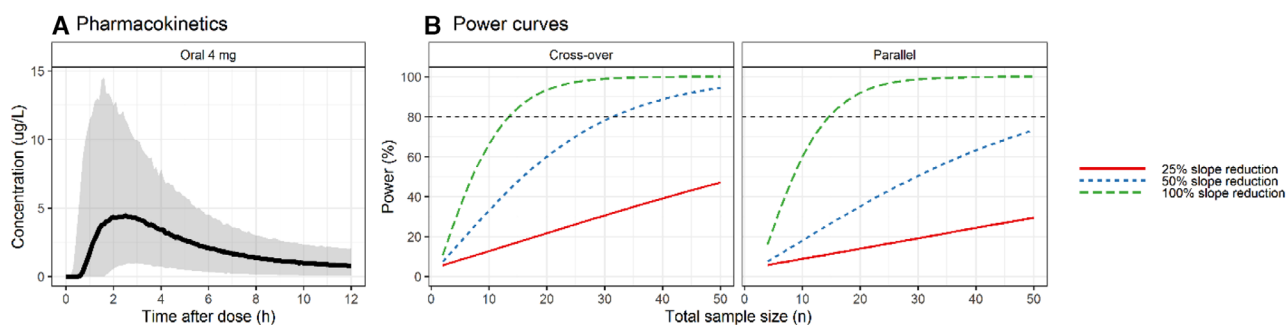


Figure 5. (A) Simulated ($n = 1000$) PK profiles after oral administration of biperiden hydrochloride 4 mg. Solid black line, median prediction; grey ribbon, 90% prediction interval. (B) Model-derived statistical power versus total sample size to detect a 25%, 50%, or 100% reduction of the estimated concentration-effect relationship on the adaptive tracking task in a crossover and parallel study design.

measured. Furthermore, only a single dose level was investigated in these studies. We investigated the PK and PD effects of both 2 and 4 mg of the competitive M_1 mAChR antagonist biperiden on frequently repeated cognitive and neurophysiological tests in healthy elderly individuals. Biperiden plasma concentrations were measured, and the relationship between the PK and PD was modeled in a 2-compartment population PK-PD model with linear elimination and corresponding concentration-effect relationships. This population PK-PD model was used to inform the design of future studies regarding sample size and can be further extended with the biperiden dose level and timing of PK and PD measurements.

The PD results reflect an effect on a wide range of CNS domains following biperiden administration. Most of the significant effects were observed after 4 mg biperiden. The PD effects were consistent with literature, especially the effects on the adaptive tracking test,¹¹ verbal memory,^{11,12,14,15,40} n-back test reaction time,^{11,40,41} and the pupil/iris ratio.^{40,42} The consistency with literature demonstrating the repeatability of the PD effects and the low variability of the PD effects is required for a reliable challenge model.

The PK of biperiden was well characterized in this study, even though high variability was present. The median T_{max} was comparable with previously reported

T_{max} ,^{42,43} suggesting no relevant effect of the overencapsulation. In the population PK model, the IIVs of the central volume of distribution (79.5%) and clearance (172%) were high in comparison with the results of previous studies.^{43,44} However, the quantified level of variability most likely partially originated from variability in the bioavailability after oral administration. In our population PK model, no information on this bioavailability could be quantified because no intravenous PK data were available. The variability in these structural model parameters may therefore be overpredicted. The model, including the identified IIV and between-occasion variability, can be used for simulations of oral administration but should be adapted when simulating intravenous administration of biperiden.

The results indicate that the majority of the variability originates from the PK (CV% ranging from 12% to 172%), with only low to moderate CV% present on the studied PD effects (CV% up to 76.4%). Therefore, to improve the statistical power of a challenge study with biperiden, this variability could be reduced by intravenous dosing of biperiden. With an assumed bioavailability of approximately 33%,¹⁸ an intravenous dose of 1.25-1.5 mg would reach similar peak concentrations. The exact intravenous dose required in this population should be investigated in future research.

However, even though high variability was present in this population, sufficient (80%+) statistical power could already be obtained with moderate sample sizes after oral administration of 4 mg biperiden.

To optimize the quantification of the reversal of biperiden-induced effect, the maximum PD effect of biperiden should occur at around the same time as the maximum PD effect of the experimental compound, which requires accurate planning of dosing on the study day. This timing might be improved by administering the experimental drugs when biperiden is at steady state. This could lead to stable PD-effects throughout the challenge experiment, which would simplify the interpretation of antagonistic effects of a concomitantly administered M₁ mAChR agonist. Continuous or repeated administration could raise the possibility of tolerance.⁴⁵ In the current crossover study, there was no evidence of tolerance after the washout period of 1 week.

Both doses of biperiden were well tolerated, with a limited number of mild and transient side effects. A benign side effect profile is important when investigating new drugs in this challenge model as adverse effects may negatively influence the quantification of PD effects and may negatively affect the safety profile of a new drug. In this respect, biperiden was much better tolerated by elderly individuals than scopolamine in previous studies. In addition, this nonselective mAChR antagonist has shown an age-dependent decline in clearance.⁴⁶ Considering the tolerability and the PK-PD results, the 4-mg dose is preferable over the 2-mg biperiden dose based on tolerability and PD effects. The quantified concentration-effect relationships suggest that increasing the dose will result in larger PD effects. However, a higher dose of biperiden might come with more side effects, but this is not clearly documented in the literature.

The observed effects on n-back, VVLT, and adaptive tracking can be explained by the pharmacological mechanism of biperiden because the brain areas involved in these tests comprise a high density of M₁ mAChRs. The n-back test is a working memory task associated with prefrontal function,^{47,48} the VVLT is associated with hippocampus (right anterior), prefrontal cortex (right dorsolateral), and left medial temporal lobe activity,⁴⁹ and sustained attention measured by the adaptive tracking test is associated with activity of the basal forebrain, prefrontal cortex, and parietal cortical regions.⁵⁰ Thus, in these tests, the prefrontal cortex or hippocampus plays an important role. The M₁ mAChR is the most abundant receptor of all mAChRs in the hippocampus (47%-60%) and in the cortex (34%-55%),^{51,52} and antagonizing the M₁ mAChR will hamper cortical and hippocampal functioning. Dilatation of the pupil is caused by blocking parasympathetic

contraction of the iris sphincter muscle. In the human iris, the M₃ mAChR is the most expressed receptor. The M₁ mAChR only comprises 7% of the total number of expressed mAChRs,⁵³ which may explain why only a relatively small effect on pupil size is observed.

The impaired adaptive tracking suggests a reduction in sustained attention. The adaptive tracking test is also a psychomotor task and can therefore be influenced by effects on motor coordination; however, no effect of biperiden on finger-tapping test performance was observed. Therefore, not impaired motor function, but reduced sustained attention is a likely explanation for the observed effects. Muscarinic activity plays an important role in sustained focused (visual) attention.⁵⁴

Body sway was not normalized 22 hours postdose. Delayed recovery of balance could be because of binding to the M₁ mAChRs in the vestibular system, where clearance might be slower than clearance from the plasma.⁵⁵ Just like disturbed body balance, pupil enlargement was still present 22 hours after administration of 4 mg biperiden. It could be that clearance of biperiden from the peripheral M₁ mAChRs in the iris and ciliary body is slower than that from the plasma, although it has been assumed that clearance from the vitreous is similar to plasma.⁵⁶ A long duration of pupillary dilation has also been observed with scopolamine.⁵⁷

When comparing the biperiden effects observed in the current study with scopolamine effects described in the literature, the biperiden effects seem smaller. For example, the decrease in adaptive tracking in the current study was 2.1 percentage point, compared with 9% to 10 percentage point after scopolamine.⁵⁷⁻⁶⁰ The impairment in verbal memory (2-3 fewer words correctly recalled) was also smaller than the effects of scopolamine (2-7 fewer words recalled).^{3,57,58,60,61} It could be that the dose of biperiden is relatively lower than the scopolamine dose used, or it is because of the difference in pharmacological targets of both compounds. It is also possible that different mAChR-subtypes contribute to the functional domains that were tested in this study. Scopolamine antagonizes M₁-M₅ mAChRs, whereas biperiden is a relatively specific M₁ mAChR antagonist. The M₁ mAChR plays a major role in cognitive function⁷ and represent 35% to 60% of the total mAChRs in areas related to cognitive function: the neocortex and the hippocampus.^{51,52,62} However, the M₁ mAChR is not associated with all hippocampus-dependent learning tasks,⁷ and the remaining 40% to 65% of the total mAChRs consists of M₂-M₅ mAChRs. These other mAChRs are also involved in learning and memory,⁶³⁻⁶⁸ although the role of the M₃ mAChR in cognitive function could not be demonstrated in humans.⁶⁹ Body sway was increased to a greater extent after scopolamine (increase of 150-162 mm^{58,60}) than

after biperiden administration (increase of 79.7 mm after 4 mg biperiden). Besides the M₁ mAChR, the M₂ and M₅ mAChRs are expressed in the afferent vestibular ganglia and the vestibular end organs.⁷⁰ Consequently, antagonism of M₂ and M₅ mAChRs can contribute to a disturbed balance. Also, the M₃ mAChR antagonist darifenacin has been shown to increase body sway.⁶⁹

In addition to antagonism of the M₂-M₅ mAChRs in the brain structures involved in cognition, the sedative effect of scopolamine might also have contributed to the impaired performance of PD tests. Saccadic eye movement is a very sensitive marker for sedative effects.²⁹ Changes in saccadic eye movements are often attributed to suppression of the brain stem reticular formation by stimulation of gamma-aminobutyric acid (GABA) type A receptors with subunit $\alpha 1$.^{71,72} Nonetheless, a concentration-related decrease in peak saccadic velocity was also observed after scopolamine,⁵⁷⁻⁶⁰ suggesting a role for mAChRs in sedation. An interaction between mAChRs and GABA receptors has been described⁷³; however, the exact contribution of each type of mAChR to sedative effects has not been well established. In the brain stem, the M₂ mAChR represents 80% of all mAChRs,⁵² and GABAergic neurons in the reticular formation also contain M₂ mAChRs.⁷⁴ In other brain areas, activation of the M₂ and M₄ mAChRs decreased the release of GABA.^{73,75} The latter might suggest that inhibition of M₂ mAChRs results in an increase of GABA and consequently a sedative effect. As the M₁ mAChR is barely present in the brain stem and the sedative effect of mAChR stimulation seems to be mediated by agonism of the M₂ mAChR, saccadic peak velocity was not decreased, and the score on VAS measuring alertness did not change after biperiden administration in this study. We believe it is safe to conclude that scopolamine has a larger sedative effect than biperiden.

Because of the effects of M₂-M₅ antagonism by scopolamine on cognitive performance and sedation, it is expected that an M₁ mAChR agonist can reverse the effects only to a limited extent. As a consequence, the reversal might get lost in the margins of variability, and therefore the biperiden challenge model seems favorable over the scopolamine model to demonstrate effects of selective M₁ receptor agonists.

Conclusions

Biperiden doses of 2 and 4 mg were very well tolerated and especially 4 mg biperiden caused clear temporary PD effects in different CNS domains, including decline in cognitive function. The PD effects are concentration related and are therefore explained by the pharmacological mechanism of biperiden, making this model

a tool to proof pharmacology and a tool to provide support for the cognitive-enhancing effects of the M₁ mAChR agonist.

Conflicts of Interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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Data Availability

Data and material: the data sets generated during and/or analyzed during the current study are available on request. Code availability: the model code is available on request.

References

1. Caulfield MP, Birdsall NJ. International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol Rev.* 1998;50(2):279-290.
2. Nathan PJ, Watson J, Lund J, et al. The potent M1 receptor allosteric agonist GSK1034702 improves episodic memory in humans in the nicotine abstinence model of cognitive dysfunction. *Int J Neuropsychopharmacol.* 2013;16(4):721-731.
3. Koller G, Satzger W, Adam M, et al. Effects of scopolamine on matching to sample paradigm and related tests in human subjects. *Neuropsychobiology.* 2003;48(2):87-94.
4. Lanctot KL, Herrmann N, Yau KK, et al. Efficacy and safety of cholinesterase inhibitors in Alzheimer's disease: a meta-analysis. *CMAJ.* 2003;169(6):557-564.
5. Tan CC, Yu JT, Wang HF, et al. Efficacy and safety of donepezil, galantamine, rivastigmine, and memantine for the treatment of Alzheimer's disease: a systematic review and meta-analysis. *J Alzheimers Dis.* 2014;41(2):615-631.
6. Buckley JS, Salpeter SR. A risk-benefit assessment of dementia medications: systematic review of the evidence. *Drugs Aging.* 2015;32(6):453-467.
7. Anagnostaras SG, Murphy GG, Hamilton SE, et al. Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. *Nat Neurosci.* 2003;6(1):51-58.
8. Pergolizzi JV, Jr., Philip BK, Leslie JB, Taylor R, Jr., Raffa RB. Perspectives on transdermal scopolamine for the treatment of postoperative nausea and vomiting. *J Clin Anesth.* 2012;24(4):334-345.
9. Bolden C, Cusack B, Richelson E. Antagonism by antimuscarinic and neuroleptic compounds at the five cloned human muscarinic cholinergic receptors expressed in Chinese hamster ovary cells. *J Pharmacol Exp Ther.* 1992;260(2):576-580.
10. Veselinovic T, Vernaleken I, Janouschek H, et al. Effects of anticholinergic challenge on psychopathology and cognition in drug-free patients with schizophrenia and healthy volunteers. *Psychopharmacology (Berl).* 2015;232(9):1607-1617.
11. Wezenberg E, Verkes RJ, Sabbe BG, Ruigt GS, Hulstijn W. Modulation of memory and visuospatial processes by biperiden and rivastigmine in elderly healthy subjects. *Psychopharmacology (Berl).* 2005;181(3):582-594.
12. Sambeth A, Riedel WJ, Klinkenberg I, Kahkonen S, Blokland A. Biperiden selectively induces memory impairment in healthy volunteers: no interaction with citalopram. *Psychopharmacology (Berl).* 2015;232(11):1887-1897.

13. Danielmeier C, Allen EA, Jocham G, Onur OA, Eichele T, Ullsperger M. Acetylcholine mediates behavioral and neural post-error control. *Curr Biol*. 2015;25(11):1461-1468.
14. Guthrie SK, Manzey L, Scott D, Giordani B, Tandon R. Comparison of central and peripheral pharmacologic effects of biperiden and trihexyphenidyl in human volunteers. *J Clin Psychopharmacol*. 2000;20(1):77-83.
15. Borghans LG, Blokland A, Sambeth A. Effects of biperiden and acute tryptophan depletion and their combination on verbal word memory and EEG. *Psychopharmacology (Berl)*. 2017;234(7):1135-1143.
16. Reinke W, Westphal KP, Diekmann V, Grozinger B, Kornhuber HH. Changes in functionally determined order processes of the EEG in a motor task due to various dosages of biperidene. *Neuropsychobiology*. 1994;29(4):194-201.
17. Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood-brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Tech*. 2016;20:27-34.
18. Geneesmiddelen CtBv. SAMENVATTING VAN DE PRODUCTKENMERKEN Akineton, tabletten 2 mg RVG02196. 2018. https://www.geneesmiddeleninformatiebank.nl/ords/f?p=111:3::SEARCH:NO::P0_DOMAIN,P0_LANG,P3_RVG1:H,NL,02196. Accessed June 24, 2020.
19. Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. *Br J Clin Pharmacol*. 1984;18(Suppl 1):69S-72S.
20. van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther*. 1991;50(2):172-180.
21. van Steveninck AL, Gieschke R, Schoemaker HC, et al. Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. *Psychopharmacology (Berl)*. 1993;110(4):471-478.
22. Gijsman HJ, Van Gerven JM, Tieleman MC, et al. Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol*. 1998;18(4):289-295.
23. Hart EP, Alvarez-Jimenez R, Davidse E, et al. A computerized test battery to study pharmacodynamic effects on the central nervous system of cholinergic drugs in early phase drug development. *J Vis Exp*. 2019(144):e56569.
24. Lim HK, Juh R, Pae CU, et al. Altered verbal working memory process in patients with Alzheimer's disease: an fMRI investigation. *Neuropsychobiology*. 2008;57(4):181-187.
25. Rombouts SA, Barkhof F, Van Meel CS, Scheltens P. Alterations in brain activation during cholinergic enhancement with rivastigmine in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2002;73(6):665-671.
26. Sweet LH, Rao SM, Primeau M, Durgerian S, Cohen RA. Functional magnetic resonance imaging response to increased verbal working memory demands among patients with multiple sclerosis. *Hum Brain Mapp*. 2006;27(1):28-36.
27. de Haas SL, Franson KL, Schmitt JA, et al. The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A alpha2,3 selective agonist, in comparison with lorazepam in healthy volunteers. *J Psychopharmacol*. 2009;23(6):625-632.
28. Twa MD, Bailey MD, Hayes J, Bullimore M. Estimation of pupil size by digital photography. *J Cataract Refract Surg*. 2004;30(2):381-389.
29. van Steveninck AL, Verver S, Schoemaker HC, et al. Effects of temazepam on saccadic eye movements: concentration-effect relationships in individual volunteers. *Clin Pharmacol Ther*. 1992;52(4):402-408.
30. Bittencourt PR, Wade P, Smith AT, Richens A. Benzodiazepines impair smooth pursuit eye movements. *Br J Clin Pharmacol*. 1983;15(2):259-262.
31. Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. *Neurology*. 1975;25(11):1065-1070.
32. Jobert M, Wilson FJ, Ruigt GS, Brunovsky M, Prichep LS, Drinkenburg WH. Guidelines for the recording and evaluation of pharmaco-EEG data in man: the International Pharmaco-EEG Society (IPEG). *Neuropsychobiology*. 2012;66(4):201-220.
33. Bartha-Doering L, Deuster D, Giordano V, am Zehnhoff-Dinnesen A, Dobel C. A systematic review of the mismatch negativity as an index for auditory sensory memory: from basic research to clinical and developmental perspectives. *Psychophysiology*. 2015;52(9):1115-1130.
34. Norris H. The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology*. 1971;10(21):181-191.
35. Bond A, Lader M. The use of analogue scales in rating subjective feelings. *Br J Med Psychol*. 1974;47(3):211-218.
36. de Visser SJ, van der Post J, Pieters MS, Cohen AF, van Gerven JM. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. *Br J Clin Pharmacol*. 2001;51(2):119-132.
37. de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. *Br J Clin Pharmacol*. 2003;55(1):39-50.
38. Andrew JM. Delinquents and the Tapping Test. *J Clin Psychol*. 1977;33(3):786-791.
39. Bauer JR. *NONMEM Users Guide: Introduction to NONMEM 7.3.0*. Ellicott City, MD: ICON Development Solutions; 2013.
40. Borghans L, Sambeth A, Prickaerts J, Ramaekers JG, Blokland A. The effects of the soluble guanylate cyclase stimulator riociguat on memory performance in healthy volunteers with a biperiden-induced memory impairment. *Psychopharmacology (Berl)*. 2018;235(8):2407-2416.
41. Borghans L, Sambeth A, Blokland A. Biperiden selectively impairs verbal episodic memory in a dose- and time-dependent manner in healthy subjects. *J Clin Psychopharmacol*. 2020;40(1):30-37.
42. Hollmann M, Brode E, Greger G, Muller-Peltzer H, Wetzelsberger N. Biperiden effects and plasma levels in volunteers. *Eur J Clin Pharmacol*. 1984;27(5):619-621.
43. Grimaldi R, Perucca E, Ruberto G, et al. Pharmacokinetic and pharmacodynamic studies following the intravenous and oral administration of the antiparkinsonian drug biperiden to normal subjects. *Eur J Clin Pharmacol*. 1986;29(6):735-737.
44. Hollmann M, Müller-Peltzer H, Greger G, et al. Pharmacokinetic-dynamic study on different oral biperiden formulations in volunteers. *Pharmacopsychiatry*. 1987;20(2):72-77.
45. Salin-Pascual RJ, Granados-Fuentes D, Galicia-Polo L, Nieves E, Gillin JC. Development of tolerance after repeated administration of a selective muscarinic M1 antagonist biperiden in healthy human volunteers. *Biol Psychiatry*. 1993;33(3):188-193.
46. Alvarez-Jimenez R, Groeneveld GJ, van Gerven JM, et al. Model-based exposure-response analysis to quantify age related differences in the response to scopolamine in healthy subjects. *Br J Clin Pharmacol*. 2016;82(4):1011-1021.
47. Tsuchida A, Fellows LK. Lesion evidence that two distinct regions within prefrontal cortex are critical for n-back performance in humans. *J Cogn Neurosci*. 2009;21(12):2263-2275.
48. Yapple ZA, Stevens WD, Arsalidou M. Meta-analyses of the n-back working memory task: fMRI evidence of age-related changes in prefrontal cortex involvement across the adult lifespan. *NeuroImage*. 2019;196:16-31.
49. Johnson SC, Saykin AJ, Flashman LA, McAllister TW, Sparling MB. Brain activation on fMRI and verbal memory ability:

- functional neuroanatomic correlates of CVLT performance. *J Int Neuropsychol Soc.* 2001;7(1):55-62.
50. Sarter M, Givens B, Bruno JP. The cognitive neuroscience of sustained attention: where top-down meets bottom-up. *Brain Res Brain Res Rev.* 2001;35(2):146-160.
51. Flynn DD, Ferrari-DiLeo G, Mash DC, Levey AI. Differential regulation of molecular subtypes of muscarinic receptors in Alzheimer's disease. *J Neurochem.* 1995;64(4):1888-1891.
52. Levey AI. Immunological localization of m1-m5 muscarinic acetylcholine receptors in peripheral tissues and brain. *Life Sci.* 1993;52(5-6):441-448.
53. Gil DW, Krauss HA, Bogardus AM, WoldeMussie E. Muscarinic receptor subtypes in human iris-ciliary body measured by immunoprecipitation. *Invest Ophthalmol Visual Sci.* 1997;38(7):1434-1442.
54. Thiele A, Bellgrove MA. Neuromodulation of attention. *Neuron.* 2018;97(4):769-785.
55. Salt AN. Pharmacokinetics of drug entry into cochlear fluids. *Volta Rev.* 2005;105(3):277-298.
56. Del Amo EM, Rimpela AK, Heikkinen E, et al. Pharmacokinetic aspects of retinal drug delivery. *Prog Retinal Eye Res.* 2017;57:134-185.
57. Liem-Moolenaar M, de Boer P, Timmers M, et al. Pharmacokinetic-pharmacodynamic relationships of central nervous system effects of scopolamine in healthy subjects. *Br J Clin Pharmacol.* 2011;71(6):886-898.
58. Liem-Moolenaar M, Zoethout RW, de Boer P, et al. The effects of a glycine reuptake inhibitor R231857 on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. *J Psychopharmacol.* 2010;24(11):1681-1687.
59. Baakman AC, Alvarez-Jimenez R, Rissmann R, et al. An anti-nicotinic cognitive challenge model using mecamylamine in comparison with the anti-muscarinic cognitive challenge using scopolamine. *Br J Clin Pharmacol.* 2017;83(8):1676-1687.
60. Liem-Moolenaar M, Zoethout RW, de Boer P, et al. The effects of the glycine reuptake inhibitor R213129 on the central nervous system and on scopolamine-induced impairments in psychomotor and cognitive function in healthy subjects. *J Psychopharmacol.* 2010;24(11):1671-1679.
61. Ellis JR, Ellis KA, Bartholomew CF, et al. Muscarinic and nicotinic receptors synergistically modulate working memory and attention in humans. *Int J Neuropsychopharmacol.* 2006;9(2):175-189.
62. Weinberger DR, Gibson R, Coppola R, et al. The distribution of cerebral muscarinic acetylcholine receptors in vivo in patients with dementia. A controlled study with 123IQNB and single photon emission computed tomography. *Arch Neurol.* 1991;48(2):169-176.
63. Seeger T, Fedorova I, Zheng F, et al. M2 muscarinic acetylcholine receptor knock-out mice show deficits in behavioral flexibility, working memory, and hippocampal plasticity. *J Neurosci.* 2004;24(45):10117-10127.
64. Tzavara ET, Bymaster FP, Felder CC, et al. Dysregulated hippocampal acetylcholine neurotransmission and impaired cognition in M2, M4 and M2/M4 muscarinic receptor knockout mice. *Mol Psychiatry.* 2003;8(7):673-679.
65. Poulin B, Butcher A, McWilliams P, et al. The M3-muscarinic receptor regulates learning and memory in a receptor phosphorylation/arrestin-dependent manner. *Proc Natl Acad Sci U S A.* 2010;107(20):9440-9445.
66. Bodick NC, Offen WW, Levey AI, et al. Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. *A Neurol.* 1997;54(4):465-473.
67. Araya R, Noguchi T, Yuhki M, et al. Loss of M5 muscarinic acetylcholine receptors leads to cerebrovascular and neuronal abnormalities and cognitive deficits in mice. *Neurobiol Dis.* 2006;24(2):334-344.
68. Leaderbrand K, Chen HJ, Corcoran KA, et al. Muscarinic acetylcholine receptors act in synergy to facilitate learning and memory. *Learning Memory (Cold Spring Harbor, NY).* 2016;23(11):631-638.
69. Golding JF, Wesnes KA, Leaker BR. The effects of the selective muscarinic M3 receptor antagonist darifenacin, and of hyoscine (scopolamine), on motion sickness, skin conductance & cognitive function. *Br J Clin Pharmacol.* 2018;84(7):1535-1543.
70. Wackym PA, Chen CT, Ishiyama A, Pettis RM, Lopez IA, Hoffman L. Muscarinic acetylcholine receptor subtype mRNAs in the human and rat vestibular periphery. *Cell Biol Int.* 1996;20(3):187-192.
71. Busetini C, Frölich MA. Effects of mild to moderate sedation on saccadic eye movements. *Behav Brain Res.* 2014;272:286-302.
72. McKernan RM, Rosahl TW, Reynolds DS, et al. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. *Nat Neurosci.* 2000;3(6):587-592.
73. Grilli M, Zappettini S, Raiteri L, Marchi M. Nicotinic and muscarinic cholinergic receptors coexist on GABAergic nerve endings in the mouse striatum and interact in modulating GABA release. *Neuropharmacology.* 2009;56(3):610-614.
74. Toossi H, Del Cid-Pellitero E, Jones BE. Homeostatic changes in GABA and acetylcholine muscarinic receptors on GABAergic neurons in the mesencephalic reticular formation following sleep deprivation. *eNeuro.* 2017;4(6). ENEURO.0269-17.2017
75. Salgado H, Bellay T, Nichols JA, et al. Muscarinic M2 and M1 receptors reduce GABA release by Ca2+ channel modulation through activation of PI3K/Ca2+ -independent and PLC/Ca2+ -dependent PKC. *J Neurophysiol.* 2007;98(2):952-965.

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