Innovative cholinergic compounds for the treatment of cognitive dysfunction

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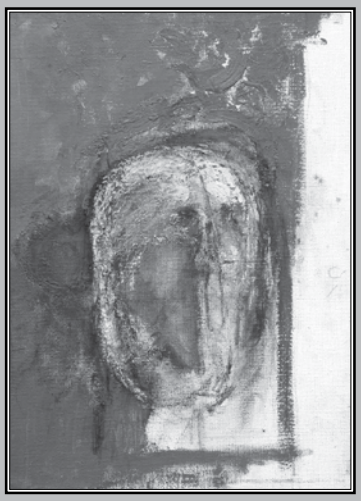
CHAPTER VII



BIPERIDEN CHALLENGE MODEL IN HEALTHY ELDERLY AS PROOF-OF-PHARMACOLOGY TOOL; A RANDOMIZED PLACEBO-CONTROLLED TRIAL

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ABSTRACT

Selective M_1 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 M_1 MAChR antagonist biperiden could be used to demonstrate procognitive and pharmacological effects of selective M_1 MAChR agonists. The aim of this study was to develop such a model. To this end, twelve healthy elderly subjects participated in a randomized, placebo controlled, three-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 mg biperiden showed significant impairment of sustained attention (-2.1% point in adaptive tracking (95% CI [-3.043; -1.148])), verbal memory (2-3 words fewer recalled (95% CI [-5.9; -0.2])), and working memory (up to 50 ms increase in the n-back task reaction time (95% CI [21.854; 77.882])) compared with placebo. The PK data was best fitted by a 2-compartment model and showed a high inter-occasion and inter-subject variability. Population PK-PD analysis quantified significant concentration-effect relationships for the n-back reaction times, n-back accuracies and adaptive tracking. In conclusion, biperiden caused M_1 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.

INTRODUCTION

Acetylcholine is one of the main neurotransmitters 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 currently mainly available (i.e. registered) therapies for the treatment of cognitive dysfunction in patients with mild to moderate Alzheimer's disease are acetylcholinesterase inhibitors (AChEIs) such as donepezil and galantamine. However, these drugs are only effective in a limited number of patients, and are associated with significant (gastro-intestinal) 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 are limited[4-6]. In response to these limitations, selective M_1 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-specifically acting cholinesterase inhibitors. The M_1 muscarinic acetylcholine receptor (MAChR) is an potential target of a selective muscarinic drug as this receptor plays a major role in cognitive function[7].

Several anti-cholinergic 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 anti-cholinergic challenge is that this induces temporary (reversibly) cognitive defects, which involve the same neurobiological mechanisms as are targeted by pro-cholinergic drugs. Scopolamine is a competitive MAChR antagonist that is non-selective 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 M_1 MAChR agonists which 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 M_1 MAChR antagonist (equilibrium dissociation constant (Kd) for M_1 0.48 ± 0.02; for M_2 6.3 ± 0.5; for M_3 3.9 ± 0.1; for M_4 2.4 ± 0.03; for M_5 6.3 ± 0.1)[9]. Administration of biperiden has been shown to lead to impairments in episodic and working memory[10-12], attention[11] and post-error control[13]. Because of the higher M_1 selectivity of biperiden, a biperiden challenge model would be more appropriate to use in early phase clinical studies of M_1 specific MAChR agonists. Several studies have investigated biperiden as a cognitive challenge model in healthy young [13, 12, 14-16], healthy elderly[11], and schizophrenia patients[10]. These studies have, however, significant design-related limitations: only one session of testing was performed post dose, in most cases around the T_{max} of

biperiden (approximately 1 hour post dose); a single dose level was investigated; 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 analysed. 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. Additionally, biperiden has only been studied in few elderly subjects. Since M_1 MAChR agonists are under development for the treatment of Alzheimer's disease, it is useful to already know about the behaviour 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 M_1 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 SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

TRIAL DESIGN AND SUBJECTS This was a randomized, double-blind, placebo-controlled, three-way cross-over study in which biperiden 2 mg, 4 mg and placebo were orally administered to 12 healthy elderly subjects. Akineton® 2 mg tablets (Laboratorio Farmaceutico) and placebo tablets were over-encapsulated in Swedish orange capsules size 00 at Leiden University Medical Centre Pharmacy in accordance with local regulations. The treatment phase consisted of three identical treatment periods separated by a washout period of 1 week between the medication administrations. 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 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 65-80 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 exam (MMSE) 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 (TEAEs), safety laboratory, vital signs and electrocardiogram (ECG).

PK ASSESSMENTS To assess the pharmacokinetic characteristics of biperiden, venous blood samples were obtained pre-dose and at 0.5, 1, 1.5, 2, 2.5, 4, 7, 10 and 22 hours post dose. Plasma concentrations of biperiden were determined by Ardena Bioanalytical Laboratory (Assen, the Netherlands). Extraction of biperiden from human K2EDTA plasma samples was performed using Liquid Liquid Extraction and followed by analysis using a Shimadzu Prominence / Nexera liquid chromatography system, equipped with a Sciex API 4000 tandem mass spectrometer (LC-MS/MS). Biperiden-D_5 was used as internal standard. Separation was established on a XBridge Phenyl LC column (4.6 x 100 mm, 3.5µm) using isocratic elution with 0.025% NH_4OH in 67% acetonitrile at a flow of 1.0 ml/min. The mass spectrometer was equipped with a Turbo Ion Spray (TIS) probe operated in the positive Multiple Reaction Monitoring (MRM) mode. The mass transitions for biperiden was 312 à 143 (m/z) and 317 à 148 for the internal standard. The analytical range of the assay was 0.100–10.0 ng/mL. The accuracy and precision of the assay were monitored during all analysis runs using Quality Control samples (QCs) 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% (level Low), 99.2% (level Medium) and 102.1% (level High). The between-day variability, expressed as CV% was 6.5% (level Low), 3.1% (level Medium) and 2.1% (level High). Non-compartmental analysis was performed in R, version 2.12.0 for Windows (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria, 2010).

PD ASSESSMENTS To assess the effects of biperiden on central nervous system (CNS) functioning, PD tests were performed repeatedly using the NeuroCart, 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 at 1, 2.5, 4, 7 and 22 hours post dose. The duration of one PD testing round was 1 hour. The visual verbal learning test (VVLT) immediate part was only performed 1.5 hour post dose and the delayed recall/recognition condition was performed 40 minutes thereafter. 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 hour after administration and mean half-life of 24-37 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:non-target rate was 1:3. Subjects were required to press a key for both targets and non-targets. 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 'correct responses –incorrect responses/total responses' (accuracy measure) and reaction time[25].

- **Visual verbal learning test**

For the visual verbal learning test (VVLT) 30 words were presented. By recalling immediately, acquisition was assessed, by recalling after 30 minutes recall active retrieval from long term memory was assessed, by recognition memory storage was assessed[27,23].

- **Pupillometry**

To determine the pupil diameter pictures were taken with a digital camera (Canon EOS1100D) and a single flash. The diameter of the pupil and the iris were determined in the number of pixels used horizontally. The 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 min. 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 have been described elsewhere[31,30,23]. The subject was requested to follow a horizontally moving target on a screen at 58 cm distance. The target moved continuously for measurement of smooth pursuit and jumps 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 min in each eye state[32]. Each recording employs alternating periods with eyes open and closed with a duration of 64-seconds for each period. EEG was continuously recorded using a 40-channel recording system (Refa-40, TMSi B.V., the Netherlands). Twenty-one electrodes were placed according to the international 10-20 system (32-lead cap, TMSi B.V.), but replacing electrodes placed at the earlobes (i.e., A1 and A2) with electrodes placed at the mastoids (i.e., M_1 and M_2). The scalp electrode impedance was kept below 5kΩ. The ground electrode was placed at AFz. Additionally, to detect ocular artefacts, vertical and horizontal EOG were also recorded. Two Ag/AgCl electrodes were placed at the outer canthi of both eyes, and two Ag/AgCl electrodes are 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 cut-off frequency at 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 (*i.e.*, fast Fourier transformation): ß-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 (ERP) 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 ms at a sound pressure level of 75 dB. All tones had a 5 ms rise and fall time. Tones were presented at a fixed rate of 2 Hz.

- **Visual analogue scales**

Visual analogue scales (VASS) according to Bond and Lader were used to subjectively assess effects on alertness, mood and calmness [34, 23, 35]. For the VAS nausea subjects were asked to indicate how nauseous they feel on a 100-mm line[36, 37, 35].

- **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 five performances of 10 seconds. The mean tapping rate and the standard deviations are 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 be analyzed with a mixed model analysis of covariance (ANCOVA) 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 (ANOVA) with treatment and period as fixed factors and subject as random factor. In these analysis models, all means are estimated. These are called the least square means (LSMS). Biperiden 2 mg and 4 mg were compared with placebo. Statistical analysis was conducted with SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). 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 concentrations and PD parameters, a population PK-PD model was developed using non-linear mixed effect modelling (NONMEM V7.3)[39].

For the PK model, one- two- and three compartmental models, with and without lag time on the absorption of biperiden and transit compartments, were explored. Inter-individual variability and between-occasion variability was included on the model parameters following a bottom-up inclusion procedure and were included if a significant ($p < 0.01$) 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. In order to capture the concentration-effect relationship, linear, EMAX and sigmoidal EMAX relationships were explored.

Age, sex, body weight, and body mass index (BMI) were tested as potential covariates for parameters on which inter-individual variability (IIV) could be identified. Covariates were stepwise introduced to the base models (PK and PD) and the covariates that were significant at $p < 0.01$ were added to the model, followed by a backward exclusion step ($p < 0.001$).

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 vs. the observations and the conditional weighted residuals with interactions vs. PRED and time.

- **Simulation of statistical power**

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

Each scenario was simulated in 1000 individuals, with 2 baseline measurements and PD measurements at 1, 2, 3, 4, and 5 hours post-dose. Simulated data was analyzed with a 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 cross-over designs, respectively. The mean of both baseline measurements was included as covariate. A significance level of $p < 0.05$ was used for the determination of the statistical power.

RESULTS

SUBJECTS A total of 12 healthy elderly (5 females, 7 males) were enrolled and completed the study. Subjects had a mean age of 71.6 (range 69-78) years, weight of 76.2 (range 56.2-88.7) kg and BMI of 26.2 (range 20.5-31.1) kg/m^2.

PHARMACOKINETICS The PK of biperiden showed high variability between occasions and high inter-subject variability after 2 mg and 4 mg dosing (Figure 1). The median T_{max} of the plasma concentration is at 2 hours post dose (range 1-4 hours). The mean c_{max} is 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 $AUC_{(0\text{-last})}$ 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% point was observed after 2 mg biperiden (95% CI [-2.31; -0.42], p= 0.0075) and of 2.10% point after 4 mg biperiden (95% CI [-3.04; -1.15], p=0.0002) (Figure 2).

- **N-back task**

Visual inspection of n-back the 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 ms, 95% CI [6.40; 68.0], p=0.0212) and 1-back condition (mean difference 49.9 ms, 95% CI [21.9; 77.9], p=0.0016). The accuracy was slightly but significantly decreased with 0.06 (95% CI[-0.12; -0.01], p=0.0209) 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 (95% CI [-4.9; -0.1], p=0.0387) fewer words were recalled; during third immediate recall round 2.9 (95% CI [-5.8; -0.1], p=0.0453) fewer words were recalled; 3.1 (95% CI [-5.9; -0.2], p=0.0344) fewer words were recalled after a delay of 30 minutes and 6.5 (95% CI [-10.8; -2.2], p=0.0053) fewer words were recognized after a delay while the reaction time was 92.2 ms (95% CI [5.1; 179.3], p=0.0390) longer.

- **N-back task**

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 being significantly different from placebo (right eye: mean difference 0.07341, 95% CI [0.02957; 0.11725], p=0.0033; left eye: mean difference 0.065, 95% CI [0.02789; 0.10211], p=0.0028)). Following the maximum mean change, the pupil/iris ratio in both eyes decreased, however it was not normalized at 22 hours post dose (Figure 2).

- **Body sway**

The body sway graphs suggested a dose related increase postural movements. Only after 4 mg biperiden, the body sway increased significantly with 27% (79.7 mm) compared with placebo (95% CI [3.4; 55.9%], p=0.025) (Figure 2).

- **Saccadic and smooth pursuit eye movements**

Smooth pursuit decreased with 3.55% point following 4 mg biperiden compared with placebo (95% CI [-5.58; -1.53], p= 0.0016). No significant effect was observed after 2 mg biperiden. No significant effects were observed on saccadic inaccuracy, peak velocity or reaction time for both dosages compared to 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 and b. In all cortical areas, alpha and theta power was decreased during the eyes closed condition after 4 mg biperiden. Beta power was decreased at 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 central location, and a diffuse increase in delta power.

- **Mismatch Negativity**

The MMN latency at Fz increased significantly with 12.1 ms after 2 mg bideriden (95% CI [3.004–21.282], p=0.0119) and with 13.9 ms after 4 mg biperiden (95% CI [5.071–22.773], p=0.0038) compared to placebo.

- **Visual analogue scales and Tapping test**

No significant changes were observed after both dosages on the tapping test performance or on the VAS Bond&Lader subscales of mood, alertness and calmness or on VAS nausea scores.

POPULATION PK-PD ANALYSIS

- **Population PK-PD model development**

The PK data was best fit by a 2-compartment model with linear elimination. Inclusion of a lag time and transit compartment were 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 is 29.5 hour.

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 on 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 % 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 ms [RSE 24.1%, IIV 37.0%], Hill coefficient=2.25 [18.9%]) was best fit for 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 the corresponding power at multiple sample sizes in a study are shown in Figure 5.

Results show that 15 subjects are required in both a parallel and cross-over study design 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) are required in a cross-over design compared to a parallel design (n=50+) to achieve a power of 80%. However, even though the group size is smaller, subjects have to participate in two study occasions. Therefore, the number of performed occasions will remain comparable between parallel and cross-over study designs. This agreement between the cross-over and parallel study design is due to the high BOV present in the model.

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

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 M_1 MAChR agonists in future studies. Previous studies investigated the effects of biperiden on cognitive functioning mainly in young subjects with only one session of testing post dose, in most cases around the expected T_{max} of biperiden, although no PK was measured. Furthermore only a single dose level was investigated in these studies. We investigated the PK and PD effects of both 2 mg and 4 mg of the competitive M_1 MAChR antagonist biperiden on frequently repeated cognitive and neurophysiological tests in healthy elderly. Biperiden plasma concentrations were measured and the relationship between the PK and PD were modelled in a two-compartment population PK-PD model with linear elimination and corresponding concentration-effect relationships. This population PK-PD model was used to inform on 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 [11,12,14,40,15], n-back test reaction time [40,41,11], and the pupil/iris ratio[42,40]. The consistency with literature demonstrating the repeatability of the PD effects and the low variability of the PD effects are required for a reliable challenge model.

The PK of biperiden was well characterized in this study even though high levels of variability were present. The median T_{max} is comparable with previously reported T_{max}[42,43] suggesting no relevant effect of the over-encapsulation. In the population PK model, the IIV of the central volume of distribution (79.5%) and clearance (172%) were high in comparison with results of previous studies[44,43]. 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 since no intravenous PK data was available.

The variability in these structural model parameters may therefore be over-predicted. The model, including the identified IIV and BOV, 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%'s ranging from 12% to 172%), with only low to moderate CV% present on the studied PD effects (CV%'s up to 76.4%). Therefore, in order 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.

In order 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 at 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 M_1 MAChR agonist. Continuous or repeated administration could raise the possibility of tolerance[45]. In the current cross-over study there were no evidence of tolerance after the wash-out period of 1 week.

Both dose levels 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 than scopolamine in previous studies, also because this non-selective MAChR antagonist shows an age-dependent decline of clearance[46]. Considering the tolerability and the PK-PD-results 4 mg dose is preferable over a 2 mg biperiden dose based on tolerability and PD effects. The quantified concentration-effect relationships suggest that increasing the dose level will result in larger PD effects. However, a higher dose level 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, since the brain areas involved in these

tests comprise a high density of M_1 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), left medial temporal lobe activity[49], and sustained attention measured by the adaptive tracking test is associated with basal forebrain, prefrontal cortex, and parietal cortical regions activity[50]. Thus in these tests the prefrontal cortex or hippocampus play an important role. The M_1 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 M_1 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 M_3 MAChR is the most expressed receptor. The M_1 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 the finger tapping test performance was observed. Therefore not impaired motor function, but reduced sustained attention is a likely explanation of the observed effects. Muscarinic activity plays an important role in sustained focused (visual) attention[54].

The body sway was not normalized at 22 hours post dose. A delayed recovery of the balance could be due to binding to the M_1 MAChRs in the vestibular system, where the clearance might be slower than clearance from the plasma[55]. Just like the disturbed body balance, the pupil enlargement was still present 22 hours after 4 mg biperiden administration. It could be that clearance of biperiden from the peripheral M_1 MAChRs in the iris and ciliary body is slower than 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 to scopolamine effects described in literature, the biperiden effects seem smaller. For example the decrease in adaptive tracking in the current study was 2.1%-point, compared to 9-10%-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 words fewer recalled) [61, 57, 58, 60, 3]. It could be that the dose level of biperiden is relatively lower than the used scopolamine dose levels or due to 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 M_1-M_5 MAChRs, whereas biperiden is a relatively specific M_1 MAChR antagonist. The M_1 MAChR plays a major role in cognitive function[7] and represent

35-60% of the total mAChRs in areas related to cognitive function: the neocortex and the hippocampus [51, 62, 52]. However, the M_1 mAChR is not associated with all hippocampus dependent learning tasks[7] and the remaining 40-65% of the total mAChRs consists of M_2-M_5 mAChRs . These other mAChRs are also involved in learning and memory[63-68], although the role of the M_3 mAChR in cognitive function could not be demonstrated in humans[69]. Body sway was increased into 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 M_1 mAChR, the M_2 and M_5 mAChRs are expressed in the afferent vestibular ganglia and the vestibular end-organs[70]. Consequently, antagonism of M_2 and M_5 mAChRs can contribute to a disturbed balance. Also the M_3 mAChR antagonist darifenacin has been shown to increase body sway[69].

In addition to antagonism of the M_2-M_5 mAChRs in the brain structures involved in cognition, also the sedative effect of scopolamine might have contributed to the impaired performance of PD tests. The saccadic eye movements are a very sensitive marker for sedative effects[29]. Changes in saccadic eye movements are often attributed to suppression of the brainstem reticular formation by stimulation of gamma-aminobutyric acid (GABA) type A receptors with subunit α_1[71,72]. Nonetheless, a concentration-related decrease in peak saccadic velocity was also observed after scopolamine [60, 58, 59, 57], suggesting a role for mAChRs in sedation. An interaction between mAChRs and GABA receptors has been described[73], however, the exact contribution of each type of mAChR to sedative effects has not been well established. In the brainstem, the M_2 mAChR represents 80% of all mAChRs[52] and GABAergic neurons in the reticular formation also contain M_2 mAChRs[74]. In other brain areas, the activation of the M_2 and M_4 mAChRs decreased the release of GABA[73,75]. The latter might suggest that inhibition of the M_2 mAChRs result in an increase of GABA and consequently a sedative effect. As the M_1 mAChR is barely present in the brain stem and the sedative effect of mAChR stimulation seems to be mediated by agonism of the M_2 mAChR, the saccadic peak velocity was not decreased and the score on the VAS measuring alertness did not change after biperiden administration in this study, we feel it is safe to conclude that scopolamine has a larger sedative effect than biperiden.

Due to the effects of M_2-M_5 antagonism by scopolamine on cognitive performance and sedation, it is expected that an M_1 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 M_1 receptor agonists.

CONCLUSIONS

Biperiden doses of 2 mg 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 cognitive enhancing effects of M_1 mAChR agonist.

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TABLE 1 **Population PK model parameter estimates of oral biperiden.**

Parameter	Estimate (CV%)
Lag time (h)	0.54 (BOV = 75%)
Absorption rate constant (/h)	2.73 (BOV = 97.7%)
Volume of distribution - Central (L/F)	491.40 (IIV = 79.5%)
Volume of distribution - Peripheral (L/F)	1537.00
Inter-compartmental clearance (L/h/F)	79.03
Clearance (L/h/F)	78.06 (IIV = 172%, BOV = 12%)
Proportional residual error (σ2)	0.03

BOV=between occasion variability; IIV=inter individual variability; CV% calculated by sqrt(e^ω2-1); Biperiden was modelled as biperiden hydrochloride. Relative bioavailability of 1 was assumed. Covariance IIV Vd-central versus Clearance = 0.74.

FIGURE 1 **Individual biperiden plasma concentrations after 2 mg and 4 mg oral biperiden hydrochloride**

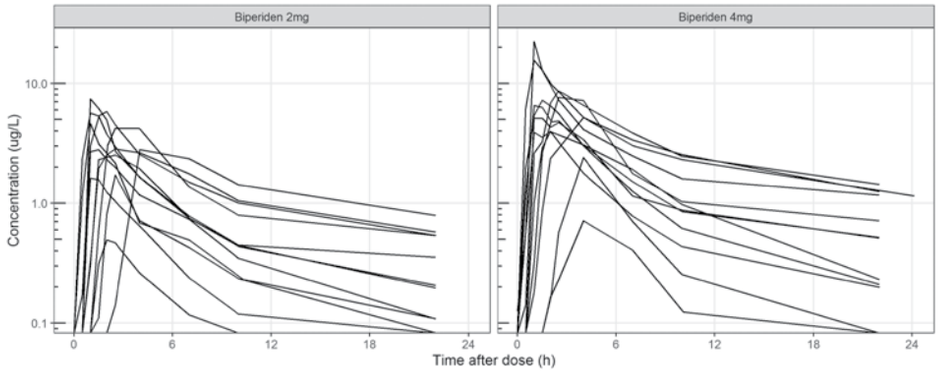

FIGURE 2 **Pharmacodynamic effects on adaptive tracking, n-back test, body sway and pupil size presented as change from baseline (mean, 95% CI error bars)** – *see inside back cover for these images in full color.*

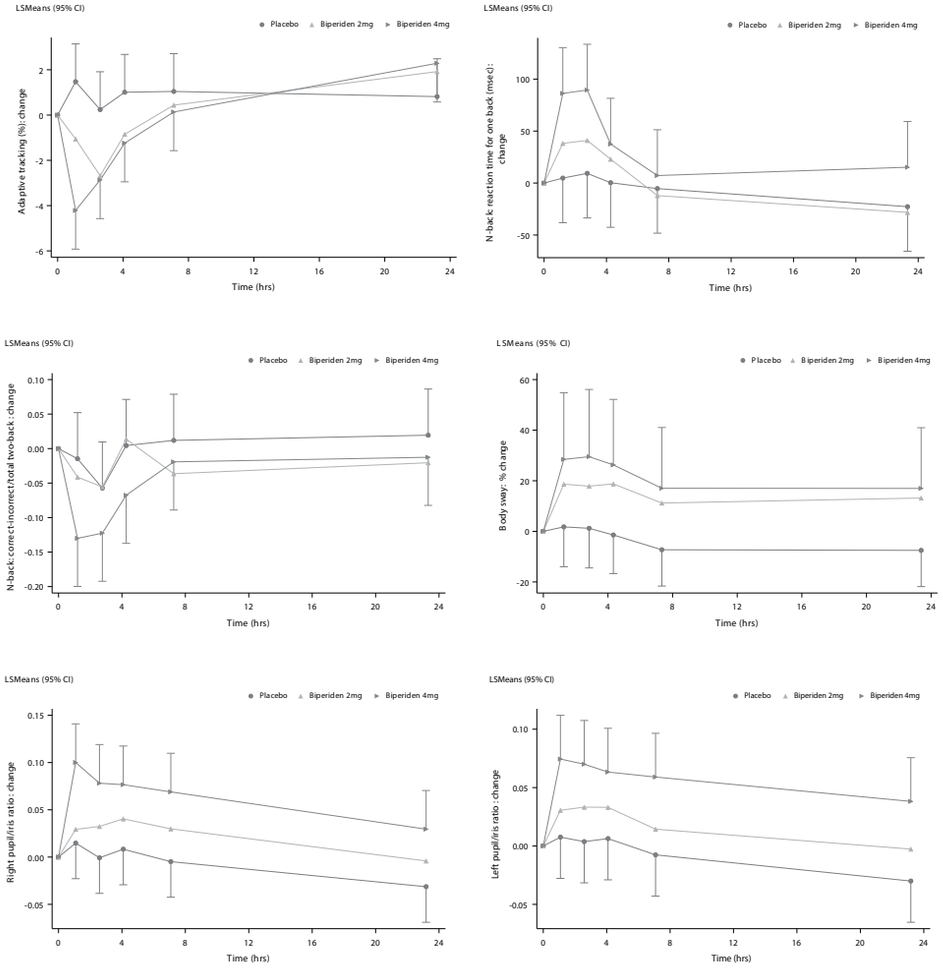

FIGURE 3A Heatplots showing the effects of 4 mg biperiden on EEG eyes closed condition. For each frequency band and each electrode (representing a cortical area) the % of change in power compared with placebo is shown. * = p<0.05; **=p<0.01 – *see inside back cover for these images in full color.*

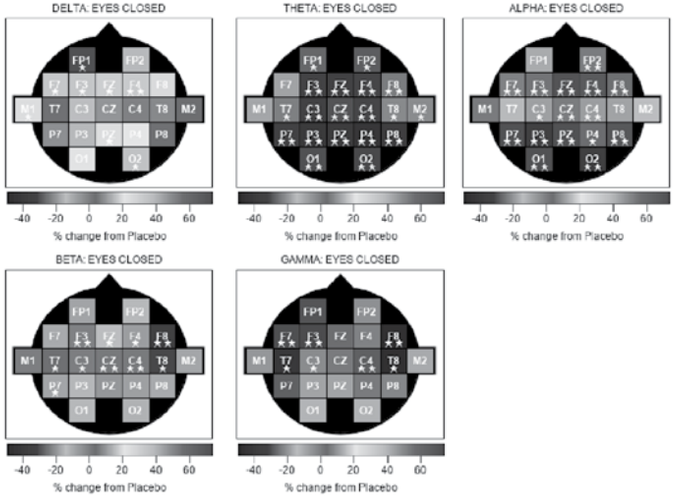

FIGURE 3B Heatplots showing the effects of 4 mg biperiden on EEG eyes open condition. For each frequency band and each electrode (representing a cortical area) the% of change in power compared with placebo is shown. * = p<0.05; **=p<0.01 – *see inside back cover for these images in full color.*

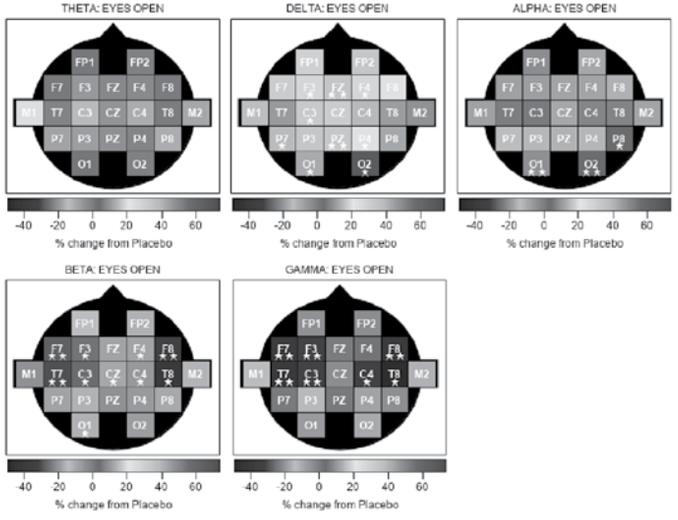

FIGURE 4 Visualization of the typical concentration-effect relationships for the n-back (A) and the adaptive tracking task (B).

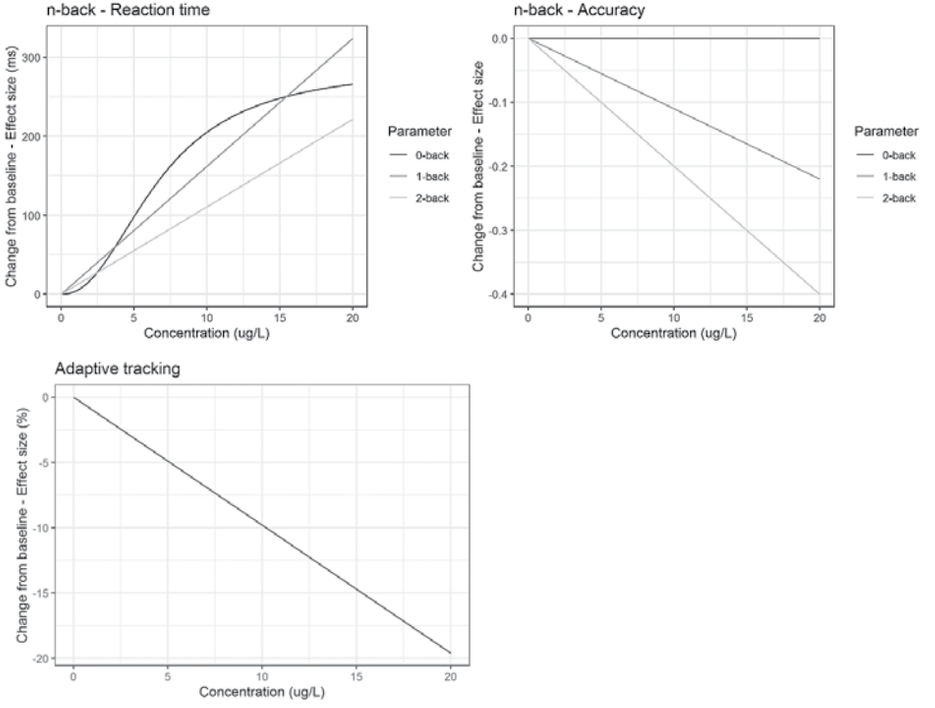

FIGURE 5A Simulated (n=1000) PK profiles after 4 mg oral administration of biperiden hydrochloride. Solid black line = median prediction, grey ribbon = 90% prediction interval.

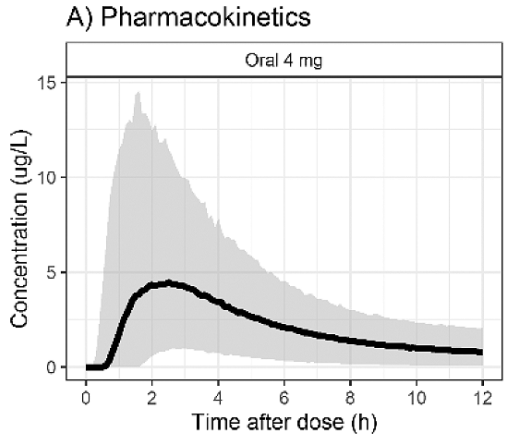

FIGURE 5B Model-derived statistical power versus total sample size to detect a 25%, 50%, or 100% reduction of the estimated concentration-effect relationship on the adaptive tracking task in a cross-over and parallel study design.