Innovation in cholinergic enhancement for Alzheimer's Disease

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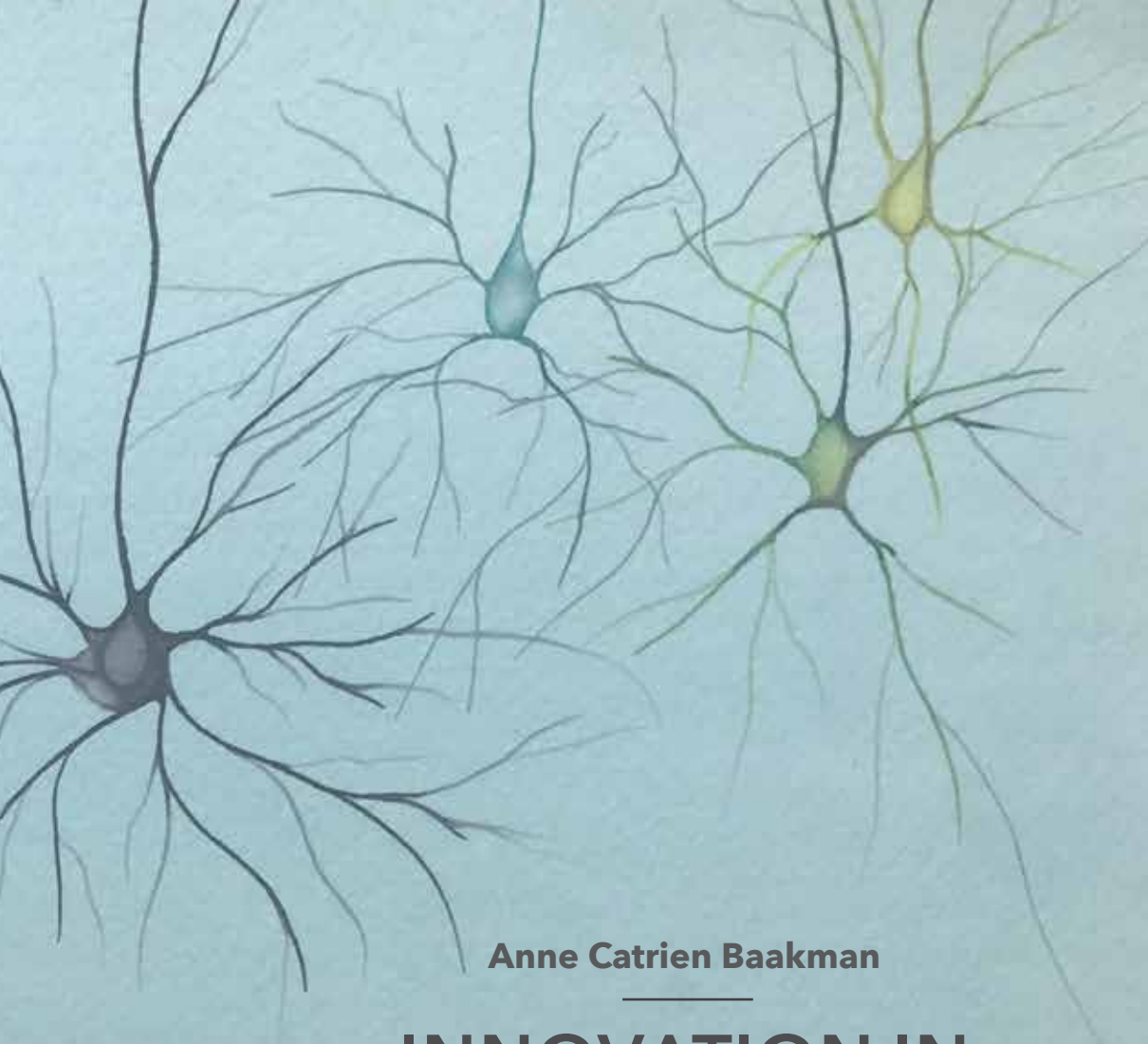

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INNOVATION IN CHOLINERGIC ENHANCEMENT FOR ALZHEIMER'S DISEASE

Innovation in cholinergic enhancement in Alzheimer's Disease

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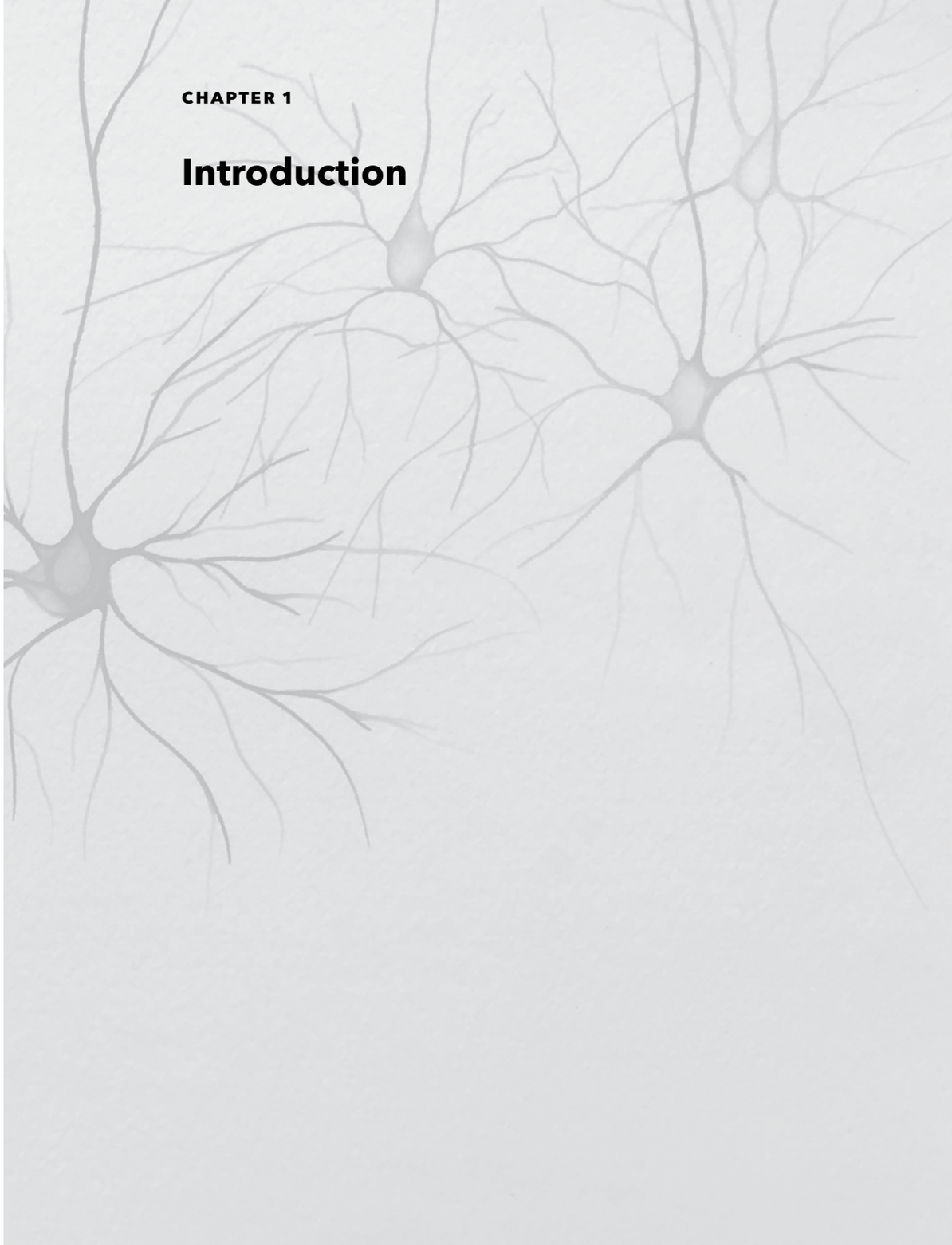
CHAPTER 1

Introduction



HISTORICAL CONTEXT

In 1906, the German psychiatrist Alois Alzheimer studied the case of Auguste D, a woman suffering from cognitive impairment in her early fifties. He described her symptoms in detail, including aphasia, apraxia, agnosia, disorientation, paranoia and memory deficits. After her death, he examined her brain and described the now well-known triad of neurofibrillary tangles, amyloid plaques and atrophy.[1,2] The combination of symptoms and pathological findings was later named Alzheimer's Disease (AD) by Emil Kraepelin, one of his colleagues. Although this was a new disease, it took over half a century before any progress was made in diagnosis and research. In the sixties and seventies, as a result of the success of the levodopa treatment for Parkinson's disease, it was discovered that certain neurotransmitter deficits were the central feature of a degenerative neurological disease and it became commonly assumed that a clearly defined neurochemical abnormality could also be identified in AD, which would provide the basis for the development of therapeutic interventions.[3] Post-mortem studies in the early seventies with brains of AD patients confirmed a substantial presynaptic cholinergic deficit, reduced choline uptake, reduced acetylcholine (ACh) release and loss of cholinergic neurons from the nucleus basalis of Meynert.[3] Additionally, other studies found a reduced choline acetyltransferase activity especially in those areas containing high density of neurofibrillary tangles, confirming a selective neurodegenerative process.[4] Clinical research in animals and humans confirmed that administration of anticholinergics induced memory loss and impairment in attention that were in some aspects comparable to the deficits occuring in aging.[5–8]

The positive effect of cholinesterase inhibitors like physostigmine on cognitive functioning supported this hypothesis.[7,9] All those discoveries resulted in the 'cholinergic deficit hypothesis' that at least some of the cognitive and behavioural symptoms of AD are explained by the lack of ACh, which was the dominant theory in AD in the early eighties.

Acetylcholine

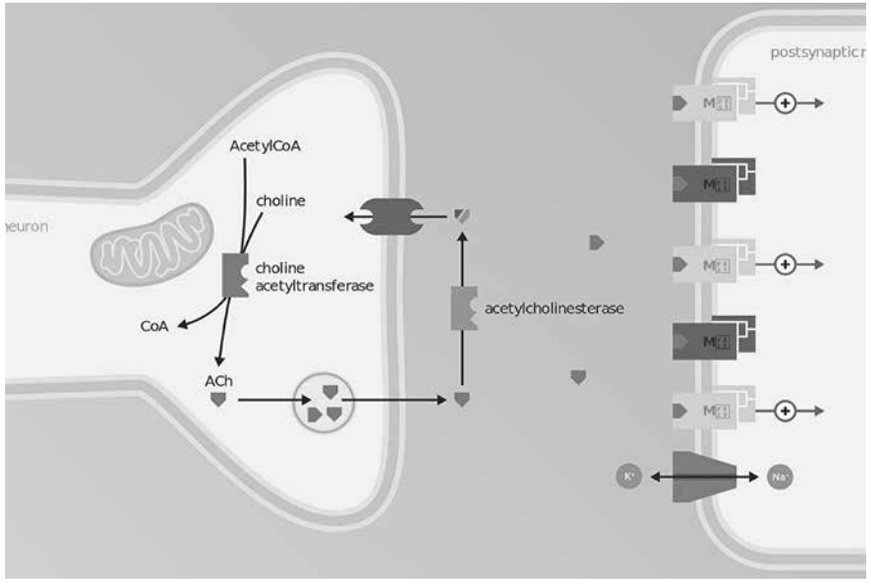
Acetylcholine is synthesized from choline and acetyl co-enzyme A by choline acetyltransferase. After depolarization of the presynaptic neuron, it is released in the synaptic cleft and binds to the postsynaptic receptors. To limit its action, acetylcholine is degraded by acetylcholinesterase in the synaptic cleft. The cholinergic system consists of the neurons using acetylcholine as neurotransmitter for transsynaptic communication. The highest density of these neurons is found in the basal forebrain (including the nucleus basalis of Meynert and the substantia innominata) and the brain stem, with a widespread projection to the cerebral cortex.[10] Cholinergic neurons can also be found in the peripheral nervous system and are crucial for neuromuscular signal transduction. The autonomic nervous system also contains many cholinergic neurons, influencing for example heart rate, blood pressure and bowel movements. Acetylcholine exerts its action by binding to the postsynaptic receptors, divided in two subtypes. The muscarinic AChRs in the central nervous system (CNS), especially the M1 subtype, are mainly located in the cerebral cortex, hippocampus, striatum, nucleus accumbens, dentate gyrus and brainstem and associated with memory, arousal and sleep.[11–15] Nicotinic AChRs are mostly found in the cerebral cortex, thalamus, hippocampus, dentate gyrus and striatum and associated with memory.[16,17]

There are several possible pharmacological approaches for stimulating the cholinergic system to accomplish a potential procognitive effect. To increase the level of ACh in the synaptic cleft, the break-down can be diminished by cholinesterase inhibitors. Another option is to stimulate the postsynaptic neuron with a direct AChR agonist. With these principals in mind, several compounds were developed to influence the cholinergic system.

In the second half of the eighties, several M1 AChR agonists were evaluated because of the high density of M1 AChRs in the hippocampus. In general, they had positive cognitive effects, but the cholinergic side effects overshadowed the benefits. This might have been due to a lack of selectivity leading to dominant peripheral cholinergic stimulation.[18] This strategy was therefore soon abandoned. The other approach, inhibiting ACh breakdown appeared more hopeful and several cholinesterase inhibitors were developed.[19–23] The cholinesterase inhibitor (CEI) tacrine was the first to acquire registration in 1995. The registration was supported by mitigating effects of tacrine on the scopolamine model of cognitive impairment in healthy volunteers.[24] However, this did not lead to widespread acceptance of the scopolamine model, or more generally of CNS testing, in healthy volunteers during the development of drugs for dementia. This may have been due to the disappointing effects of tacrine, which did not fulfill its huge expectations as a first-in-class anti-dementia drug. Soon after launch, tacrine was withdrawn because of limited clinical effects in combination with hepatotoxicity and gastrointestinal side effects (over 70% on the highest dose).[22,23] Consequently, other CEIs were developed, aiming to at least equal the clinical efficacy of tacrine, while inducing fewer side effects – but without using neuropharmacodynamic testing in healthy subjects. This led to donepezil and a few years later rivastigmine and galantamine entering the market. The pre-registration studies of these drugs were quite promising, indicating a positive effect on cognition in several

patient studies.[25,26] These drugs have also shown effects on cognitive tests in healthy (young and/or elderly) subjects, but this was only demonstrated late during development or after launch.[27] This thesis explores the possibility to use CNS pharmacodynamics much earlier during development of drugs in dementia, to provide proof of pharmacological activity and support dose selection. To understand the relevance of this approach, it is first important to summarize the more traditional development trajectories of these drugs.

FIGURE 1 Cholinergic synaps.



Rivastigmine

After careful evaluation in preclinical studies, further study of the new compound ENA 713 (rivastigmine) was carried forward to human subjects.[28] This started with a first in human study, investigating pharmacokinetics (PK), safety and tolerability of single doses up to 3 mg in 80 young healthy male subjects. These doses were well tolerated, but no information was obtained about cognitive function.[29] PK and safety studies were also performed in healthy elderly volunteers, although these data were never published.[28]

As a next step, safety, tolerability and efficacy were tested in a 13 week trial in 402 AD patients with doses of 2 and 3 mg of rivastigmine twice daily compared to placebo.[30] In the highest dose group, a beneficial effect on the Clinical Global Impression of Change (CGIC – a now rarely used impression of the change in overall clinical state of the patients) at week 13 was found for 43%, compared to 30% in the placebo group (p=0.05) and for DSS (Dementia Signs and Symptoms) at 7 and 13 weeks (p=0.005 and p=0.05 respectively). Adverse events (AEs) were generally mild, short, predominantly gastrointestinal and did not result in dose reduction or discontinuation. The low incidence of AEs and good tolerability suggested that higher dose of rivastigmine could be given. Since tolerability differences between patients and healthy volunteers had been previously reported after administration of CEIs, the possibility was considered that AD patients could tolerate doses of rivastigmine higher than 6 mg/day. This hypothesis was supported by a study assessing plasma PK and AChE inhibition in cerebrospinal fluid, indicating a much higher AChE inhibition centrally compared to the periphery.[31]

Consequently, a study was designed to investigate the maximum tolerated dose (MTD) in AD patients in order to test if higher doses could demonstrate greater efficacy in subsequent clinical trials. Fifty AD patients were randomized to receive 12 mg/day in two or three doses per day or placebo, for a 9-week dose escalation period followed by 1-week washout. In this study, the highest dose permitted was 12 mg/day, based on 50% of the no-toxic-effect level determined in animals. A MTD could not be established. Doses up to 12 mg/day were well tolerated, with the majority of patients experiencing only mild to moderate AEs. Surprisingly, but perhaps exemplary for the relatively uninformed protocols of the time measures of pharmacodynamics were not measured in this study.[32]

Next, a double-blind, randomized study in 114 AD patients was conducted to again assess adverse events and tolerability, this time combined with assessment of efficacy.[33] This study aimed to determine the MTD as well, while evaluating the tolerability of the same dose in a BID compared with a TID, but also to assess the efficacy of the individual MTD compared with placebo. In order to do that, patients received increasing doses of either rivastigmine BID, TID or placebo until they reached their MTD over the 10-week titration period, followed by an 8-week maintenance phase. The MTD was approximately 10 mg/day (both BID and TID); mild gastrointestinal complaints were the most frequent AEs. With respect to efficacy, a clear improvement in global function (CIBIC+), in cognition (ADAS-cog), and in activities of daily living (ADL) was seen. Nonetheless, the main goal of this study was to optimize dosage rather than testing efficacy. As a consequence, it has been criticized for that the sample size was too small and the duration of the trial too short.

These studies were followed by several double-blind, placebo-controlled clinical trials in AD patients, testing doses of 1-12 mg/day.[30,34–36] These studies showed a statistically significant improvements in the 6-12 mg/day group

compared with placebo on ADAS-cog, MMSE scales, CIBIC+ and ADL and led to the market approval of rivastigmine.

By providing more continuous delivery of drug into the bloodstream, it was expected that peak-dose fluctuations in drug plasma concentration would be reduced and, therefore, tolerability would be improved. After unsuccessfully developing an extended-release rivastigmine formulation Novartis started working on the development of a rivastigmine transdermal patch. The first study compared the efficacy, safety and tolerability of rivastigmine patches with capsules during 6 months in 1195 AD patients.[37,38] Patients were randomized to placebo, 10 cm² rivastigmine patch (delivering 9.5 mg/24 hours), 20 cm² rivastigmine patch (17.4 mg/24 hours), or 6 mg BID rivastigmine capsules (12 mg/day). The study established the 5 cm² patch as the recommended initial dose and the 10 cm² patch as the maintenance dose, becoming the first transdermal treatment for patients with mild-to-moderate AD in July 2007. As the study showed that 10 cm² of rivastigmine was safer but 20 cm² was more efficacious, Novartis planned to analyze the potential of a 15 cm² rivastigmine patch (delivering 13.3 mg/24 h) with the OPTIMA (OPtimizing Transdermal Exelon In Mild-to-moderate Alzheimer's Disease) study. OPTIMA demonstrated higher efficacy of the 15 cm² rivastigmine patch on functional outcomes compared to 10 cm², without compromising safety and tolerability.[39] Subsequently in 2012 the 15 cm² rivastigmine patch received approval by the FDA for the treatment of severe AD after the positive results of the ACTION (activities of daily living and cognition) study became public.[40] In this study, the 15 cm² patch demonstrated superior efficacy on ADL and cognition when compared to a 5 cm² patch for 24 weeks in patients with severe AD. The high-dose patch was generally well tolerated, with no unexpected safety concerns.[41] An open-label extension of the ACTION study showed greater decline in severe AD patients with delayed up-titration to high-dose 15 cm² patch compared to patients who received it since the beginning of the trial. In addition, no clinically relevant differences in safety and tolerability were observed.[42]

Donepezil

In 1983 Eisai Japan started with tacrine derivatives until they discovered a highly selective and reversible AChE inhibitor donepezil hydrochloride (E2020, Aricept®).[43,44] Donepezil was entered in clinical trial investigation in 1989. Phase I trials designed by Eisai used the erythrocyte membrane acetylcholinesterase (rbc-AChE) inhibition as measurement of pharmacodynamic (PD) activity. This was an improvement over rivastigmine, as an early evaluation of pharmacodynamics was done in Phase I, rather than just side effect incidence. A direct correlation was observed between plasma donepezil concentrations and rbc-AChE inhibition, with the obvious caveat that this may not be a direct measure of central inhibition. Later, phase II trials testing donepezil started to use several other clinical outcomes after a minimum of 8 weeks as a measurement of PD activity. Specifically, an open phase II study found that 9% of the patients taking 1 mg and 21% of those taking 2 mg improved their functioning, and 56% in the 1 mg group and 57% in the 2 mg group were slightly improved. Another phase II open trial found that in 22% of the cases the administration of donepezil was rated as more than useful, in 67% it was slightly useful, and there was no case were it was not useful at all.[45]

Phase III studies showed improvements in ADAS-cog, Clinician Interview-Based Impression of Change Plus (CIBIC+), and MMSE.[46]

Based on those pivotal phase III trials, in 1997 donepezil received marketing approval by the Food and Drug Administration (FDA) and was commercialized under the name of Aricept® by Eisai and Pfizer. Currently, donepezil (10 mg/day) is the most prescribed cholinesterase inhibitor worldwide.

After the FDA approval, a considerable number of studies have continued with this drug. Greenberg and Homma confirmed an improvement in cognition after 24 weeks of taking 5 mg/day of donepezil, and Krishnan and Burns confirmed this in patients taking 10 mg/day of donepezil for 24 weeks.[47–50]

Galantamine

Throughout the Caucasus Mountains, extracts from the snowdrop (*Galanthus spp*) have been used for hundreds of years to treat painful neurological conditions and poliomyelitis.[51] Once its synthetic production was established, galantamine was registered under the trade name Nivalin® and became commercially available in Bulgaria in the '50s for the treatment of post-poliomyelitis paralysis, myasthenia gravis and to reverse neuromuscular blockade in anesthesia.[52,53]

In the 1980s, researchers studying AD in Western Europe turned their attention to galantamine, because this does not only have beneficial effects on neuromuscular cholinergic function, but is also able to penetrate the blood–brain barrier.[51] For instance, galantamine injections reduced scopolamine-induced learning and memory deficits in rats, inhibited scopolamine-induced passive avoidance in rats, and improved spatial memory in mice with lesions to the nucleus basalis of Meynert.[54–56]

The 90s witnessed the clinical development of galantamine into a medication for AD. During this same period, the dual mechanism of action of galantamine was discovered, that is, galantamine inhibits AChE and at the same time potentiates nicotinic neurotransmission by allosteric modulation on nAChR.[57]

In vitro studies showed 50-fold selectivity for AChE, as opposed to cholinesterase confined to the peripheral circulation. In vivo administration of galantamine in a healthy volunteer and in a patient who underwent long-term treatment confirmed the high selectivity of galantamine for AChE.[58]

In 1989, the first modern phase I clinical study of galantamine was performed in 8 healthy volunteers.[59] The obtained pharmacokinetics suggest that the sc and oral Nivalin® formulations are bioequivalent. Thus, there seems to be no reason to prefer the sc instead of the oral route of administration. Additionally, the typical scheme of application at that moment used to begin with 2.5 mg/day, and it was eventually increased to 25-30 mg/day. However, an initial daily dose of 2.5 mg was too small to quickly reach biologically relevant steady state levels. Since this study showed that a single dose of 10 mg galantamine did not increase side effects, 10-15 mg as a loading daily dose at the very beginning of the galantamine treatment seemed preferable.

Three open pilot studies were published between 1989 and 1993.[60] The first study involved 10 AD patients receiving 30 mg/day of galantamine for 8 weeks. The results for CGIC, two psychometric tests and tolerability were promising, but in view of the low numbers understandably, statistically not significant for MMSE. In the second open pilot study, 18 AD patients received 30 mg/day galantamine. Although no statistically significant differences in neuropsychological tests were seen after 8 weeks, 6 patients with a favourable drug response continued for 13 to 16 months. This means that 30-60 % of these patients benefited from a sustained response to the drug. In the third trial, 19 AD patients received a low galantamine dose (30-40 mg/day) for 6 weeks and a high dose (45-60 mg/day) for 6 additional weeks, with a 3-week washout period between the two treatment phases. ADAS-cog and MMSE scores improved in the low dose regiment. Nevertheless, in this study the dropout rate was 37% due to adverse drug reactions. According to the Cochrane Review, there were some more early-phase clinical trials, although these are not publicly available.[61]

In 1993, the first galantamine study to employ a double-blind design was published.[61] The design of the study was the following: 3 weeks of starting washout period, followed by 3 weeks of single-blind 10 mg/day, then the dose was titrated up to 50 mg/day, and finally there were 10 weeks of double-blind maintenance phase. The 141 patients who completed the 3-week single-blind phase showed a dose-related improvement in ADAS-cog. Responders who had been randomized to continue in the treated group significantly improved in ADAS-cog, MMSE, SBT and CGIC, while placebo deteriorated. However, this study has been criticized for its too selective inclusion criteria, which makes the outcome reflect the potential of the drug, rather than its clinical efficacy.

In 1996, a placebo-controlled phase II trial studied the efficacy and safety of three doses of galantamine: 22.5, 30 and 40 mg/day. Patients treated with the 30 mg/day dose showed statistically significant improvements in the ADAS-cog scale.[62]

Phase III clinical trials studied the efficacy and safety of galantamine compared to placebo in patients with mild to moderate AD, providing evidence of efficacy and tolerability of the drug.[63–67] All those trials were published in the year the drug was launched or soon thereafter. Two different studies showed that after 6 months the cognitive function and clinical outcome of patients treated with both 24 and 32 mg/day of galantamine is maintained above baseline [63,65] with the same results after 3 months of treatment in another study.[66,67] Moreover, in one trial benefits were also seen in ADL and in a preserved functional performance.[67] In a 5-month trial was shown that patients receiving 16 and 24 mg/day of galantamine experienced a significant benefit in ADAS-cog and CIBIC+. Doses of 8 mg/day did not provide significant results. Since the 32 mg/day group did not generally provide significantly superior scores on cognition over the 24 mg/day dose but it did increase the AEs, daily 24 mg of galantamine was considered to be the optimal therapeutic dose.[64] All those trials were carried out for a maximum of 1 year, and longer powerful trials testing the long-term effect of galantamine in AD-treatment are still lacking.

CONCLUSION

The development of the cholinesterase inhibitors for Alzheimer's disease are a long and winding road of trials that are generally geared towards an effect on questionnaire-based outcome measurements. Whilst this is currently the only biomarker for clinical improvement in the disease, it is also relatively limited and difficult to assess. The human pharmacology of these medicines is easier to evaluate but does of course not necessarily relate to the clinical outcome. This is the dilemma for any future development of drugs for AD. If the early phase human pharmacology is ignored, the development will be done with relatively unsupported dose levels and a non-differentiating outcome measurement. The upper side of the dose range is then determined by an increase of subjective side effects. This has led to dosages that are registered but the approach is long and expensive and is difficult to use for new compounds. Alternatively more use can be made of early human pharmacology which is the approach that is practiced in the experiments in this thesis.

After the demise of tacrine, the development of all three currently registered CEIs started with traditional single and multiple ascending dose safety and

tolerability studies, without any cognitive outcome measures to assess efficacy in an early stage. So, the knowledge derived from these studies was about pharmacokinetics and side effects. Only during the development of donepezil, the effect on red blood cells was investigated as measure for pharmacological effects. Cognitive effects were first measured in phase III studies. In these large trials in AD patients, multiple doses compared to placebo were administered and efficacy was assessed after weeks to months, using clinical outcome measures, such as ADAS-cog, MMSE and clinician's global impression of change (CGIC). In some studies in patients with AD being treated with galantamine or donepezil, a computerized test battery was used at several time points between 1 and 52 weeks of treatment.[68,69] Thus, both the effective dose and efficacy on clinically relevant outcome measures were only studied in a later phase of clinical development, in heterogeneous patient groups over a relatively long treatment period. A different approach to development of neuropharmacological compounds is to combine the assessment of safety and tolerability, efficacy and dose finding in early phase I and II studies in healthy volunteers or small, well selected groups of patients. When sensitive, frequently conducted pharmacodynamic tests are used, in combination with pharmacokinetic measurements, proof of concept and an impression of the effective dose range can be acquired, even in small groups of healthy volunteers. This approach goes from the assumption that such early phase effects after a single dose may better reflect the pharmacological -and ultimately clinical effects than just dosing to a level where side effects become prohibitive. The value of such an approach has been shown on numerous occasions by us and others for an orexine receptor antagonist, a subunit selectieve GABA A agonist, neublastin in patients with sciatica and a compound reducing growth hormone release.[70-73]

Preview of this thesis

The central question of this thesis is if the integration of pharmacokinetics, CNS pharmacodynamics and clinical assessments in early phase drug development is feasible for drugs for Alzheimer's disease. AD affects global cognitive functioning and early studies – often in optimally functioning subjects may not be able to show improvement. The room for improvement is greater in patients (with a larger decline) but the potential for improvement may be less. Additionally, practical and ethical objections exist when groups of affected patients are included in early studies with inherent large burden for the cognitively impaired subject.

An obvious intermediate approach would be to induce a temporary state of cognitive dysfunction in subjects, preferably affecting the cholinergic system. For this purpose, the anti-muscarinic drug scopolamine is often used. However, scopolamine administration also induces a considerable level of sedation, which may obscure potential improvements induced by co-administration of a new procognitive compound. This thesis describes several examples of the above mentioned study designs and suggestions for further improvement.

Chapter 2 describes a study of the effects of several doses of the α7 nAChR partial agonist EVP-6124, alone and in combination with two doses of donepezil in healthy elderly subjects, receiving a scopolamine challenge prior to administration of EVP-6124 and/or donepezil.

Chapter 3 is an extensive exploration of another anticholinergic challenge model with the nAChR specific antagonist mecamylamine. Although this challenge model has been used before, its PD and PK characteristics were not well described and a detailed comparison with scopolamine was never done.

Chapter 4 delineates a PKPD model of mecamylamine and explains its use in study design.

Chapter 5 is a first in human trial with Gln-1062, a prodrug of the CEI galantamine, started on the standard starting dose of 10% of the level of no adverse effects (NOAEL) in animal studies. Since no PD effects were expected at the two lowest doses to be administered, these were given to healthy young male volunteers. In accordance with the pharmacodynamic approach that is central to this thesis, PK, PD and safety were measured.

Chapter 6 is a study in which we attempt to see if an early pharmacodynamic test battery after a single dose of galantamine predicts the response after 6 months. Such a study design could serve as an example to connect early phase pharmacodynamics to longer term clinical effects.

Chapter 7, a different approach to improve cognition was chosen. In this study, the histamine 3 receptor (H_3R) inverse agonist CEP-26401 was investigated. As histamine has an indirect effect on several neurotransmitter systems, including the cholinergic system, this might also be a target for procognitive medication. Based on previous studies with this compound, low doses were administered and its effects were compared to placebo, donepezil and modafinil.

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CHAPTER 2

No synergistic effect of sub-therapeutic doses of donepezil and EVP-6124 in healthy elderly subjects in a scopolamine challenge model

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ABSTRACT

INTRODUCTION Donepezil is a widely used cholinesterase inhibitor in the management of Alzheimer's disease. Despite large-scaled evidence for its efficacy, elevated peripheral ACh levels often lead to side effects and are dose limiting. The present study is designed to test whether administering EVP-6124, an α-7 nicotinic agonist, either alone or in combination with donepezil can reduce scopolamine-induced cognitive deficits in healthy elderly subjects. Secondary objectives are to explore safety and pharmacokinetic and pharmacodynamics effects of EVP-6124 alone and in combination with donepezil compared to placebo.

METHODS A phase I randomised, single-centre, placebo-controlled, double-blind, 5 way, partial cross-over study was performed with donepezil 2.5, 5 mg or placebo combined with EVP-6124 0.3, 1, 2, 4 mg or placebo in 3 cohorts of healthy elderly subjects in a scopolamine (0.3 mg i.v.) challenge test. Safety, pharmacokinetic and pharmacodynamics outcomes were assessed.

RESULTS A total of 36 subjects completed the study. Effective dose combinations were donepezil/EVP-6124 (5/2 mg) and donepezil/EVP-6124 (5/0.3 mg) and showed significant improvements of the delayed recall of the VVLT (1.2; CI=0.1,2.3) and reaction time during the 2-back condition of the N-back (-42; CI=-77,-8) respectively. Overall, no marked reversal of scopolamine effects was observed. Donepezil pharmacokinetic parameters were similar with and without EVP-6124.

DISCUSSION This study shows no synergistic effect of sub-therapeutic doses of donepezil and EVP-6124 in a scopolamine challenge model in healthy elderly subjects. Dosing of scopolamine and the combination of donepezil and EVP-6124 requires further study.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia. As the world population ages, prevalence and economic costs are estimated to increase at a rapid pace. Disease prevalence will increase to approximately 75 million AD patients in 2030 and costs will approach ~1.1% of the gross domestic product.[12] Acetylcholinesterase inhibitors (AChEIs) are the most widely prescribed class of drugs for the symptomatic treatment of mild-to-moderate AD. Clinical trials demonstrate that AChEIs donepezil, galantamine or rivastigmine at recommended dosage show significant improvements in cognitive and functional capacities and deceleration of the AD pathogenesis in people with mild, moderate or severe AD.[3-5] However, despite the widely use of AChEIs and the large-scaled evidence for its efficacy, elevated peripheral ACh levels often lead to peripheral side effects such as vomiting and/or nausea.[3] These elevated ACh levels are dose limiting while central AChE inhibition is suboptimal.

The nicotinic acetylcholine receptor agonist (nAChR) EVP-6124 might be a candidate for the treatment of AD in combination with AChEIs, as it potentiates the effect of acetylcholine by occupying one of the two available ACh binding sites on the α7 nAChR.[6,7] Occupation of only one binding site will prevent desensitization, but at the same time, lower acetylcholine levels will be able to activate the receptor. Co-administration with an AChEI would therefore require lower doses to achieve the same effect in AD patients, thereby reducing the severity and number of peripheral ACh side effects due to AChEI. In addition to expansion of the therapeutic window of AChEIs, this 'potentiation' of the nACh receptor may also lead to a more effective improvement of cognitive functions, and postsynaptic receptor activation may have a positive pro-cognitive effect even if (presynaptic) cholinergic neurons are mostly degenerated. In a pre-clinical animal model, Prickaerts and colleagues indicated a potential synergistic effect of donepezil and EVP-6124, as co-administration of sub-therapeutic dosages of donepezil and EVP-6124 showed similar effects as either donepezil or EVP-6124 at higher dosages.[8] Data from phase I and II trials involving EVP-6124 confirmed these findings in subjects with mild-to-moderate AD and showed that the treatment with donepezil and EVP-6124 was well-tolerated [9,10], which prompted the further investigation of EVP-6124 in phase III trials. Two phase III trials aiming to assess the efficacy and tolerability of EVP-6124 in patients with mild-to-moderate Alzheimer's disease were initiated but halted in 2015 due to gastrointestinal adverse events.[11-13] Since then, evidence on the suggested synergistic effects of donepezil and EVP-6124 have not been pursued.

This study was designed to determine whether the strong potentiation of the effects of donepezil by co-treatment with EVP-6124 that was observed in rats, can also be observed in healthy elderly volunteers during cognition deficits induced by scopolamine administration. Since it is difficult to demonstrate improvement of cholinergic neuronal functioning in healthy volunteers, scopolamine hydrobromide, a muscarinic acetylcholine receptor antagonist, was administered in order to induce a temporary cholinergic deficiency leading to impairment of some cognitive functions.[14] Secondary objectives of this study were to explore pharmacokinetic and pharmacodynamics effects and safety of EVP-6124 alone and in combination with donepezil compared to placebo.

METHODS

Trial design and subjects

A randomised, single centre, placebo controlled, double blind, five-way partial cross-over study was performed with four dose levels of EVP-6124 or placebo and two dose levels of donepezil or placebo in a scopolamine challenge cognitive impairment model. Subjects were non-smoking, healthy, elderly (65+) subjects. Main exclusion criteria were a Mini Mental State Examination score lower than 27, impaired renal or liver function, prolonged QTc and use of interfering concomitant medication. Subjects were randomised to one of three cohorts. Subjects in cohort 1 received either double placebo or donepezil placebo in combination with EVP-6124 (0.3, 1, 2 or 4 mg). Subjects in cohort 2 received either double placebo or donepezil 2.5 mg in combination with EVP-6124 (placebo, 0.3, 1 or 2 mg). Subjects in cohort 3 received either double placebo or donepezil 5 mg in combination with EVP-6124 (placebo, 0.3 mg, 1 mg and 2 mg). Treatments were orally administered in a randomised order. Each treatment period was separated by a 14-day washout period. The study cohorts and treatment periods are summarised in Table 1. All subjects received scopolamine 0.3 mg intravenously on each occasion. In order to reach the expected T_{max} of all treatments at approximately the same time point, scopolamine was administered 6 hours after administration of EVP-6124 and 4 hours after administration of donepezil. All subjects gave written informed consent for participation in the study. The study was approved by the ethics committee of the Leiden University Medical Center, the Netherlands. The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. The trial was registered in the European Union Clinical Trials Register (2011-006016-31).

TABLE 1 **Overview of study cohorts and treatment periods.**

	Cohort 1 (n=12)		Cohort 2 (n=12)		Cohort 3 (n=12)	
Treatment period†	DPZ	EVP-6124	DPZ	EVP-6124	DPZ	EVP-6124
1	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
2	Placebo	0.3 mg	2.5 mg	Placebo	5 mg	Placebo
3	Placebo	1 mg	2.5 mg	0.3 mg	5 mg	0.3 mg
4	Placebo	2 mg	2.5 mg	1 mg	5 mg	1 mg
5	Placebo	4 mg	2.5 mg	2 mg	5 mg	2 mg

† The order of the treatment periods was randomised for each subject; Each treatment period was separated by a 14-day washout period; All subjects received scopolamine 0.3 mg i.v.; DPZ=donepezil.

Dosing rationale

DONEPEZIL In previous studies, oral donepezil 5 mg partially reversed the effect of scopolamine 0.3 mg administered subcutaneously to healthy elderly volunteers.[15] In a pre-clinical animal model, Prickaerts and colleagues reported a potential synergistic effect of donepezil and EVP-6124, as co-administration of sub-therapeutic dosages of donepezil and EVP-6124 showed similar effects as either donepezil or EVP-6124 alone at higher dosages.[8] Data from phase I and II trials involving EVP-6124 confirmed these findings in subjects with mild-to-moderate AD and showed that the treatment with donepezil and EVP-6124 was well-tolerated [9,10], which prompted the further investigation of EVP-6124 in phase III trials. Two phase III trials aiming to assess the efficacy and tolerability of EVP-6124 in patients with mild-to-moderate Alzheimer's disease were initiated but halted in 2015 due to gastrointestinal adverse events, perhaps due to the 5-HT3 antagonist activity of EVP-6124 and gastrointestinal motility effects.[11-13] Since then, evidence on the suggested synergistic effects of donepezil and EVP-6124 have not been pursued. As the combination of sub-therapeutic doses of EVP-6124 and donepezil is expected to lead to enhanced efficacy, a 2.5 mg dose of donepezil was chosen in the current study to determine enhancement of the donepezil effect in the presence of EVP-6124. Additionally, a 5.0 mg dose of donepezil was chosen to determine if any further improvement beyond the presumed maximal donepezil effect could be induced by EVP-6124.

EVP-6124 Single oral doses ranging from 1–180 mg showed linear pharmacokinetics with C_{max} values from 0.6–100 ng/ml (1.8–312 nM) achieved 5–8 hours after dosing in healthy volunteers. Effects on the Digit Symbol Substitution Test were most prevalent at 20 mg.[16] In the current study, a single oral dose of EVP-6124 0.3,

1.0, 2.0 and 4.0 mg was studied. The relatively low dose range of EVP-6124 was chosen on purpose, as pre-clinical studies showed a synergistic effect of donepezil and EVP-6124, when given at sub-therapeutic dosages (0.3 and 1.0 mg/kg).[8]

SCOPOLAMINE The muscarinic M1-5 acetylcholine receptor antagonist scopolamine is known to induce temporary impairment in cholinergic-dependent cognitive function. The application of the scopolamine challenge model is the most extensively used pharmacological model of cognitive impairment.[17] Previous studies have shown that a dose of 0.5 mg intravenously induces significant cognitive deficits in healthy young volunteers, while in healthy elderly volunteers a subcutaneous dose of 0.3 mg resulted in quantifiable and reproducible cognitive deficits.[14,15,18] Because intravenous dosing was expected to lead to a shorter duration of effect with only a slightly higher C_{max}, it was decided to administer a dose of 0.3 mg scopolamine intravenously to the healthy elderly volunteers in this study.[19]

Pharmacokinetic assessment

Venous blood samples were obtained via an indwelling catheter before administration of EVP-6124 and at 5 hours, 6.15 hours (immediately after scopolamine infusion), 7, 8, 9, 10 and 12 hours after administration. Plasma concentrations of EVP-6124, donepezil and scopolamine were determined (PRA, Assen, The Netherlands) by a validated method using high performance liquid chromatography coupled to tandem-mass spectrometry (LC/MS-MS). Pharmacokinetic non-compartmental data analysis was performed to determine Tmax, C_{max}, $AUC_{0\rightarrow t}$ by cohort per treatment. AUC was determined using the trapezoidal method. For scopolamine $AUC_{0\rightarrow inf}$, lambda and the elimination half-life ($t_{1/2}$) was also calculated.

Pharmacodynamic assessment

The 'NeuroCart' is a battery of sensitive tests for a wide range of CNS domains that was developed to examine different kinds of CNS-active drugs.[20] The N-back test and the symbol digit substitution test were used to evaluate working memory,[21-26] the Stroop test evaluated inhibition, interference and controlled versus automatic processing,[27] adaptive tracking measured attention and eye-hand coordination,[28-33] the single reaction time task measured reaction time,[34] finger tapping measured motor speed,[35] the visual analogue scale according to Bond & Lader was used to assess subjective states,[36,37] pharmaco-electroencephalography (p-EEG), eye movements and pupil size were used to monitor any drug effects, which can be interpreted as evidence of penetration and activity in the brain,[32,33,38,39] body movements were measured with the body sway meter[40] and the Visual Verbal Learning Test (VVLT) measured the whole scope of learning behaviour (i.e., acquisition, consolidation, storage and retrieval).[41]

All tests were performed twice before administration of scopolamine, and repeated immediately and at 1, 2, 3, 4 and 6 hours after administration of scopolamine. Pre-dose test scores were averaged. The only exception was VVLT, which was only performed 1 hour after dosing of scopolamine. Measurements were performed in a quiet room with ambient illumination with only one subject per session in the same room.

Safety assessments

All subjects underwent medical screening, including medical history, physical examination, vital signs measurement, 12-lead electrocardiogram (ECG), urinalysis, drug screen and safety chemistry and hematology blood sampling. During treatment periods, safety was assessed using monitoring of adverse events (AEs), vital signs, ECG and safety chemistry and hematology blood sampling.

Sample size calculation and statistics

A sample size of 36 patients was defined to have 80% power to detect a 80% reduction of scopolamine effects due to the combination of donepezil and EVP-6124. Pharmacodynamic endpoints were summarised (mean and standard deviation of the mean, median, minimum and maximum values) by treatment and time. For cohort 1 the EVP-6124 treatments were compared to the placebo treatment. For cohort 2 and 3 the EVP-6124 treatments plus donepezil treatments were compared to the EVP-6124 placebo and donepezil treatment. To establish whether significant treatment effects could be detected, repeatedly measured variables were analysed with a mixed model analysis of variance with treatment, time and treatment by time as fixed factors and subject, subject by treatment and subject by time as random factor and the (average) baseline measurement as covariate. The change compared to the scopolamine challenge alone (with double oral placebo) was analysed. A $p<0.05$ (two-sided) was considered statistically significant. Non-compartmental pharmacokinetic analyses (NCA) were performed on the plasma concentration data following oral administration of EVP-6124, donepezil and scopolamine. Statistical summaries, descriptive statistics and frequency tables were generated using SAS software (version 9.1.3). Pharmacokinetic analysis was performed using R (version 2.12.0).

RESULTS

Subjects

Overall, 38 subjects were enrolled in the study. One subject retracted informed consent shortly after administration of EVP-6124 or placebo and did not perform any post-dose measurements. Data of this drop-out subject was only included in the safety analysis. One subject discontinued the study after receiving EVP-6124 placebo and donepezil placebo during period 2, because of urinary retention due to prostate hypertrophia. All 37 dosed subjects were included in the safety analyses; 36 subjects were analysed for pharmacokinetic and pharmacodynamic outcomes. Subject demographics and baseline characteristics are summarised in table 2. Despite randomization, cohort 3 had a relatively high percentage of male subjects. There were no relevant differences in other parameters between the cohorts.

Safety

All but one subject who received at least one dose of study medication (n=36, 97.3%) reported at least one treatment related adverse event (AE) during the study. The most frequently reported drug related AEs were somnolence, dry mouth, dizziness, headache, disturbance in attention and gait disturbance (see table 3). Most events were mild in intensity and self-limiting. One subject discontinued the study after receiving EVP-6124 placebo and donepezil placebo, because of urinary retention due to prostate hypertrophia, requiring transurethral prostatectomy 12 days after his second study period. This AE was classified as unrelated to the study drugs. There were no relevant changes in ECG, vital signs or laboratory values.

TABLE 2 Subject demographics and baseline characteristics

	Cohort 1 (n=12)	Cohort 2 (n=12)	Cohort 3 (n=12)	All (n=36)
Age (years)	69.3 (65-77)	68.1 (65-75)	69.7 (65-78)	69.0 (65-78)
Sex (% male)	41.7	66.7	83.3	63.9
Weight (kg)	74.1 (54.9-95.8)	79.2 (54.7-100.9)	80.1 (64.2-93.6)	77.8 (54.7-100.9)
BMI (kg/m²)	25.5 (21.4-28.7)	25.6 (21.5-29.8)	26.7 (22.3-31.0)	25.9 (21.4-31.0)
MMSE	29.1 (28-30)	28.7 (27-30)	29.1 (28-30)	28.9 (27-30)

Means and ranges are presented; BMI=body mass index; MMSE=Mini Mental State Examination.

TABLE 3 Most frequent occurring treatment related adverse events for all dose combinations

		N†	Somnolence	Dry mouth	Dizziness	Headache	Distur-bance in attention	Gait disturbance
DPZ	**EVP-6124**							
Placebo	Placebo	35	22 (62.9%)	25 (71.4%)	19 (54.3%)	4 (11.4%)	-	4 (11.4%)
Placebo	0.3 mg	12	6 (50.0%)	8 (66.7%)	6 (50.0%)	1 (8.3%)	2 (16.7%)	1 (8.3%)
Placebo	1 mg	11	5 (45.5%)	8 (72.2%)	3 (27.3%)	3 (27.3%)	2 (18.2%)	1 (9.1%)
Placebo	2 mg	12	8 (66.7%)	10 (83.3%)	4 (33.3%)	-	1 (8.3%)	1 (8.3%)
Placebo	4 mg	12	7 (58.3%)	10 (83.3%)	5 (41.7%)	1 (8.3%)	2 (16.7%)	3 (25.0%)
2.5 mg	Placebo	11	7 (63.6%)	6 (54.4%)	6 (54.4%)	1 (9.1%)	-	-
5.0 mg	Placebo	10	6 (60.0%)	6 (60.0%)	6 (60.0%)	1 (10.0%)	1 (10.0%)	-
2.5 mg	0.3 mg	11	9 (81.8%)	5 (45.5%)	6 (54.5%)	2 (18.2%)	2 (18.2%)	1 (9.1%)
2.5 mg	1 mg	11	9 (81.1%)	9 (81.1%)	6 (54.5%)	3 (27.3%)	1 (9.1%)	-
2.5 mg	2 mg	12	11 (91.7%)	7 (58.3%)	5 (41.7%)	4 (33.3%)	1 (8.3%)	2 (16.7%)
5.0 mg	0.3 mg	11	6 (54.5%)	4 (36.4%)	5 (45.5%)	-	-	-
5.0 mg	1 mg	11	8 (72.7%)	5 (45.5%)	6 (54.5%)	1 (9.1%)	1 (9.1%)	-
5.0 mg	2 mg	11	8 (72.7%)	6 (54.5%)	7 (63.6%)	1 (9.1%)	2 (18.2%)	-
All		37	31 (83.3%)	32 (86.5%)	32 (86.5%)	11 (29.7%)	12 (32.4%)	11 (29.7%)

† All subjects received scopolamine 0.3 mg i.v. on each occasion; DPZ=donepezil.

Pharmacodynamics

Pharmacodynamic effects for all different combinations of donepezil and EVP-6124 are summarised in Table 4. The accuracy on the N-back deteriorated after administration of donepezil/EVP-6124 (5/2 mg) for the 1-back paradigm, and administration of donepezil/EVP-6124 (2.5/2 mg) for the 2-back paradigm. Further, reaction time on the 2-back paradigm of the N-back improved after administration of donepezil/EVP-6124 (5/0.3 mg). None of the other combinations of donepezil and EVP-6124 affected N-back accuracy or reaction time. The administration of donepezil/EVP-6124 (5/2 mg) led to improvement of the delayed word recall of the VVLT. Outcomes on the saccadic inaccuracy worsened after administration of donepezil/EVP-6124 (2.5/0.3 mg) and after administration of donepezil/EVP-6124 (2.5/1 mg). Saccadic reaction time worsened after administration of donepezil/EVP-6124 (5/1 mg), but none of the other combinations of EVP-6124 and donepezil affected saccadic eye movements. None of the other tests were significantly affected by any combination of EVP-6124 and donepezil.

TABLE 4 Pharmacodynamic effects compared to placebo

	Cohort 1 (n=12)				Cohort 2 (n=12)				Cohort 3 (n=12)			
	DPZ Placebo + EVP-6124 0.3 mg	DPZ Placebo + EVP-6124 1 mg	DPZ Placebo + EVP-6124 2 mg	DPZ Placebo + EVP-6124 4 mg	DPZ 2.5 mg + EVP-6124 Placebo	DPZ 2.5 mg + EVP-6124 0.3 mg	DPZ 2.5 mg + EVP-6124 1 mg	DPZ 2.5 mg + EVP-6124 2 mg	DPZ 5 mg + EVP-Placebo	DPZ 5 mg + EVP-6124 0.3	DPZ 5 mg + EVP-6124 1 mg	DPZ 5 mg + EVP-6124 2 mg
Adaptive tracking (%)	-1.23 (-2.80, 0.34) p=0.1200	0.03 (-1.56, 1.63) p=0.9671	-0.83 (-2.33, 0.67) p=0.2691	-0.26 (-1.76, 1.24) p=0.7281	1.49 (0.20, 2.77) p=0.0247	-1.12 (-2.41, 0.17) p=0.0855	-1.00 (-2.31, 0.31) p=0.1312	-1.14 (-2.43, 0.14) p=0.0796	0.72 (-0.76, 2.20) p=0.3297	-0.23 (-1.74, 1.28) p=0.7616	-0.02 (-1.52, 1.48) p=0.9777	-0.00 (-1.49, 1.48) p=0.9985
Body sway (mm)†	7.1 (3.2, 18.6) p=0.1796	-0.3 (10.1, 10.5) p=0.9471	-1.7 (-11.3, 8.8) p=0.7290	11.6 (0.7, 23.6) p=0.0363	1.9 (-10.9, 16.6) p=0.7767	-4.4 (-16.0, 8.7) p=0.4792	-0.0 (-12.3, 13.9) p=0.9957	4.7 (-8.0, 19.1) p=0.4786	-7.6 (-16.7, 2.5) p=0.1296	2.6 (-7.7, 14.1) p=0.6260	5.4 (-5.1, 17.2) p=0.3152	-3.0 (-12.6, 7.7) p=0.5561
VVLT delayed word recall (nr of words)	0.4 (-0.9, 1.7) p=0.5508	0.1 (-1.2, 1.4) p=0.8959	-1.1 (-2.3, 0.2) p=0.0970	-0.4 (-1.6, 0.9) p=0.5755	-0.4 (-1.5, 0.7) p=0.4707	0.1 (-1.0, 1.2) p=0.654	0.5 (-0.6, 1.6) p=0.3988	0.3 (-0.7, 1.4) p=0.5417	-0.2 (-1.3, 0.8) p=0.6466	0.2 (-0.9, 1.3) p=0.7362	0.4 (-0.7, 1.5) p=0.4469	1.2 (0.1, 2.3) p=0.0327
N-back, 0-back accuracy (%)	0.02 (-0.04, 0.08) p=0.5073	0.04 (-0.03, 0.10) p=0.2318	0.05 (-0.01, 0.11) p=0.0818	0.06 (0.00, 0.12) p=0.0470	0.03 (-0.05, 0.11) p=0.4512	0.04 (-0.04, 0.12) p=0.3346	0.06 (-0.02, 0.14) p=0.1629	0.01 (-0.07, 0.09) p=0.8116	0.04 (-0.02, 0.10) p=0.1614	-0.05 (-0.11, 0.01) p=0.1030	0.01 (-0.05, 0.07) p=0.7496	-0.03 (-0.10, 0.03) p=0.2730
N-back, 0-back reaction time (msec)	8 (-12, 29) p=0.4004	-17 (-37, 4) p=0.1085	-5 (-25, 15) p=0.6209	-16 (-36, 4) p=0.1151	-24 (-48, 1) p=0.0615	-9 (-31, 13) p=0.3889	13 (-9, 35) p=0.2366	11 (-11, 33) p=0.3189	-18 (-36, -1) p=0.0386	-7 (-24, 11) p=0.4388	-6 (-24, 12) p=0.5046	3 (-15, 20) p=0.7511
N-back, 1-back accuracy (%)	0.02 (-0.05, 0.10) p=0.5558	0.04 (-0.04, 0.12) p=0.3062	0.08 (0.01, 0.16) p=0.0337	0.05 (-0.03, 0.12) p=0.2385	-0.00 (-0.12, 0.11) p=0.9598	0.04 (-0.08, 0.16) p=0.5014	0.01 (-0.11, 0.12) p=0.9269	-0.01 (-0.13, 0.10) p=0.8351	0.03 (-0.05, 0.11) p=0.4286	-0.04 (-0.12, 0.04) p=0.3158	-0.03 (-0.11, 0.05) p=0.5004	-0.09 (-0.17, -0.01) p=0.0273
N-back, 2-back accuracy (%)	-0.02 (-0.11, 0.07) p=0.6878	0.01 (-0.08, 0.10) p=0.8796	0.03 (-0.06, 0.13) p=0.4557	-0.02 (-0.11, 0.07) p=0.7181	0.04 (-0.08, 0.17) p=0.5034	0.01 (-0.11, 0.14) p=0.8346	-0.06 (-0.19, 0.06) p=0.3162	-0.14 (-0.26, -0.01) p=0.0336	0.02 (-0.08, 0.11) p=0.7469	-0.03 (-0.12, 0.07) p=0.5790	-0.04 (-0.14, 0.05) p=0.3887	-0.08 (-0.18, 0.01) p=0.0800
N-back, 2-back reaction time (msec)	-5 (-36, 25) p=0.7210	-3 (-33, 28) p=0.8693	1 (-29, 31) p=0.9305	-8 (-39, 22) p=0.5733	-27 (-70, 16) p=0.2115	27 (-16, 70) p=0.2057	35 (-8, 79) p=0.1070	41 (-2, 85) p=0.0625	35 (-1, 70) p=0.0541	-42 (-77, -8) p=0.0187	-20 (-55, 15) p=0.2513	-15 (-49, 19) p=0.3722
Simple reaction time test (%)†	3.3 (-7.0, 14.6) p=0.5380	-1.5 (-11.5, 9.8) p=0.7844	-1.1 (-10.9, 9.8) p=0.8319	5.2 (-5.2, 16.7) p=0.3279	-14.6 (-26.2,-1.3) p=0.0336	2.7 (-10.5, 17.9) p=0.6933	2.5 (-10.9, 17.8) p=0.7246	6.4 (-7.6, 22.6) p=0.3786	-3.1 (-9.2, 3.4) p=0.3379	0.0 (-6.3, 6.8) p=0.9947	-0.2 (-6.5, 6.6) p=0.9632	0.0 (-6.3, 6.7) p=0.9999
EEG alpha Fz-Cz (uV)†	6.8 (-1.4, 15.7) p=0.1053	12.0 (3.1, 21.7) p=0.0087	7.3 (-0.9, 16.2) p=0.0818	5.3 (-2.8, 14.0) p=0.1987	5.8 (-7.5, 21.0) p=0.4030	-4.4 (-16.5, 9.5) p=0.5065	3.1 (-10.1, 18.3) p=0.6505	-6.6 (-18.4, 6.7) p=0.3051	4.0 (-13.2, 24.6) p=0.6615	-9.6 (-25.2, 9.4) p=0.2909	-5.3 (-21.4,14.1) p=0.5556	-15.2 (-29.3,1.8) p=0.0759
Saccadic inaccuracy (%)	0.6 (-0.2,1.4) p=0.1224	0.0 (-0.8,0.8) p=0.9525	-0.1 (-0.9,0.6) p=0.7196	0.3 (-0.5,1.1) p=0.4213	-1.4 (-2.7,-0.1) p=0.0311	1.3 (0.1, 2.6) p=0.0388	1.4 (0.0, 2.7) p=0.0474	0.8 (-0.5,2.0) p=0.2132	0.0 (-0.9,1.0) p=0.9433	-0.2 (-1.2,0.8) p=0.7336	0.5 (-0.5,1.5) p=0.3449	0.2 (-0.7,1.2) p=0.6470
Saccadic reaction time (msec)	-4 (-13,5) p=0.4018	-2 (-12,7) p=0.5871	-3 (-12, 6) p=0.4605	3 (-6, 11) p=0.5707	-4 (-20, 11) p=0.5539	8 (-6,23) p=0.2495	3 (-12, 18) p=0.6847	11 (-4,26) p=0.1466	-11 (-20,-2) p=0.0190	-4 (13,5) p=0.3548	12 (3,22) p=0.0190	1 (-8,10) p=0.8674

†Back translated from log; effect parameter and 95% confidence intervals are presented; DPZ=donepezil. Significant p-values (<0.05) are highlighted in bold/italics.

EVP-6124 alone had a dose-dependent positive effect on the 0-back accuracy, which only reached significance for the 4 mg dose. EVP-6124 2 mg had a positive effect on 1-back accuracy, none of the other combinations of EVP-6124 and donepezil significantly affected the N-back parameters (see Table 4). EVP-6124 4 mg induced an increase in body sway and EVP-6124 1 mg induced an increase in power in the EEG alpha frequency. None of the other tests were affected by any dose of EVP-6124 alone.

Administration of donepezil 2.5 mg alone led to an improvement on adaptive tracking, SRT and saccadic inaccuracy (see table 4). Administration of donepezil 5 mg led to an improve of saccadic reaction time and reaction time of the 0-back paradigm of the N-back, but to an increased reaction time on the 2-back paradigm. None of the other tests were affected by donepezil 2.5 or 5 mg.

Administration of scopolamine alone led to a worsened performance on adaptive tracking, N-back, SDST, Stroop test, SRT, saccadic eye movements, body sway, finger tapping and VAS alertness, as well as a decrease in EEG alpha frequency and an increase in EEG delta frequency. Scopolamine did not affect EEG beta and theta frequencies, smooth pursuit eye movements and VAS composite scores for calmness and mood.

Pharmacokinetics

Table 5 shows the pharmacokinetic parameters of donepezil and EVP-6124. Based on the non-compartmental analysis, donepezil pharmacokinetic parameters were similar with or without EVP-6124, suggesting that EVP-6124 did not affect the pharmacokinetic profile of donepezil. Conversely, EVP-6124 pharmacokinetic parameters were similar with or without donepezil suggesting that donepezil did not affect the pharmacokinetic profile of EVP-6124. Because all subjects received scopolamine, the study design does not allow an investigation of any potential pharmacokinetic interactions between scopolamine and donepezil or EVP-6124.

DISCUSSION

Pre-clinical experiments have shown a synergistic effect of EVP-6124 and donepezil in reducing the -- effects of scopolamine on short term memory observed in rats using the Morris water maze task. A complete reversal of scopolamine-induced effects was observed when both donepezil and EVP-6124 were given at approximately 1/10th of the dose at which each of the compounds alone fully reversed the effects of scopolamine.[8] The current study was designed to reproduce the

synergistic effect in humans observed in the animal model where sub-therapeutic doses of both EVP-6124 and donepezil did not lead to full reduction of scopolamine induced cognitive deficits when given alone, but did lead to full reversal when co-administered. However, this study did not demonstrate synergy between donepezil and EVP-6124 when these drugs were given at sub-therapeutic dose levels.

The dose combinations of donepezil/EVP-6124 (5 mg/2 mg) and donepezil/EVP-6124 5 mg/0.3 mg were effective, with significant improvements of the delayed recall of the VVLT and reaction time during the 2-back condition of the N-back respectively. A pharmacokinetic interaction was excluded, as pharmacokinetic parameters suggest that the pharmacokinetic profile of EVP-6124 did not affect the profile of donepezil and vice versa. The NeuroCart battery of CNS tests was sufficiently sensitive to detect scopolamine-induced deficits in cognition and other CNS functions. Although both donepezil and EVP-6124 alone and the combination of both compounds did reduce the (cognitive) deficits induced by scopolamine administration in some of the neurophysiological and cognitive tests performed, an obvious reversal of scopolamine effects was not observed.

When given separately, both compounds produced inconsistent effects. The highest doses of EVP-6124 showed an effect on the accuracy of the 0-back condition of the N-back working memory task, but had no effect on learning, recall or recognition of the VVLT. Donepezil 2.5 mg had an effect on SRT, adaptive tracking and saccadic inaccuracy, but these effects were not confirmed when dosed at 5.0 mg. The ability of the NeuroCart battery to detect reversal of scopolamine induced cognitive impairment may not have been optimal.

TABLE 5 Pharmacokinetic parameters.

	Treatment group	AUC_{0-t} ($pg \cdot hr \cdot mL^{-1}$)	T_{max} (hr)	C_{max} ($pg \cdot mL^{-1}$)
EVP-6124 0.3 mg	DZP placebo + EVP-6124 0.3 mg	2474 ± 572.4	5.82 ± 0.939	281.2 ± 70.48
	DZP 2.5 mg + EVP-6124 0.3 mg	1781 ± 347.2	5.81 ± 1.008	205.0 ± 39.21
	DZP 5 mg + EVP-6124 0.3 mg	2176 ± 723.0	5.79 ± 0.88	249.6 ± 81.94
EVP-6124 1 mg	DZP placebo + EVP-6124 1 mg	7412 ± 1379.0	5.61 ± 0.672	852.6 ± 153.50
	DZP 2.5 mg + EVP-6124 1 mg	5760 ± 1296.0	6.88 ± 1.789	659.9 ± 140.60
	DZP 5 mg + EVP-6124 1 mg	6496 ± 1907.0	5.71 ± 1.270	773.5 ± 198.80
EVP-6124 2 mg	DZP placebo + EVP-6124 2 mg	14600 ± 3310.0	5.49 ± 0.911	1671.0 ± 360.20
	DZP 2.5 mg + EVP-6124 2 mg	11220 ± 2002.0	5.92 ± 1.35	1402.0 ± 252.70
	DZP 5 mg + EVP-6124 2 mg	12920 ± 4474.0	6.25 ± 1.919	1493.0 ± 447.10
EVP-6124 4 mg	DZP placebo + EVP-6124 4 mg	27960 ± 5020.0	5.99 ± 1.122	3249.00 ± 680.200

Means ± SD are presented; DZP=donepezil

There are several possible explanations for our findings. First, the dose of scopolamine could have been too high in the elderly subjects in this study. The intravenous dose of 0.3 mg scopolamine resulted in a mean C_{max} of 3772.9 pg/ml and an AUC_{0-inf} 3431.3 pg*hr/ml, which is at least 25% higher than reported in other studies in younger healthy subjects.[42,43] In combination with slight age-related cholinergic deficiency, this might have led to detrimental effects of scopolamine on most of the cognitive tests. EVP-6124, donepezil or any combination did produce some reversal of the scopolamine-induced cognitive deficits. However, subtle effects might have been overshadowed by the robust scopolamine effects. While other studies showed a decrease of cognitive impairment due to the combination of donepezil and EVP-6124 without use of the scopolamine challenge model, it remains under debate whether the challenge model was suitable to show the expected synergy in this study. The scopolamine challenge test has been successfully used in drug development to demonstrate the pharmacological activity of cognition-enhancing compounds by reversal of scopolamine-induced cognitive deficits in healthy volunteers.[15,42-48] Evidence also suggests that low concentrations of scopolamine (0.3 mg subcutaneous) can already induce a measurable significant decline in visuomotor speed and spatial working memory in healthy older people.[15] Altogether, the scopolamine challenge model has the potential to show the expected synergistic effect in the elderly, but dose selection and dosage form require careful reconsideration.[49]

Another reason for the lack of synergistic effect of donepezil and EVP-6124 in this study might be insufficient dosing of donepezil and/or EVP-6124. Although oral donepezil (5 mg) was previously demonstrated to reverse the effects of scopolamine (0.3 mg administered subcutaneously) in healthy elderly volunteers,[15] other studies only suggest effects of donepezil at a higher dose of 10 mg or when given in a paradigm where scopolamine is administered subcutaneously to healthy elderly volunteers, which could be expected to lead to lower C_{max}.[15,48] The low dose range of EVP-6124 in this study was obviously chosen on purpose, as pre-clinical studies showed a synergistic effect of donepezil and EVP-6124, when given at sub-therapeutic dosages. These studies also indicated that desensitization would occur at higher doses.[8,9,10] In the current study, only the two highest doses of 2 mg and 4 mg EVP-6124 without co-administration of donepezil gave an increased accuracy on the N-back task for working memory. When given together with donepezil, only the combination of the highest doses (EVP-6124 2 mg and donepezil 5 mg) led to an increased delayed recall on VVLT and decrease in reaction time during N-back. These data show no signs of desensitization.

Overall, treatment with sub-therapeutic dose levels of donepezil and EVP-6124, in combination with scopolamine, was well tolerated in this study. Comparable to

other studies investigating the combination of donepezil and EVP-6124, 98 percent experienced at least one adverse event of which the majority was anticholinergic.[15] The three most frequently reported adverse events (somnolence, dry mouth, and dizziness) each occurred in 80% of subjects. The majority of adverse events had an anticholinergic nature and was therefore most likely related to the administration of scopolamine.

In conclusion, while administration of EVP-6124 alone and donepezil alone led to some reduction of scopolamine-induced effects in some of the measured pharmacodynamic variables, there were no clear indications of synergistic effects of EVP-6124 and donepezil in the scopolamine challenge model in healthy elderly subjects.

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CHAPTER 3

An anti-nicotinic cognitive challenge model using mecamylamine in comparison with the anti-muscarinic cognitive challenge using scopolamine

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ABSTRACT

INTRODUCTION The muscarinic acetylcholine receptor antagonist scopolamine is often used for proof-of-pharmacology studies with pro-cognitive compounds. From a pharmacological point of view, it would seem more rational to use a nicotinic rather than a muscarinic anticholinergic challenge to prove pharmacology of a nicotinic acetylcholine receptor agonist. This study aims to characterize a nicotinic anticholinergic challenge model using mecamylamine and to compare it to the scopolamine model.

METHODS In this double blind, placebo controlled, four way cross-over trial 12 healthy male subjects received oral mecamylamine 10 and 20 mg, intravenous scopolamine hydrobromide 0.5 mg and placebo. Pharmacokinetics were explored using non-compartmental analysis. Pharmacodynamic effects were measured with a multidimensional test battery that includes neurophysiological, subjective, (visuo)motor and cognitive measurements.

RESULTS All treatments were safe and well tolerated. Mecamylamine had a T_{max} of 2.5 hours and a C_{max} of 64.5 $ng \cdot ml^{-1}$ for the 20 mg dose. Mecamylamine had a dose dependent effect which decreased the adaptive tracking performance, VAS alertness, finger tapping time and performance in the visual verbal learning task. No effects were seen on the simple reaction time test or saccadic peak velocity. Scopolamine significantly affected almost all pharmacodynamic tests.

CONCLUSION This study demonstrated that mecamylamine causes nicotinic receptor specific temporary decline in cognitive functioning. Compared with the scopolamine model, pharmacodynamic effects were less pronounced at the dose levels tested, but mecamylamine caused less sedation. The cognitive effects of scopolamine might at least partly be caused by sedation. Whether the mecamylamine model can be used for proof-of-pharmacology of nicotinic acetylcholine receptor agonists remains to be established.

INTRODUCTION

Alzheimer's Disease (AD) is the most common form of dementia, with a prevalence of 3–7% in the Western European population (Takizawa *et al*, 2015). AD causes significant burden for the patients and their caregivers and high health care costs for society. Even though many research groups aim to unravel the pathophysiology and many pharmaceutical companies are searching for pharmacological targets for a curative treatment, no new drugs have been registered for this indication since 2003. The only approved therapy for mild to moderate AD is symptomatic treatment with cholinesterase inhibitors (CEIs), increasing the acetylcholine level in the synaptic cleft of cholinergic neurons. The cholinergic system is hypothesized to play an important role in several cognitive processes such as attention and memory (Drachman and Leavitt, 1974). Also, pathology studies have shown decreased levels of acetylcholine levels in the brains of patients with AD. Nevertheless, treatment with CEIs is only effective in about 14–36% of the AD patients and the dose is limited by peripheral side effects such as nausea, vomiting and diarrhoea (Birks, 2006; Birks *et al*, 2009; Olin and Schneider, 2002; Rösler *et al*, 1999; Tariot *et al*, 2000). CEIs inhibit esterases peripherally and in the central nervous system (CNS) so they will not only enhance functioning of cholinergic neuronal system, but will also induce peripheral cholinergic side effects, mainly via autonomic parasympathetic neurons. These peripheral side effects could be avoided with agonists that are more selective for AChRs with a higher presence in the cns than peripherally, such as the α7 and α4β2 nicotinic acetylcholine receptor (nAChR). nAChR are mainly located in the hippocampus, thalamus, amygdala, striatum, entorhinal, frontal and pre-frontal cortex. Based on the localization of nAChR in the human brain, nicotinergic blockade could be expected to result in an impairment of cognitive functions such as acquisition, processing and recall of information (Paterson and Nordberg, 2000). Accumulating evidence suggests that α7 nAChRs play an important role in the pathophysiology of neuropsychiatric diseases, including schizophrenia and AD. Hence, a number of pharmaceutical industries have developed selective and high affinity α7 nAChR agonists as therapeutic drugs for these neuropsychiatric diseases (Toyohara and Hashimoto, 2010). Therefore, specific agonists targeting nAChR are currently being developed.

Proof-of-pharmacology studies with cholinergic compounds are often performed in healthy subjects after administration of scopolamine (Blin *et al*, 2009; Buccafusco, 2009; Cho *et al*, 2011; Deiana *et al*, 2009; Lee *et al*, 2009; Liem-Moolenaar *et al*, 2010; van Ruitenbeek *et al*, 2008; Snyder *et al*, 2005).

Scopolamine is a competitive muscarinic acetylcholine receptor (mAChR) antagonist with similar binding to all five known muscarinic receptor subtypes. From a pharmacological point of view, it seems more rational to use a nicotinic rather than a muscarinic anticholinergic challenge in a proof of pharmacology study of a nicotinic acetylcholine receptor agonist.

Mecamylamine is a nAChR antagonist that has been used for the treatment of severe hypertension since the 1950s. In 2009 it was withdrawn from the market because of its unfavourable risk-benefit profile compared with many other available antihypertensives. Mecamylamine's antihypertensive effects are mediated through nAChR in peripheral autonomic ganglia. However, it also binds to nAChR present in the CNS (Stone *et al*, 1956). Previous studies have confirmed that mecamylamine, temporarily and reversibly, perturbs the above-mentioned cognitive processes in healthy volunteers (Little *et al*, 1998; Newhouse *et al*, 1992, 1994; Thompson *et al*, 2000; Voss *et al*, 2010).

With this study we aimed to better characterize the pharmacodynamic and pharmacokinetic effects of mecamylamine compared to scopolamine in order to improve the knowledge about a nAChR specific anti-cholinergic challenge and to develop a challenge model that may be suitable for proof-of-pharmacology studies with nAChR agonists.

METHODS

Trial design and subjects

This double blind, double dummy, placebo controlled, four-way cross-over study was performed in healthy, non-smoker, young male subjects. On four different occasions with a wash-out of 7 days in between, all subjects received an oral dose of mecamylamine 10 mg with intravenous placebo, an oral dose of mecamylamine 20 mg with intravenous placebo, an intravenous dose of scopolamine hydrobromide 0.5 mg with oral placebo and both oral and intravenous placebo. The expected T_{max} of scopolamine was 15 minutes after the start of the infusion, while the expected T_{max} of mecamylamine was 3 hours after oral administration (Liem-Moolenaar *et al*, 2011; Young *et al*, 2001). Therefore, the intravenous dose of scopolamine or placebo was given 2.45 hours after administration of mecamylamine or placebo with infusion duration of 15 minutes in order to have a T_{max} of both drugs at approximately the same time point. All subjects gave written informed consent for participation in the study. The ethics committee of the Leiden University Medical Center (The Netherlands) approved the study.

Dosing rationale

For the treatment of hypertension, the approved starting dose of mecamylamine was 25 mg per day and in various cognitive studies, a maximum of 20 mg orally produced few adverse effects, other than mild hypotension (Dumas *et al*, 2006, 2008, 2010; Ellis *et al*, 2006; Erskine *et al*, 2004; Ford *et al*, 1956; Green *et al*, 2005; Little *et al*, 1998; Newhouse *et al*, 1992, 1994; Thienel *et al*, 2009; Thompson *et al*, 2000; Voss *et al*, 2010; Young *et al*, 2001). Cognitive impairments are observed at dose levels of 15 mg and higher (Little *et al*, 1998; Newhouse *et al*, 1992, 1994; Thompson *et al*, 2000). For the pharmacological challenge in this study a lower (10 mg) and higher (20 mg) dose were chosen in order to better determine concentration-effect relationships. Mecamylamine uptake is characterized by complete absorption from the gastrointestinal tract (Young *et al*, 2001).

Scopolamine has been validated and frequently used as a pharmacological challenge in previously published studies with minimal adverse effects and demonstrable cognitive impairments at 0.5 mg scopolamine intravenously dosed (Liem-Moolenaar *et al*, 2011).

Pharmacokinetics

Venous blood samples were obtained via an indwelling catheter before administration of mecamylamine or placebo and at 0.5, 1.0, 2.0, 3.0, 3.25, 4.0, 6.0, 8.0, 10.0 and 22.0 hours after drug administration. Plasma concentrations of mecamylamine and scopolamine were determined at the department of Clinical Pharmacology and Pharmacy at the vu University Medical Centre (Amsterdam, The Netherlands) by a validated method using high performance liquid chromatography coupled to tandem-mass spectrometry (LC-MS/MS).

The LC-MS/MS consisted of a Waters Alliance 2795 separation module and a Quattro Micro tandem mass spectrometer from Waters (Watford, UK). System control, data acquisition and data processing were performed using MassLynx v4.1. Chromatography was performed on a Kinetex C18 analytical column from Phenomenex. The particle size was 2.6 µM, column length was 150 mm and column diameter was 3.0 mm. The mobile phase ratio of 70% mobile phase A and 30% mobile phase B was run with a flow of 0.5 $mL \cdot min^{-1}$. Both mobile phases contained 0.05 % (v/v) trifluoretic acid and 5 mM ammoniumformate, whereas mobile phase A was prepared in purified water and mobile phase B was prepared in methanol. Ionization of the drugs was achieved in the positive electrospray modus. The respective MRM transitions were 168.1 > 137.1 m/z for mecamylamine, 304.2 > 138.1 m/z for scopolamine, 171.2 > 137.1 m/z for mecamylamine-D3 and 307.1 >

141.1 m/z for scopolamine-D3. For sample preparation, 100 µL of an aqueous solution containing 1 M zinc sulphate was added to 40 µL plasma and short vortexed. Hereafter 100 µL of the internal standard was added containing 100 $\mu g \cdot L^{-1}$ of mecamylamine-D3 and scopolamine-D3 in methanol. After vortexing for 3 minutes the samples were centrifuged at 10900 g for 3 minutes. The clear supernatant was transferred to vials and 25 µL was injected on the LC-MS/MS.

Pharmacodynamic assessments

To determine the pharmacodynamic effects of mecamylamine, a battery of tests (NeuroCart®) with a previously shown sensitivity to drug effects on a wide range of CNS domains was used (Liem-Moolenaar *et al*, 2011; van Steveninck *et al*, 1991, 1999; de Visser *et al*, 2003). All tests were performed twice at baseline, and repeated at 1.0, 2.0, 3.25, 4.0, 6.0, 8.0 and 10.0 hours after administration of mecamylamine or placebo. The only exception was the visual verbal learning test, which was performed 3.5 hours after dosing (immediate recall) and 5 hours after dosing (delayed recall and recognition). Measurements were performed in a quiet room with ambient illumination with only one subject per session in the same room.

FINGER TAPPING This test evaluates motor activation and fluency and has been adapted from the Halstead Reitan Test Battery (Andrew, 1977). The volunteer was instructed to tap as quickly as possible with the index finger of the dominant hand. Each session contained 5 performances of 10 seconds. Feedback on performance was given by a counter in the centre of the screen, while the amount of taps of each 10 second trial was shown on the screen in between the trials. The mean tapping rate of five trials per time point was used for statistical analysis.

N-BACK This test evaluates the working memory and requires buffering and updating consonants, matching, encoding and responding. The N-back test consists of three conditions, with increased working memory load. Letters were presented consecutively on the screen with a speed of 30 letters per minute. In the first condition subjects had to indicate whether the letter on the screen was an 'x'. In the second condition, subjects indicated whether the letter seen was identical to the previous letter. In the third condition, subjects were asked to indicate whether the letter was identical to two letters before the letter seen (Lim *et al*, 2008; Rombouts *et al*, 2002; Sweet *et al*, 2006).

ADAPTIVE TRACKING Adaptive tracking is a pursuit-tracking task, measuring attention and eye-hand coordination. A circle moves pseudo-randomly about a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average performance scores over a three-minute period was used for analysis. Before study participation, subjects performed three training sessions and at each occasion two baseline measurements were done (Gijsman *et al*, 1998; van Steveninck *et al*, 1991, 1993, 1999).

SACCADIC PEAK VELOCITY Saccadic peak velocity (SPV) is one of the most sensitive parameters for sedation. The use of a computer for measurement of saccadic eye movements has been described elsewhere (Baloh *et al*, 1975; van Steveninck *et al*, 1991, 1999). Average values of latency (reaction time), saccadic peak velocity of all correct saccades and inaccuracy of all saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

SMOOTH PURSUIT EYE MOVEMENTS The same system as used for saccadic eye movements was also used for measurement of smooth pursuit. For smooth pursuit eye movements, the target moves at a frequency ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5 degrees eyeball rotation to both sides. Four cycles are recorded for each stimulus frequency. The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as parameter (Baloh *et al*, 1975; Bittencourt *et al*, 1983).

PHARMACO-ELECTROENCEPHALOGRAPHY Pharmacoelectroencephalography (p-EEG) was used to monitor any drug effects, which can be interpreted as evidence of penetration and activity in the brain (Cohen *et al*, 1985; Van Steveninck *et al*, 1993). EEG recordings were made using gold electrodes, fixed with EC2 paste (Astromed) at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). The signals were amplified by use of a Grass 15LT series Amplifier Systems with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using customized CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was ad-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK). Data blocks

containing artefacts were identified and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the very low (0.5–2 Hz), δ (2–4 Hz), θ (4–7.5 Hz), α (7.5–13.5 Hz), *β* (13.5–35 Hz), and γ (35–48.9 Hz) frequency ranges. The duration of EEG measurements was 64 seconds per session.

PUPIL SIZE Pupil diameter was determined using a digital camera (Canon powershot A620) and a flash. The subject was instructed to look into the lens. A sharp picture of the eyes was taken using a camera with flash. All pictures were stored digitally. The diameters of the pupil and the iris were determined in the number of pixels used horizontally. For each eye, these values were recorded on data collection forms, and the pupil / iris ratio was subsequently calculated as a measure of pupil size.

BODY SWAY The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway was measured with a pot string meter (celesco) based on the Wright ataxia meter (Wright, 1971). This method has been used to demonstrate effects of sleep deprivation (van Steveninck *et al*, 1999), alcohol (van Steveninck *et al*, 1993) and benzodiazepines (van Steveninck *et al*, 1993; Van Steveninck *et al*, 1997). With a string attached to the waist, all body movements over a period of time were integrated and expressed as mm sway. The total period of body-sway measurement was two minutes.

STROOP The Stroop test mainly investigates inhibition, interference and controlled versus automatic processing. A two trial version of the colour-word Stroop task was presented to the subjects. In the first trial, six coloured items in green, red or blue were presented at random and subjects indicated which colour they saw. In the second trial, 34 colour and word pairs were presented randomly to the subject, forming either congruent or incongruent matches. The subjects were asked to indicate the colour of the word (for example: if the word blue was written in red, the correct answer was 'red') (Laeng *et al*, 2005).

SIMPLE REACTION TIME TASK The Simple Reaction Time Task (SRTT) measures the attention and speed of information processing of the participant. In this task, participants view a black computer screen. At random intervals (0.5–1.5 seconds), a white circle appears in the centre of the computer screen. Participants were instructed to press the space bar with the index finger of their dominant hand each time the circle appears. They were instructed to respond as quickly as possible after appearance of the circle. A total of 40 circles were presented, and the duration of the task was approximately 1 minute. The outcome of the task is the time between stimulus display and response. It has been shown to respond to several classes of sedative drugs (Wezenberg *et al*, 2007).

VISUAL ANALOGUE SCALE Changes in subjective conditions are important aspects of drug effects, and a visual analogue scale (VAS) is one of the most commonly used ways to assess subjective states. It is a psychometric response scale, which is particularly suited to repeatedly quantify present subjective states. In the VAS according to Bond & Lader, the 'directions' of different scales on a form were alternated, to avoid 'habitual scoring' by subjects. Composite scores were derived for alertness, mood and calmness (Norris, 1971).

VISUAL VERBAL LEARNING TEST The Visual Verbal Learning Test (VVLT) contains three different subtests that cover almost the whole scope of learning behaviour (i.e., acquisition, consolidation, storage and retrieval) (de Haas *et al*, 2009). Subjects were presented 30 words in three consecutive word trials. Each trial ended with a free recall of the presented words (Immediate Recall). Approximately thirty minutes after start of the first trial, the volunteers were asked to recall as many words as possible (Delayed Recall). Immediately thereafter, the volunteers underwent memory recognition test, which consisted of 15 presented words and 15 'distractors' (Recognition).

Safety assessments

All subjects underwent medical screening, including medical history, physical examination, vital signs measurement in supine and standing position, 12-lead electrocardiogram (ECG), urinalysis, drug screen and safety chemistry and haematology blood sampling. During study periods, safety was assessed using monitoring of adverse events, vital signs, ECG and safety chemistry and haematology blood sampling.

Pharmacokinetic and statistical analysis

The graphs and the pharmacokinetic parameters for mecamylamine were calculated by non-compartmental analysis in R (R Core Team, 2013). Primary pharmacokinetic endpoints were: maximum plasma concentration (C_{max}), time of maximum plasma concentration (T_{max}), area under the plasma concentration vs. time curve ($AUC_{0\text{-last}}$), area under the plasma concentration vs. time curve extrapolated to infinity ($AUC_{0\text{-}\infty}$), apparent terminal half-life, apparent clearance (Cl/F) and apparent volume of distribution (Vd/F).

A mixed model analysis of covariance using sas 9.1.3 for Windows (sas Institute Inc., Cary, NC, USA) was used for analyses of pharmacodynamic effects, with subject, subject by treatment and subject by time as random effects; treatment, study period and by treatment by time as fixed effects; and the average baseline value as covariate. VVLT was analysed using a mixed model analysis of variance with fixed factors treatment and period, random factor subject and, if available, the (average) baseline. As this was an exploratory study, no formal adjustment for multiple testing was used. A *p* value below 0.05 was considered statistically significant. In order to properly compare scopolamine and mecamylamine effects, two timepoints before scopolamine administration (1 and 2 hours after mecamylamine administration) were not included in the LSM graphs.

RESULTS

A total of 15 healthy male subjects participated in the trial. During execution of the study, three subjects stopped prematurely, due to personal circumstances (1), difficulties in blood sampling (1) and because of adverse events (nausea; 1). A total of 14 subjects completed at least one study period with treatment of mecamylamine and 12 subjects completed all study occasions. Subjects had a mean age of 25.9 (range 19–36) years, weight of 80.9 (range 59.9–90.0) kg and BMI of 24.4 (range 18.6–30.3) kg·m².

Safety

All subjects reported at least one treatment emergent adverse event. Most frequent occurring adverse events were somnolence, dizziness, fatigue, nausea, dry mouth and headache (table 1). Adverse effects were mild and occasionally moderate and all disappeared spontaneously within a few hours. 3 of 14 subjects reported postural dizziness at the 20 mg mecamylamine dose. This coincided in all cases with measurable orthostatic hypotension.

The difference between standing and supine blood pressure significantly increased on the 20 mg mecamylamine dose, compared to placebo, while heart rate was significantly higher (table 2). Also, the difference in blood pressure between supine and standing position was significantly higher on the 20 mg mecamylamine dose, compared to placebo. On the 10 mg dose of mecamylamine, only the increase in supine and standing heart rate was statistically significant compared to placebo. There were no other consistent changes in ECG or laboratory safety parameters.

TABLE 1 **Most frequent treatment emergent adverse events.** Number of adverse events and percentage from the subjects experiencing the adverse events.

	Placebo n=14	Mecamylamine 10 mg n=12	Mecamylamine 20 mg n=14	Scopolamine 0.5 mg n=13
Subjects with at least 1 AE	7 (50.0%)	8 (66.7%)	12 (85.7%)	13 (100%)
Number of different AEs	8	9	33	19
Somnolence	2 (14.3%)	6 (50.0%)	9 (64.3%)	7 (53.8%)
Dizziness	-	2 (16.7%)	4 (28.6%)	10 (76.9%)
Fatigue	2 (14.3%)	2 (16.7%)	5 (35.7%)	4 (30.8%)
Nausea	2 (14.3%)	1 (8.3%)	5 (35.7%)	3 (23.1%)
Dry mouth	1 (7.1%)	-	1 (7.1%)	5 (38.5 %)
Headache	2 (14.3%)	2 (16.7%)	1 (7.1%)	2 (15.4%)
Disturbance in attention	-	1 (8.3%)	2 (14.3%)	1 (7.7%)
Dysgeusia	1 (7.1%)	-	2 (14.3%)	1 (7.7%)
Diplopia	-	-	1 (7.1%)	2 (15.4%)
Dizziness postural	-	-	3 (21.4%)	-

TABLE 2 **Vital signs per treatment group.** Per group the difference estimate and in parenthesis the confidence interval is presented.

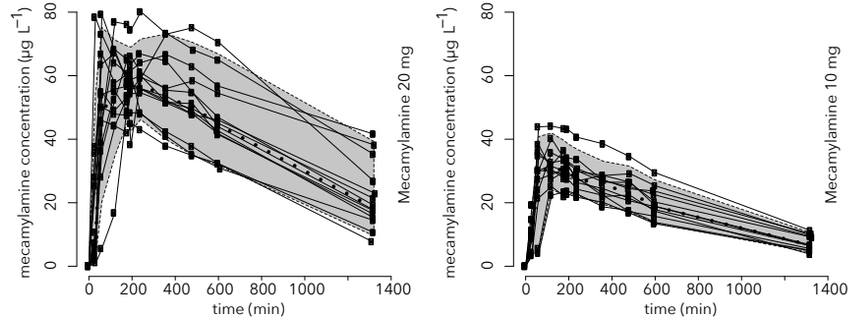
	Treatment effect	Mecamylamine 10 mg n=12	Mecamylamine 20 mg n=14	Scopolamine 0.5 mg n=13
Diastolic BP (supine) (mmHg)	p = 0.1372	1.5 (-1.2, 4.2) p=0.2674	-0.6 (-3.1, 2.0) p=0.6652	-1.7 (-4.3, 1.0) p=0.2067
Diastolic BP (standing) (mmHg)	p = 0.0021	0.1 (-3.4, 3.5) p=0.9682	-6.2 (-9.5,-2.8) p=0.0007	-2.2 (-5.7, 1.2) p=0.1995
Diastolic BP (standing-supine) (mmHg)	p = 0.0028	-1.0 (-4.3, 2.3) p=0.5428	-5.5 (-8.6,-2.5) p=0.0009	-0.3 (-3.4, 2.9) p=0.8698
Systolic BP (supine) (mmHg)	p = 0.0379	-0.4 (-4.0, 3.3) p=0.8436	-4.5 (-8.0,-0.9) p=0.0149	-3.4 (-7.0, 0.2) p=0.0632
Systolic BP (standing) (mmHg)	p = 0.0030	-1.7 (-6.0, 2.6) p=0.4277	-7.8 (-12.0,-3.7) p=0.0005	-1.6 (-5.9, 2.7) p=0.4507
Systolic BP (standing-supine) (mmHg)	p = 0.0129	-1.7 (-5.3, 1.9) p=0.3445	-4.9 (-8.4,-1.3) p=0.0090	0.8 (-2.8, 4.5) p=0.6441
Heart rate (supine) (BPM)	p < 0.0001	6.9 (3.4,10.3) p=0.0003	9.4 (6.3,12.6) p<0.0001	-4.5 (-7.8,-1.2) p=0.0099
Heart rate (standing) (BPM)	p < 0.0001	8.7 (2.9,14.5) p=0.0042	16.0 (10.4,21.5) p<0.0001	-4.4 (-10.3, 1.5) p=0.1390

Pharmacokinetics

The mean T_{max} of mecamylamine was 2.1 hours (range 1–3.3) with a C_{max} of 33.9 ng·ml^{-1} (range 23.4–44.1) for the 10 mg dose and 2.5 hours (range 0.5–6) with a C_{max} of 64.5 ng·ml^{-1} (range 45.9–80.1) for the 20 mg dose (table 3). When analysing the individual plots the terminal half-life was estimated to be 8.5 hours for 10 mg and 11.7 hours for 20 mg mecamylamine (Figure 1). This difference was not statistically significant. Other pharmacokinetic parameters were estimated as follows: Cl/F = 17.9 L·h^{-1} (range 15.1–20.7) and Vd/F = 283 L (range 260–307).

Scopolamine pharmacokinetics could not be described in detail due to the low sample frequency after administration of scopolamine. The mean C_{max} of scopolamine was 2549 pg·ml^{-1} (range 1349–4835) measured 15 minutes after the start of scopolamine infusion in all subjects. This is consistent with a previously published PK model of scopolamine (Liem-Moolenaar *et al*, 2011).

FIGURE 1 Mecamylamine plasma concentrations vs. time per dose group. Dots represent the measured mecamylamine concentrations. The dotted line represents the mean and the schaded polygon the lower and upper 95% confidence intervals.



Pharmacodynamics

The main outcome parameters of the pharmacodynamic effects are summarized in table 4 and figure 3; more detailed information is reported in the supplementary material online. Both administration of scopolamine and the 20 mg dose of mecamylamine led to a significant decrease compared to placebo in performance on adaptive tracking, the second and third trial of the immediate recall and the delayed recall of the visual verbal learning test (figure 2), finger tapping, body sway and VAS alertness. The effects of scopolamine were significantly stronger than those of mecamylamine on all these parameters, except for finger tapping and body sway. In contrast to mecamylamine, scopolamine administration resulted in an increase in reaction time and an increased score on the VAS for calmness compared to placebo. Scopolamine also induced a decrease in performance on all N-back parameters, a decrease in alpha and beta power on the p-EEG, and a decreased performance on the first immediate recall and the delayed recognition of the VVLT, the SRT and saccadic peak velocity and accuracy and smooth pursuit eye movements, while mecamylamine administration did not affect these tests. On the Stroop test, mecamylamine administration led to a decrease in reaction time compared to placebo, while scopolamine led to an increase in performance. Saccadic reaction time only increased after administration mecamylamine. No consistent differences between mecamylamine and placebo could be observed for N-back, SRT, p-EEG, saccadic inaccuracy, saccadic peak velocity, smooth pursuit eye movements and VAS Calmness. Reaction time on the VVLT recognition, pupil size and VAS mood were not affected by either scopolamine or mecamylamine compared to placebo.

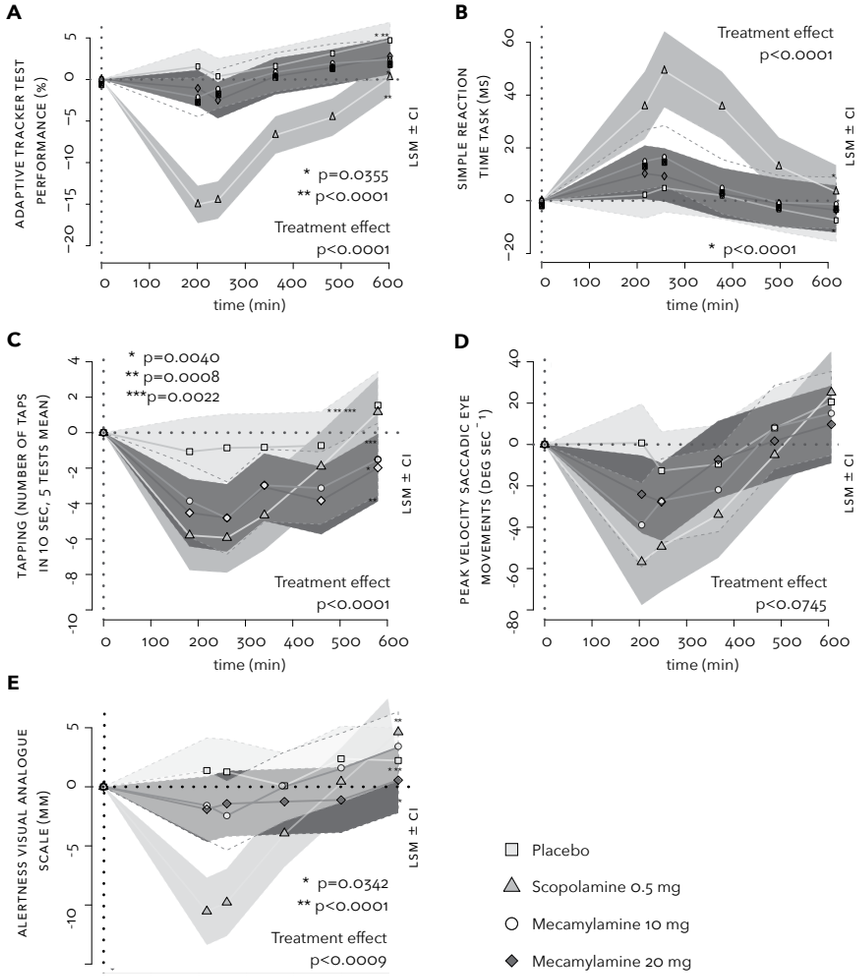
TABLE 3 Summary of mecamylamine pharmacokinetic parameters.

	Mecamylamine 10 mg (n=12)				Mecamylamine 20 mg (n=14)			
Characteristic	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Cmax (ng/ml)	33.9	5.96	23.4	44.1	64.5	10.9	45.9	80.1
Tmax (hr)	2.05	0.92	1	3.28	2.57	1.61	0.5	6
Terminal half life (hr)	8.48	1.47	5.44	11.22	11.66	5.41	6.16	23.9
AUC$_{0\text{-inf}}$	503.8	126.3	332.9	746.1	1346.1	564.7	672.3	2621.8
AUC$_{0\text{-last}}$	410.1	90.0	277.7	607.0	913.8	187.3	603.5	1260.6

TABLE 4 Pharmacodynamic effects on cognitive tests. Per group the difference estimate and in parenthesis the confidence interval is presented.

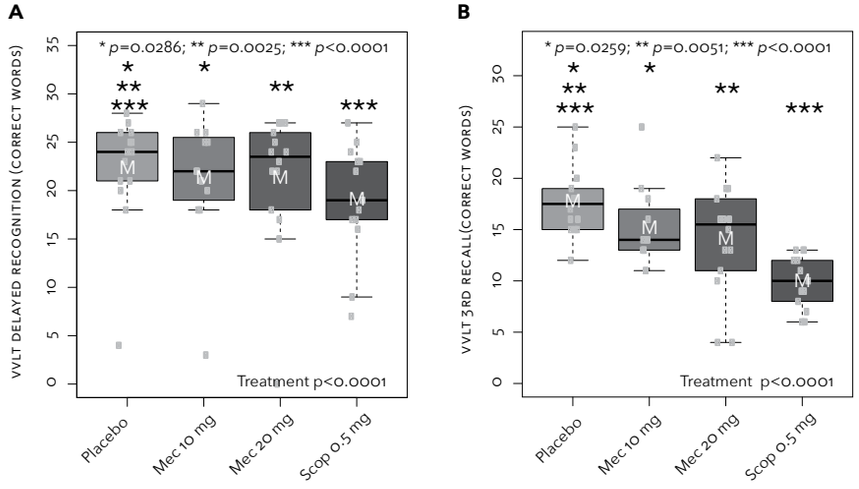
	Treatment effect	Mecamylamine 10 mg n=12	Mecamylamine 20 mg n=14	Scopolamine 0.5 mg n=13
Adaptive tracking (%)	p < 0.0001	-1.89 (-3.90, 0.12) p=0.0647	-2.06 (-3.97,-0.15) p=0.0355	-10.4 (-12.4,-8.39) p<0.0001
VAS alertness (mm)	p = 0.0009	-1.3 (-3.7, 1.2) p=0.2962	-2.5 (-4.8,-0.2) p=0.0342	-5.3 (-7.7, -2.9) p<0.0001
Finger tapping (taps in 10 sec)	p = 0.0025	-2.87 (-4.75,-0.99) p=0.0040	-3.25 (-5.05,-1.46) p=0.0008	-3.04 (-4.89,-1.18) p=0.0022
VVLT 3rd recall (number of words)	p < 0.0001	-2.7 (-5.1, -0.3) p=0.0286	-3.6 (-5.9,-1.4) p=0.0025	-7.7 (-10.1, -5.4) p<0.0001
VVLT delayed recall (number of words)	p < 0.0001	-3.1 (-5.8, -0.4) p=0.0259	-3.8 (-6.4,-1.2) p=0.0051	-7.1 (-9.8, -4.5) p<0.0001
Simple reaction time task (% change)	p < 0.0001	7.0% (-0.8%, 15.5%) p=0.0786	3.8% (-3.5%, 11.7%) p=0.3080	26.8% (17.6%, 36.8%) p<0.0001
Saccadic peak velocity (deg·sec^{-1})	p = 0.0745	-14.3 (-33.5, 4.8) p=0.1367	-10.9 (-29.0, 7.1) p=0.2232	-25.4 (-44.2, -6.6) p=0.0098

FIGURE 2 Effect on Tests Evaluating Fine Coordination, Reaction Time, Alertness, Motor Fluency and Eye Movements. Mecamylamine 10 mg, mecamylamine 20 mg, scopolamine 0.5 mg or placebo effect versus time during the Adaptive Tracking test, Simple Reaction Time Task, Tapping, Peak Velocity of the Saccadic Eye Movements and the Visual Analogue Scale evaluating Alertness.



Symbols represent the least square means per treatment group and the polygon (shaded area around the mean) the predicted confidence interval. Asterisks represent significance between groups (p value is mentioned per overall treatment effect and per group, when applicable). Vertical discontinuous line represents time point zero and the horizontal line represents zero.

FIGURE 3 Effect on Tests Evaluating Retrieval. Mecamylamine 10 mg, mecamylamine 20 mg, scopolamine 0.5 mg or placebo effect versus time during the Delayed Word Recognition and the number of correct answers during the third Recall condition of the Verbal Visual Learning Test. The box plots represent the first and third quartile, the middle line the group mean and the 'M' represents the median. The vertical lines the confidence interval. Individual observations are plotted as well.



DISCUSSION

In this study, we investigated the pharmacodynamic and pharmacokinetic profile over time of mecamylamine using an extensive CNS test battery that included cognitive as well as visuomotor and neurophysiological measures. Two oral doses of mecamylamine were compared to intravenously administered scopolamine and placebo in order to determine the profile of a nAChR specific anti-cholinergic pharmacological challenge model. All treatments administered were considered safe and well tolerated, since all adverse events were transient and mild to moderate in severity. Pharmacokinetics of scopolamine are in line with previously described results (Liem-Moolenaar *et al*, 2011). The plasma concentrations of mecamylamine almost doubled with the doubling of the dose, which suggests dose-proportionality, as has been described before (Young *et al*, 2001).

Mecamylamine showed a dose dependent decrease in performance on several tests that represent different cognitive domains. The decline in performance on adaptive tracking and reduced VAS alertness reflected a deficiency in sustained attention. The decrease on the third trial of the immediate and the delayed recall

of the VVLT represents a reduction in learning ability and memory retrieval. This mecamylamine induced impairment in acquisition and recall of information was expected, based on the localisation of nAChRs in the brain (Paterson and Nordberg, 2000). These effects last up to 10 hours after drug administration. Mecamylamine did not have any significant effects on measures for sedation (SRTT and saccadic peak velocity).

The cognitive effects of mecamylamine found in this study are consistent with previous research, where mecamylamine was administered at doses of 5, 10 and 20 mg to healthy young and elderly volunteers (Newhouse *et al*, 1992, 1994). In these studies, the effects on cognition were studied one and two hours after dosing. A dose-dependent decrease in learning ability and reaction time was reported, which was more pronounced in elderly volunteers. There was no effect on subjective scales for drowsiness. Another study reported significant decrease in learning ability and semantic memory after administration of 15 mg mecamylamine (Little *et al*, 1998) and also a decrease in inspection time after administration of 20 mg of mecamylamine was reported (Thompson *et al*, 2000). Cognitive testing was done at one (Little *et al*, 1998; Thompson *et al*, 2000) or two (Newhouse *et al*, 1992, 1994) time points after dosing and tests for sustained attention were not performed in these studies. In none of the previously mentioned studies plasma mecamylamine concentrations were measured.

Conversely, several other studies found no effects of mecamylamine on various cognitive tests (Dumas *et al*, 2008; Ellis *et al*, 2006; Erskine *et al*, 2004; Green *et al*, 2005; Thienel *et al*, 2009; Voss *et al*, 2010). However, these studies all used a dose of 15 mg and investigated the cognitive effects at only one time point after dosing. With this relatively low dose and measurements at only one time point, modest effects may have been missed. This is supported by the finding that the attentional network measured with fMRI was down regulated after administration of the same dose of mecamylamine, while cognitive tests were not influenced (Dumas *et al*, 2010; Thienel *et al*, 2009). The slightly higher dose of mecamylamine and the frequency and sensitivity of our test may have attributed to the positive results of our study.

The second aim of this study was to compare the mecamylamine model with the anti-muscarinic scopolamine model. Several previous studies attempted to do this before, but none of these studies found significant cognitive effects of mecamylamine to compare with, probably due to low doses and few measurements (Dumas *et al*, 2008; Ellis *et al*, 2006; Erskine *et al*, 2004; Green *et al*, 2005; Little *et al*, 1998; Voss *et al*, 2010). In this study, scopolamine had a significant effect on all cognitive domains measured, including inhibition and working memory, as has been described before (Broks *et al*, 1988; Ellis *et al*, 2006; Green *et al*, 2005; Liem-Moolenaar *et al*, 2011; Little *et al*, 1998). The increase in reaction time and decrease in saccadic peak velocity, which was not observed after mecamylamine administration, and the larger reduction of VAS alertness, suggest that scopolamine has a strong sedative effect. These sedative effects of scopolamine have been previously reported (Kamboj and Curran, 2006; Koller *et al*, 2003; Pergolizzi *et al*, 2012). It is unlikely that this is related to relative dose differences between the doses of mecamylamine and scopolamine given in this study, since sedation is also reported after lower doses of scopolamine (Koller *et al*, 2003) and mecamylamine has been given as antihypertensive in doses up to 80 mg in the past without any relevant sedation. The brainstem and basal brain areas controlling arousal and wakefulness contain more mAChR than nAChR (Brown *et al*, 2012), which is a likely explanation for the difference in sedative effects between mecamylamine and scopolamine. The scopolamine induced sedation may contribute to the cognitive effects of scopolamine in this study which are more pronounced than those of mecamylamine (Ford *et al*, 1956; Mcqueen and Smirk, 1957). The larger magnitude of the effects of scopolamine may seem attractive, but smaller, though still relevant effects of a new compound might get lost in the margins of variability or get overshadowed by the sedation caused by scopolamine. Due to the absence of sedation, the mecamylamine challenge may not only be more suitable for proof of pharmacology studies with a nAChR agonist, but also for other procognitive compounds.

We can conclude from this study that the nicotinic anticholinergic pharmacological challenge with mecamylamine results in measurable cognitive deficits with a nAChR specific profile, which is clearly distinguishable from the profile of the mAChR antagonist scopolamine. The mecamylamine challenge could therefore be suitable for proof of pharmacology studies with nAChR agonists. Furthermore, the relevant lack of sedation is an advantage of the mecamylamine challenge, compared with the scopolamine challenge.

A PK-PD-model of mecamylamine would be helpful in designing studies with the mecamylamine challenge. However, with the results of this study, PK-PD-modelling of the neurophysiological endpoints was not possible due to the narrow range of difference in pharmacodynamic effects between the mecamylamine lower and higher dose.

In conclusion, this study demonstrated that mecamylamine causes nicotinic receptor specific temporary decline in cognitive functioning and affects different CNS domains. Compared with the scopolamine model, pharmacodynamic effects were less pronounced at the dose levels tested and caused less sedation. Whether the mecamylamine model can be used for proof-of-pharmacology of nicotinic acetylcholine receptor agonists remains to be established.

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CHAPTER 4

Pharmacokinetics and pharmacodynamics of oral mecamylamine – development of a nicotinic acetylcholine receptor antagonist cognitive challenge test using modelling and simulation

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ABSTRACT

A pharmacologic challenge model with a nicotinic antagonist could be an important tool not only to understand the complex role of the nicotinic cholinergic system in cognition, but also to develop novel compounds acting on the nicotinic acetylcholine receptor. The objective was to develop a PK-PD model using non-linear mixed effects (NLME) methods to quantitate the pharmacokinetics of three oral mecamylamine doses (10, 20 and 30 mg) and correlate the plasma concentrations to the pharmacodynamic effects on a cognitive and neurophysiologic battery of tests in healthy subjects. A one-compartment linear kinetic model best described the plasma concentrations of mecamylamine. Mecamylamine's estimated clearance was 0.28 ± 0.015 $L \cdot min^{-1}$. The peripheral volume of distribution (291 ± 5.15 L) was directly related to total body weight. Mecamylamine impaired the accuracy and increased the reaction time in tests evaluating short term working memory with a steep increase in the concentration-effect relationship at plasma concentrations below 100 $\mu g.L^{-1}$. On the other hand, mecamylamine induced a decrease in performance of tests evaluating visual and fine motor coordination at higher plasma concentrations (EC_50 97 $\mu g.L^{-1}$). Systolic and diastolic blood pressure decreased exponentially after a plasma mecamylamine concentration of 80 $\mu g.L^{-1}$, a known effect previously poorly studied in healthy subjects. The developed mecamylamine PK-PD model was used to quantify the effects of nicotinic blockade in a set of neuro-physiologic tests in humans with the goal to provide insight into the physiology and pharmacology of the nicotinic system in humans and the possibility to optimize future trials that use mecamylamine as a pharmacological challenge.

INTRODUCTION

Integrity of the cholinergic system is essential for maintaining adequate cognitive functions. Impairment of the system is seen in both neurodegenerative and psychiatric conditions such as Alzheimer's disease (AD) and schizophrenia and has become an important therapeutic target.

Scopolamine, a selective competitive muscarinic antagonist, has been widely used as a challenge drug to induce temporary disturbances resembling those of Alzheimer's disease (AD) (Ebert and Kirch, 1998). Scopolamine administration induces mainly disturbances in visuo-spatial memory and orientation, short-term verbal, numeric and episodic memory, attention and acquisition (Flicker *et al*, 1992; Molchan *et al*, 1992; Ray *et al*, 1992; Snyder *et al*, 2005; Zemishlany and Thorne, 1991). These cognitive effects were also confirmed by different methods in which scopolamine also induced a diminished hippocampal activation in the MRI (Sperling *et al*, 2002), increased slow frequency waves on EEG (Ebert and Kirch, 1998) and magnetoencephalographic band specific functional brain connectivity disturbances observed in young healthy subjects, similar to those of patients with Alzheimer's disease (Bajo *et al*, 2015). However in the last decade, interest has increased towards understanding the nicotinic acetylcholine receptor (nAChR) and its role in different cognitive functions (Levin, 2002), consequences of functional abnormalities (Court *et al*, 2000) and possible uses as therapeutic target (Hurst *et al*, 2013). The use of a muscarinic agonist as scopolamine would seem less appropriate to investigate cognitive functions involved with nicotinergic agonists and compounds with activity on the nicotinergic system in general.

Mecamylamine (a selective nicotinic acetylcholine receptor antagonist) has slowly regained attention amongst neuroscientists after being an almost obsolete and forgotten drug to treat hypertension (Shytle *et al*, 2002). In the past two decades several studies have explored the neuro-physiological effects of mecamylamine in healthy subjects. Mecamylamine 10 mg induced significant impairment in learning in healthy elderly (Newhouse *et al*, 1994a). In younger subjects, however, mecamylamine doses below 20 mg generally do not produce significant cognitive deterioration (Ellis *et al*, 2009; Little *et al*, 1998; Newhouse *et al*, 1994a; Voss *et al*, 2010). Mecamylamine 20 mg in younger healthy subjects cause significant increases in the number of errors in a learning and retrieval task, and an increase in the inspection time during a visual discrimination test, effect that was partially reversed by 5 mg of donepezil (a cholinesterase inhibitor) (Thompson *et al*, 2000). Several authors have suggested that co-administration of scopolamine and mecamylamine would better resemble cognitive impairment observed in AD

patients (Ellis *et al*, 2006; Little *et al*, 1998). For a proper characterisation of nicotinic-muscarinic interactions, it is important to first quantify the neuro-physiological effects induced of either compound alone. We have previously described the concentration-effect relationships of scopolamine (Alvarez-Jimenez *et al*, 2016; Liem-Moolenaar *et al*, 2011); and we have now examined the concentrations and effects mecamylamine alone in healthy subjects, in order to determine the plasma concentration-effects (PK-PD) relationship of mecamylamine. PK-PD modelling is a widely used technique that integrates the exposure (measured using the plasma concentrations) and effects in a semi-mechanistic model approach in order to better interpret and understand experimental results and trial outcomes. The technique has gain popularity since it provides a more mechanistic explanation of the studied system and offers the possibility to create hypothetical scenarios by simulating outcomes in different situations offering a confirmatory rather than exploratory approach to clinical trials (Danhof *et al*, 2007).

A PK-PD model of mecamylamine-induced neurophysiological and cognitive effects may be used to optimise pharmacological challenge tests of this compound, to explore the effects of antagonism of the nicotinergic system and possible reversal by selective agonists.

In the current experiments, three mecamylamine doses (10, 20 and 30 mg compared to placebo) were administered to healthy subjects to further correlate the plasma mecamylamine concentrations with the effects while concentrations and effects were frequently measured. We utilized non-linear mixed effects (NLME) methods to quantitatively correlate the pharmacokinetic plasma mecamylamine concentrations to the pharmacodynamic cognitive and neurophysiologic effects in healthy subjects based on two related clinical studies.

METHODS

Study population

Forty-four healthy male subjects between 18 and 45 years of age (under and upper limits included) participated in two clinical studies performed at the Centre of Human Drug Research (Leiden, the Netherlands). Information on demographics and dose levels administered can be found in Table 1. A medical ethics committee approved the study protocols. After giving written informed consent, all subjects were medically screened prior to study participation. Exclusion criteria included the use of agents or drugs known to influence cognitive performance and evidence of relevant medical abnormalities including conditions that could cause any kind of cognitive impairment.

TABLE 1 Subject demographics.

	Exploratory (first) trial	Confirmatory (second) trial	All
Subjects (n)	14	30	44
Age ξ (years)	25.4 (19 - 36)	23.5 (19 – 35)	24.3 (19 - 36)
Weight (kg)	80.3 ± 9.14	75.2 ± 8.47	77.3 ± 9.06
Height (cm)	182.5 ± 6.22	181.6 ± 6.16	182.0 ± 6.17
BMI ($kg \cdot m^{-2}$)	24.0 ± 2.18	22.8 ± 2.44	23.3 ± 2.41
Mecamylamine doses (mg)	Placebo, 10 and 20 mg	Placebo and 30 mg	NA

Mean ± Standard Deviation. ξ Mean (minimum-maximum). NA: not applicable.

Study design

Data for this analysis were obtained from two related clinical studies. The first was an exploratory study intended to describe the cognitive effects of mecamylamine10 and 20 mg to those of scopolamine. In the second study, after performing an interim analysis to determine a safe dose increase, the dose range was expanded to 30 mg, and the effects of two cholinergic agonists (nicotine and galantamine) were examined. Galantamine was chosen as it exerts an allosteric modulatory activity on the nAChR, which other cholinesterase inhibitors lack (Coyle and Kershaw, 2001; Maelicke *et al*, 2001; Maelicke and Albuquerque, 2000). Both studies are in preparation for publication. However, since neither of the manuscripts would allow an integrated description of concentration-effect relationships for mecamylamine across the full (10-30 mg) dose range, we decided to perform a separate dedicated PK-PD analysis that is described in this article.

In both studies mecamylamine was administered orally in fasting conditions. Subjects were fasting for at least 4 hours and administration occurred with water. Mecamylamine capsules (Euticals SpA, Milan, Italy) containing mecamylamine hci and microcrystalline cellulose as filling agent (used also in the placebo capsules) were administered orally in blinded conditions. Plasma mecamylamine concentrations were determined using a validated, selective and sensitive liquid chromatography coupled to tandem-mass spectrometry (LC-MS/MS) method (Lower Limit of Quantification for the first trial was 1.54 $\mu g.L^{-1}$ and for the second trial lowered to 0.5 $ng.L^{-1}$).

The NeuroCart battery of tests evaluating different neurophysiological, psychomotor and cognitive tests was performed to quantify mecamylamine pharmacodynamic effects on different domains. The battery of tests has been previously extensively used in clinical drug development, and detailed descriptions can be found in other publications on a range of different compounds (de Haas

et al, 2008; Liem-Moolenaar *et al*, 2010a, 2010b, 2011; van Steveninck *et al*, 1999; Strougo *et al*, 2008), including anticholinergic challenge tests (Liem-Moolenaar *et al*, 2010a, 2010b, 2011). On each study day, all pharmacodynamic tests were performed frequently at different time points per occasion in a quiet room with ambient illumination with only one subject in the same room (and a research assistant) per session. During the first trial, the NeuroCart test battery was subsequently performed at time points 30, 60, 120, 180, 195, 240, 480, 600 and 1320 minutes and for the second trial at time points 30, 80, 130, 180, 230, 280, 360 and 480 minutes post-dose. Washout periods between occasions were at least one week in both studies.

All subjects were thoroughly trained and familiarized with the psychometric tests within 21 days preceding study start to minimize inter-individual variability at baseline and to make sure subjects were able to understand and perform the tests. Each baseline assessment (pre-dose battery of tests) was performed twice at the beginning of each occasion. The mean of the two pre-dose measurements was used as baseline. A combination of tests evaluating neurophysiological and cognitive variables was analysed. Tests were included in the PK-PD analysis if they showed a statistically significant effect at 30 mg when compared to placebo. The blood pressure was modelled as a secondary measure since it was also used to determine the maximum tolerable dose for the second study, to predict the tolerability of mecamylamine in healthy subjects.

Adaptive tracker test

The test evaluates attention and executive skills such as visuo-motor coordination (Borland and Nicholson, 1984; van Steveninck *et al*, 1991). Subjects were asked to use a joystick to keep a randomly moving target on the screen inside a circle. The percentage of time that the target was kept in the circle was calculated. Even though attention is a cognitive process involved in numerous functional areas and therefore can be indirectly measured via many cognitive tests, the adaptive tracker is a more specific test for attention (arousal, vigilance) as the complexity of the test resides in sustained attention since it is very simple to perform from a psychomotor performance point of view. We have shown earlier that the adaptive tracker test was very sensitive to subtle disturbances in attention caused by ethanol, sleep deprivation, and benzodiazepines, and also to subtle enhancements by e.g. caffeine and donepezil in healthy subjects (van Steveninck *et al*, 1991, 1999; de Visser *et al*, 2003).

N-back test

Subjects were instructed to remember and correlate a sequence of letters presented in a random order, thereby allowing evaluation of (short-term) working memory (Lim *et al*, 2008). Performance was expressed as the percentage of correct answers on the 0-back paradigm, and as reaction time of all answers on the 2-back paradigm. The fraction of correct answers was logit-transformed prior to model fitting.

Based on exploratory data analysis, the following NeuroCart tests were not considered in the model, because no significant effect of 30 mg of mecamylamine compared to placebo was observed: Visual Analogue Scales (evaluating alertness mood and calmness), Finger Tapping (evaluating motor fluency), Visual Verbal Learning Test (evaluating verbal working memory), Milner Maze Test (evaluating visuo-spatial working memory) and electroencephalogram (EEG). The electroencephalogram was measured tasks free during one minute with eyes closed.

Software

Pharmacokinetics and pharmacodynamics analyses were performed using non-linear mixed-effect (NLME) modelling in NONMEM v7.2 and v7.3 (Beal *et al*, 2009). The database and all graphs were created using R v2.13.1 (R Core Team, 2013). Statistical analysis and calculations were performed using sas software for windows v9.4 (sas Institute, Inc., Cary, NC, USA).

Model development and evaluation

Plasma mecamylamine concentration-time dependent data were analysed using a consecutive NLME modelling approach; once the best pharmacokinetic model was obtained, the individual pharmacokinetic parameter estimates were fixed to develop the pharmacodynamic models. The first order conditional estimation method with interaction (FOCE-I) was used. Several compartment models were explored for the pharmacokinetic model. Weight, height, age, body mass index and body surface area (calculated using DuBois's formula) were tested as potential covariates for parameters on which inter-individual variability (IIV) could be identified and were incorporated in the model as covariates if needed.

For the pharmacodynamic endpoints, several structures including direct and indirect (using an effect compartment) sigmoidal, truncated, linear, exponential

and E_{max} model structures were tested. Delay compartments were taken into consideration for the pharmacodynamics models only when an indirect model was chosen.

For all models, once the structural model was defined additive, proportional, exponential or combined error models were tested. IIV was tested in each parameter estimate and correlations between post-hoc Bayesian parameter estimates and between post-hoc Bayesian parameter estimates and potential covariates were explored using coefficient of determination (r^2). Correlations with an $r^2 \geq 0.4$ that were considered clinically relevant were taken forward in formal testing of omega block structures and covariate analysis (weight, age and height). Competing models were compared based on their Goodness of Fit (GOF) plots, decrease of the objective function value (OFV), plausibility of parameter estimates, residual error, parameter precision (in terms of residual standard error; RSE), shrinkage and parameter distribution. The OFV is a goodness of fit statistic defined as minus two times the logarithm of the likelihood and it is provided in each model's output file provided by NONMEM. A decrease in the OFV of at least 3.84 units ($p<0.05$) was considered statistically significant. GOF plots included observations vs. population and individual predictions, conditional weighted residuals with interaction (CWRESI) vs. time and CWRESI vs. observations and IIV frequency distribution, boxplots and QQ graphs. The VPCs were obtained by simulating 1000 subjects, using the population parameter estimates and the full variance-covariance matrix. Covariates were sampled from the observed population distribution.

RESULTS

Model development – Plasma mecamylamine concentrations

Shortly after oral mecamylamine administration, plasma mecamylamine concentrations increased rapidly and, once they reached the equilibration phase, plasma mecamylamine concentrations decreased gradually (Figure 1). A one-compartment (consisting of a dose and a central compartment) linear pharmacokinetic model structure best described the plasma mecamylamine pharmacokinetic data. A two-compartment linear model resulted in a negligible inter-compartmental clearance estimate (0.000022) with a gradient that approached zero and therefore the model was abandoned. Non-linear (Michaelis-Menten) kinetics was also tested. This provided no improvement in the fit or OFV and produced an estimated k_m above the measured concentrations (158 $\mu g \cdot L^{-1}$) and was therefore rejected. Inter-individual variability could be identified on the lag time related to the oral administration (ALAG time), absorption rate constant (k_12) and clearance (CL).

The estimation of the elimination rate (k20) was dependent on the clearance and the central apparent volume of distribution as showed in Equation 1. Body weight was identified as covariate on the central volume of distribution (V) (r^2=0.66, $p<0.01$) and incorporated as mean body weight-normalised covariate (ΔOFV = -27 points; Equation 2), which completely explained the inter-individual variability (IIV) on this parameter. Equation 3 and 4 show the one-compartment model differential equations and the way the lag time (ALAG) was incorporated. The rate of absorption (k_12) was negatively correlated with the lag time or ALAG (r^2 = 0.53, $p<0.01$) and an omega block structure (variance-covariance structure) was used, reducing the IIV of the ALAG (from 0.276 to 0.099) without influencing the OFV. Pharmacokinetic model graphical result estimates can be found in Table 2.

$$k_{20} = \frac{CL}{V_C} \tag{1}$$

$$\frac{V_C}{F} = e^{\left\{\left[\log(\vartheta_{V_C})\right] + \left[CWC \cdot \log\left(\frac{WGT}{77.698}\right)\right]\right\}} \tag{2}$$

$$\frac{dA_A}{dt} = -k_{12} \cdot A_A \cdot (t > ALAG1) \tag{3}$$

$$dA_C = k_{12} \cdot A_A \cdot (t > ALAG1) - k_{20} \cdot A_C \tag{4}$$

FIGURE 1 **Mecamylamine pharmacokinetics.** Plasma mecamylamine concentrations visual predictive check graphs versus time after mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the grey area the 95% predicted interval. Circles represent the observations. Red lines represent the 95% confidence interval and the dotted line in between the median of the observations.

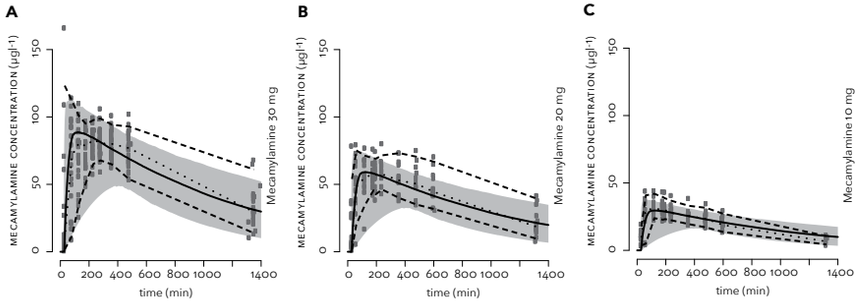


TABLE 2 Population estimates for pharmacokinetic and pharmacodynamic models for mecamylamine.
Parameters are reported as population estimate.

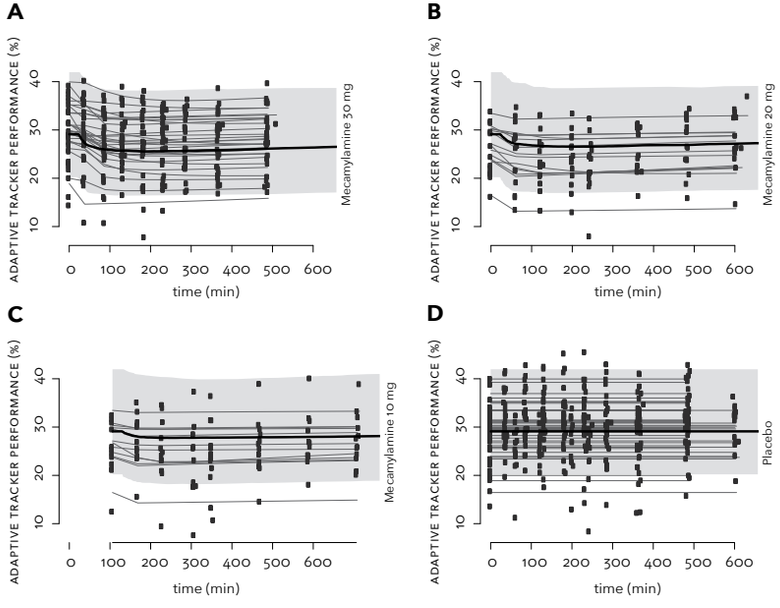
	Parameter	Units	Parameter estimate	SEM	IIV	Shrink age	Observations
Mecamylamine pharmacokinetics	$ALAG_1$	min	26.8	1.04	0.096*	19.10	Correlation -0.294
	K_{12}	min-¹	0.0335	0.0088	2.115 *	4.59	
	CL	L· min-¹	0.279	0.0154	0.326	7.63	
	v_c / f	L	291 †	5.15	-	-	
	CWV	-	0.794	0.132	-	-	
	σ^2	-	0.0328	0.00446	-	-	Proportional
Adaptive Tracker (percentage of accuracy)	EC_{50}	µg·L-¹	97.2	28.1	1.0399 *	26.7	Corr. 0.0451
	BL	-	29.0	0.82	0.182 *	0.66	
	E_{max}	%	0.27	0.053	-	-	
	γ	-	1.58	0.548	-	-	
	σ_2	-	11.5	1.2	-	-	Additive
Correct answers of the 0-back § (percentage of correct answers)	EC_{50}	µg·L-¹	8.74	17.1	-	-	
	E_{max}	%	0.377	0.163	0.885	41.3	
	BL	% correct answers	3.66	0.125	0.150	22.9	
	σ^2	-	0.00174	0.000322	-	-	Additive
Reaction Time of the 2-back paradigm (milliseconds)	BL	ms	561	12.5	0.0446◊	31.2	
	Y_{bl}	-	0.568	0.0345	-	-	
	Y_{eff}	-	0.000763	0.000219	-	-	
	σ^2	-	0.0132	0.00119	-	-	Proportional
Systolic and Diastolic Blood Pressure (mmHg)	BL_d	mmHg	70.2	0.931	0.080 *	6.91	Corr. 0.055
	BLS ‡	mmHg	121	1.26	0.064 *	6.24	
	AMP_D	mmHg	1.81	0.438	-	-	
	AMP_S	mmHg	1.12	0.422	-	-	
	PHS_D	min	8e-04	-	-	-	
	PHS_S	min	802	74.5	-	-	
	$FREQ_D$	min-1	676	-	-	-	
	$FREQ_S$	min-¹	833	-	-	-	
	$BASE_D$	-	0.0227	0.00329	-	-	
	$BASE_S$	-	0.0284	0.00318	-	-	
	$CBMI_B$	-	0.229	0.107	-	-	
	σ^2D	-	0.0109	0.001	-	-	Proportional
	σ^2S	-	0.00615	0.000598	-	-	Proportional

*IIV: Inter-individual Variability expressed as Coefficient of Variation. ‡ BMI used as a covariate. †Weight used as covariate. *Omega block structure. γ Exponent. 4 buffer compartments. § Parameters reported as natural log odds. ◊ Highest inter-occasion variability. F: oral bioavailability.*

Model development – Mecamylamine effects

PERCENTAGE OF ACCURACY OF THE ADAPTIVE TRACKER TEST Figure 2 shows the effect over time of mecamylamine administration on adaptive tracker performance (%-point accuracy). At baseline, subjects consistently scored a mean of 29 ± 0.82 %. Mecamylamine, compared to placebo, produced a decrease in performance of -1.89 %-point (confidence interval: -3.90 – 0.12; p=0.0647) after administration of 10 mg of mecamylamine, -2.06 % (-3.97 – -0.15; p=0.0355) after 20 mg of mecamylamine and -3.27 (-4.58 – -1.97; p<0.0001) after 30 mg of mecamylamine. The effect was observed promptly at the first time point after mecamylamine administration. In accordance, during PK model development, a direct E_{max} model proved similar when compared to an indirect model structure (ΔOFV = 0.6 points) and the direct model structure was therefore chosen. Equation 5 depicts the equation used to relate plasma mecamylamine concentrations (C) with the effect. The right side of the equation has as a consequence a reduction in the baseline (BL) or pre-dose value. Addition of a learning or practice effect linear and exponential function to describe the placebo data was unsuccessful since estimated OFV decreased by 12

FIGURE 2 Mecamylamine effects on the Adaptive Tracker. Performance during the Adaptive Tracker test versus time after oral mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the grey area the 95% predicted interval. The black lines represent the individual predictions. Circles represent the observations.



points. Moreover it gave negligible improvement in the fit and caused difficulties estimating the learning function (parameter with the highest gradient and covariate step aborted) and was therefore abandoned.

Adding an exponent (γ) to the E_{max} model function provided a non-significant decrease in the OFV (1 point), however it improved the shrinkage, the uncertainty of the parameters and the fit of the model and therefore was accepted. In the best model, IIV was identified for BL (ΔOFV = -809 points) and EC_{50} (ΔOFV = -152 points). An omega block was required between BL and EC_{50}.

$$Tracker = BL \cdot \left\{1 - \left[\frac{E_{MAX} \cdot C^{\gamma}}{EC_{50}{}^{\gamma} + C^{\gamma}}\right]\right\} \quad (5)$$

PERCENTAGE OF CORRECT ANSWERS IN THE 0-BACK PARADIGM OF THE N-BACK TEST Following mecamylamine administration, the number of correct answers decreased significantly with the highest dose when compared to placebo (Figure 3). Administration of mecamylamine 10 mg produced an average decrease in the 0-back ratio of correct answers of -0.03 % of correct answers (-0.08 – 0.01; p=0.1348), 20 mg -0.02 (-0.06 – 0.03; p=0.4714) and 30 mg produced a significant reduction of -0.023 (-.044 – -.003; p=0.0270). Compared to an indirect model, a direct model performed best (Equation 6). An E_{max} model proved superior compared to linear, truncated and exponential model structures (ΔOFV = 5157). A sensitivity analysis was performed to investigate the impact of one extreme outlier (Subject 6) on parameter estimation and uncertainty. Excluding this subject resulted in near identical parameter estimates and the SEM of the EC_{50} decreased from 17.1 to 4.8 µg.L^{-1}, indicating that this data point has no substantial influence of model performance. Subject 6 was included in the final model run.

$$0\ back\ correct\ answers = BL \cdot \left\{\frac{E_{MAX} \cdot C}{EC_{50} + C}\right\} \quad (6)$$

REACTION TIME IN THE 2-BACK PARADIGM OF THE N-BACK TEST Figure 4 presents how mecamylamine administration increased the reaction time of the majority of the subjects during the 2-back test. Administration of mecamylamine 10 mg produced a non-significant increase of 7 milliseconds (-37–51; p=0.7503), 20 mg -1 milliseconds (-43 – 41; p=0.9677) and 30 mg produced a significant increase of 28.3 milliseconds (2.0 – 54.6; p=0.0356) in the 2-back reaction time when compared to placebo. Addition of intra-occasion variability at baseline occurred at an early stage of model development since it was observed when fitting the data and, once implemented, resulted in a significant drop in OFV of 165 points and improved the fit of the data. The best model structure proved to be a direct model. An exponential model provided a better fit and results when compared to an E_{max} model (exponential model decreased the OFV by 22 points). The parameter estimates provided by the E_{max} model were also above the measured mecamylamine concentrations and therefore this model was rejected. Variability (inter-occasion variability) was identified only at baseline and this was sufficient to describe the data correctly. One equation was needed to correctly describe the learning or practice effect without the influence of mecamylamine (E_0), where a time-dependent function described an ascending trend seen in all subjects (Equation 7). Afterwards, this function was used in Equation 8 to characterize the effect mecamylamine exerted in the *reaction time of the 2-bask test*. Again, the concentrations (C) in the exponent multiplied by a constant (λ) related the concentrations with the effect on the test.

$$E_0(t) = BL - t^{\gamma} \quad (7)$$

$$2\ back\ RT = E_0 \cdot \left\{e^{(C \cdot \lambda)}\right\} \quad (8)$$

FIGURE 3 Mecamylamine effects on the ratio of correct answers of the 0-back paradigm. Ratio of correct answers during the 0-back paradigm versus time after oral mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the grey area the 95% predicted interval. The black lines represent the individual predictions. Circles represent the observations.

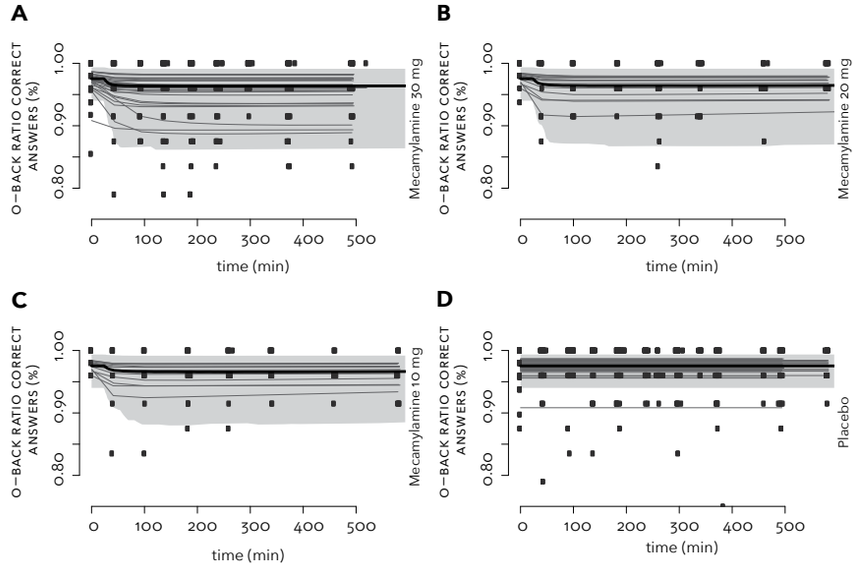
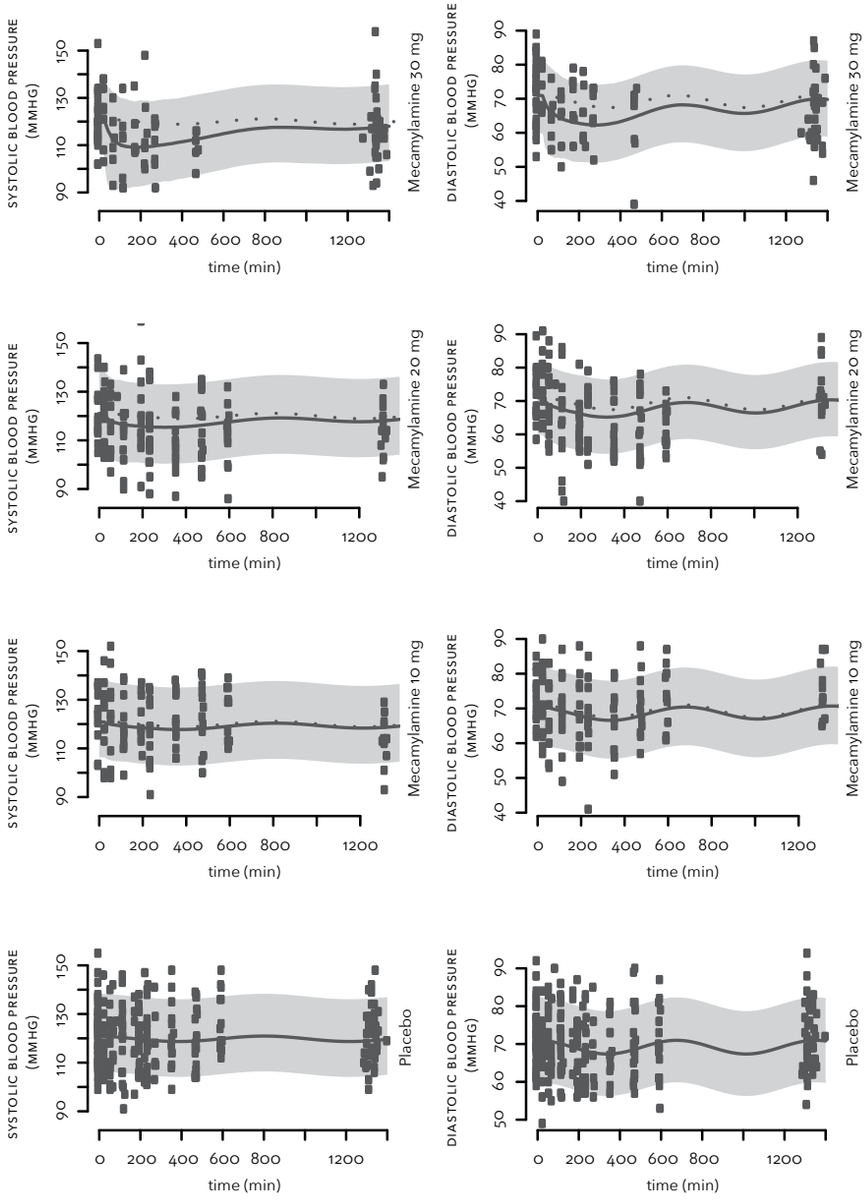


FIGURE 4 Mecamylamine effects on the blood pressure. Blood pressure versus time after oral mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the grey area the 95% predicted interval. The black lines represent the individual predictions. Circles represent the observations.



SYSTOLIC AND DIASTOLIC BLOOD PRESSURE The blood pressure decrease effect of mecamylamine was the limiting factor for the dose increase in the studies and therefore was also modelled. Figure 7 presents the time dependent graphs per mecamylamine dose. The systolic and diastolic blood pressure (SBP and DBP, respectively) were modelled simultaneously since they are intimately correlated. Rhythmic oscillations around an identity (base) line were observed in the data from the placebo group. In order to describe the baseline circadian variability, a one-cosine function was used (Van Rijn-Bikker *et al*, 2013). Shortly after mecamylamine was administered, both the SBP and DBP decreased in a dose-dependent manner. A direct truncated effect model performed better than both an E_{max} (ΔOFV = -22 points) and a linear model (ΔOFV = -12 points). A direct model structure was chosen. IIV best described the data when placed at baseline. SBP and DBP baselines were highly correlated (r^2=0.37) and physiologically plausible, therefore an omega block was placed, reducing the IIV of both parameters. The Body Mass Index (BMI) was also highly correlated to the baseline SBP (r^2= 0.49, p<0.01), adding it as a covariate produced a OFV decrease of 13 points and provided a better fit to the data. Systolic and Diastolic Blood Pressure were calculated with a cosine function of time (Equation 9), which was correlated to the plasma mecamylamine concentrations with an exponent (Equation 10). The amplitude (AMP) of the oscillations, the frequency (FREQ) and the point in the daytime that it starts (PHS) were estimated parameters. Only for the systolic blood pressure, the body mass index (BMI) was divided by the population BMI value and a constant (CBMIB) was used as correction factor to calculate the baseline as shown in Equation 11.

$$E_0(t) = BL + AMP \cdot \cos\left\{2 \cdot \Pi \cdot \left[\frac{t\text{-}PHS}{FREQ}\right]\right\} \quad (9)$$

$$BP = E_0 - (BASE \cdot e^{c}) \quad (10)$$

$$BLS = e^{\left\{\left[log(\vartheta_{BLS})\right] + \left[CBMIB \cdot \log\left(\frac{BMI}{23.25}\right)\right]\right\}} \quad (11)$$

Model evaluation

The GOF plots for all models indicate that the central and individual trend of the data is well described, and that no bias occurs over time or observations. The shrinkage was acceptable in all models except for the E_{max} estimated in the 0-back percentage of correct answers (41.3 %) and the baseline of the 2-back reaction time (31.2 %). The VPCs indicate that the variability for these parameters is well described as 95% of the data appears lie within the 95% prediction interval.

DISCUSSION

This is the first time that neurocognitive and neurophysiological effects of mecamylamine have been quantified using an exposure-effect (pharmacokinetic and pharmacodynamic) relationship approach.

Mecamylamine is a highly lipophilic secondary amine that acts by binding non-competitively and non-selectively to the nicotinic acetylcholine receptor as an antagonist to the voltage gated function of the ion channel (Varanda *et al*, 1985). Due to its chemical properties, mecamylamine distributes profusely in the body including the Blood Brain Barrier and therefore exerts its effect in the central nervous system without delay or use of an effect compartment in the model. Bioavailability of mecamylamine is unknown. Even though mecamylamine has been administered intravenously in the past, plasma concentrations have not been determined; probably due to the fact that the drug was developed more than 70 years ago when plasma concentration methods were not available (Allanby and Trounce, 1957). It has been previously reported in literature that mecamylamine bioavailability is complete, however it was determined after comparing the reduction of the systolic and diastolic blood pressure after oral and intramuscular administration in healthy subjects, without measuring plasma mecamylamine concentrations (Ford *et al*, 1956). The reported one-compartment linear-kinetic model and the estimates obtained for mecamylamine are comparable to a model developed for dexmecamylamine. Dexmecamylamine (TC-5214) is a compound with similar chemical structure, when compared to mecamylamine. The compound is currently in clinical development to treat hyperactive bladder symptoms (Xu *et al*, 2014). The authors also reported that the corrected body weight was an important covariate directly correlated to the apparent central volume of distribution, as corroborated in our model. Non-linear kinetics proved not better than zero-order clearance of plasma mecamylamine in our model. While in our model Michaelis-Menten kinetics were tested, the value estimated for the k_M, concentration at which the reaction rate is half of v_{max}, was above the measured plasma concentrations, not excluding that at higher concentrations saturation of the system may be present.

Based on previously reported work (Ellis *et al*, 2006; Little *et al*, 1998; Voss *et al*, 2010), effects induced by mecamylamine doses below 20 mg have been previously difficult to quantify in healthy subjects. In this study, we performed PK-PD modelling on statistically significant effects of mecamylamine in a dose range of 10-30 mg. This demonstrated consistent effects on all evaluated neurophysiologic tests even at dose levels as low as 10 mg. We were able to characterize the effects of administration of mecamylamine in a set of tests that were not earlier reported such as attention, vigilance and visuo-motor coordination (Adaptive Tracker) and confirm the effects previously reported in literature: impairment of learning and retrieval or working memory (N-back percentage of correct answers) and increase in reaction time (N-back reaction time).

Subjects receiving mecamylamine were more prone to commit mistakes during the 0-back paradigm compared to those in the placebo group. The estimated EC_{50} and E_{max} were low (8.7 $\mu g.L^{-1}$ and 30%, respectively) resulting in long-lasting effects (higher possibilities of making mistakes) even at low plasma concentrations. Previously reported cognitive effects after administration of 20 mg of mecamylamine (and even 10 mg of mecamylamine in elderly) include an increase in working memory errors and reaction time, compared to the placebo group (Newhouse *et al*, 1992), consistent with our findings. Nicotinic blockade in humans produces impairment in the recall and integrative brain pathways (both needed to respond correctly in the N-back paradigms), probably secondary to nAChR inactivation in the basal forebrain structures where the receptor density is high (Zoli *et al*, 2015). Despite the fact that only 18 of the total 491 (4%) plasma mecamylamine concentrations were above the EC_{50} in the Adaptive Tracker model, the E_{max} model structure described the data substantially better when compared to more simple models and was therefore accepted as most appropriate model structure. As a result, the predictive value of E_{max} for higher doses should be careful considered.

In order to use mecamylamine as a cognitive challenge model drug to explore the nicotinic central activity, and manipulate the system with drugs that exert their mechanism of action through the same nAChR receptor, it is useful to first analyse the concentration-effect relationships. The choice of the optimal mecamylamine dose should depend on the balance between desired and unwanted effects, including central and peripheral effects. Mecamylamine exerts its action in a dose-dependent manner in different brain areas, translated in an individual dose-effect relationship per cognitive area. The different evaluated effect-concentration relationships per test are shown in Figure 5. Compared with other functions, accuracy and reaction time in N-back test of working memory are relatively sensitive to mecamylamine. The decrease of the accuracy and increase in the reaction time observed in the N-back test occurs at low concentrations and reaches a steady maximum around 100 $\mu g.L^{-1}$. Above this concentration other less sensitive but potentially undesirable effects will be observed without a further clinically significant decrease in the performance on working memory. On the other hand, performance in the Adaptive Tracker (a tests evaluating attention and executive skills as visuo-motor coordination) may still decrease with higher doses

of mecamylamine, since the estimated EC_{50} concentration (97.2 µg.L^{-1}) was barely surpassed with the administration of mecamylamine 30 mg. Mecamylamine has been used in the past as a drug to treat moderately severe- to severe-hypertension due to its parasympathetic ganglionic effect. Mecamylamine effects only caused an average decrease in systolic blood pressure of 5 mmHg in our healthy subject population. This was less pronounced than the reduction of approximately 20 mmHg in hypertensive patients with hypertension, after oral administration of 20 mg of mecamylamine (Ford *et al*, 1956). Our findings further suggest, as has previously been assumed(Shytle *et al*, 2002), that one third or even less of the usual dose used to treat hypertension is enough to produce measurable central effects and higher doses than 30 mg of mecamylamine would not provide a greater decrease in tests evaluating working memory but would further decrease the blood pressure in an exponential way. Higher doses should only be considered after a careful hemodynamic risk assessment has been performed and if other cognitive areas rather than working memory are the main outcome.

FIGURE 5 Mecamylamine effects on the reaction time of the 2-back paradigm. Reaction time during the 2-back paradigm versus time after oral mecamylamine administration (time point zero) per dose. The solid line represents the model population prediction and the grey area the 95% predicted interval. The black lines represent the individual predictions. Circles represent the observations.

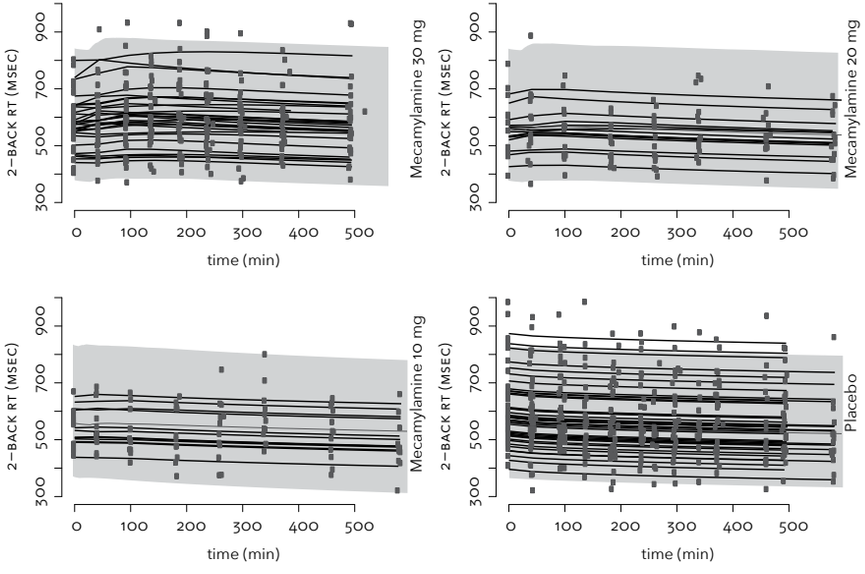
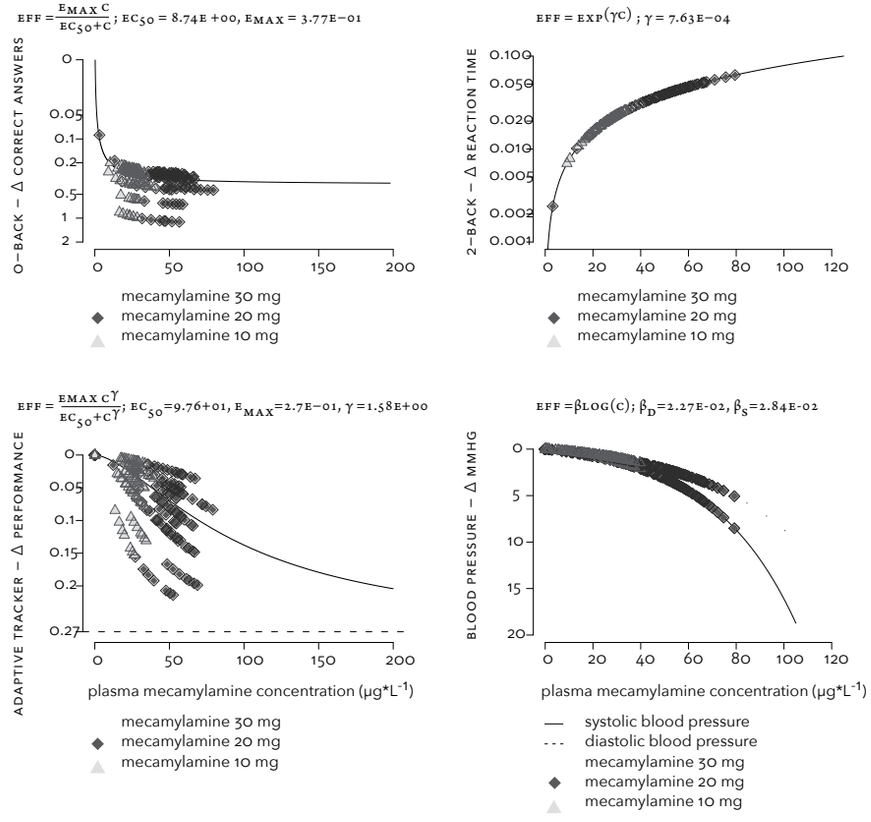


FIGURE 6 Mecamylamine concentration-effect relationships. Plasma mecamylamine concentrations versus the effect per (neuro-) physiological test (N-back percentage of correct answers and reaction time, Adaptive Tracker and Systolic and Diastolic Blood Pressure). The solid line represents the model population prediction. The dots represent the individual predictions.



Using the currently developed model could help simulate new scenarios with different mecamylamine doses based on the cognitive area of interest. A dose of 20 mg seems reasonable to induce disturbance in memory with minimum changes in the SBP as shown in Figure 5. On the other hand, the previous dose would seem insufficient to induce a decrease in visuo-spatial coordination where as shown in Figure 6, however higher doses should provide a greater decrease in performance with as consequence a more sensitive inflection point with small dose changes or co-administration of nicotinergic agonists, showed a significant effect.

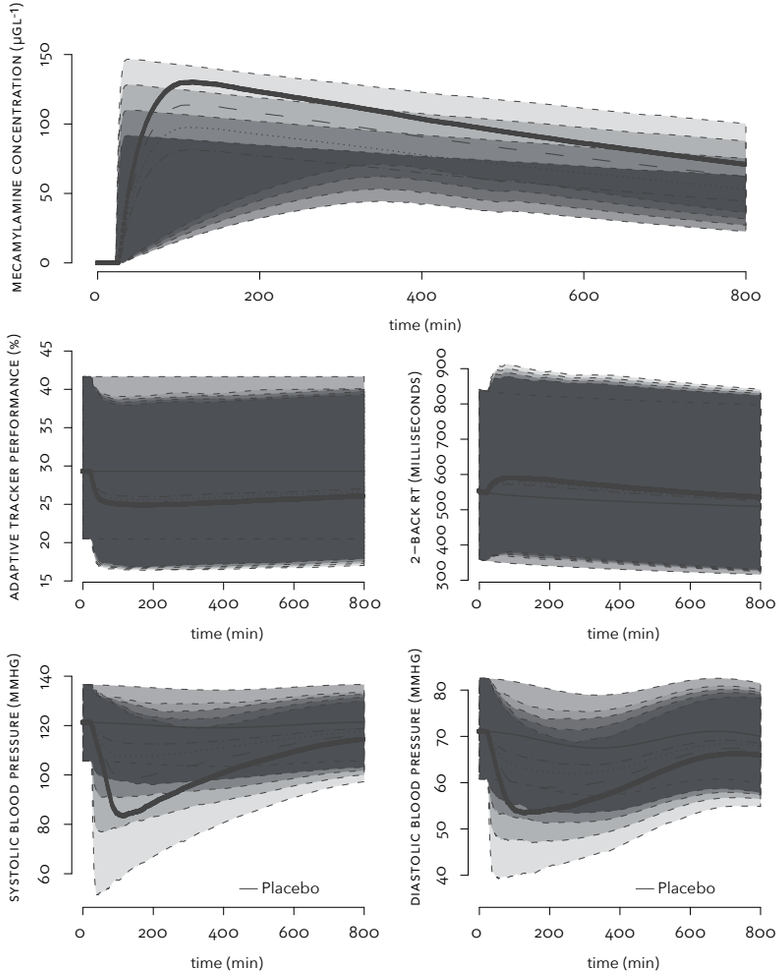
It has been proposed that elderly subjects and patients with mild cognitive impairment are more sensitive to mecamylamine effects (Newhouse *et al*, 1994a, 1994b). The developed models may be helpful to further quantitate these differences by using age as covariate in the different estimates, e.g.: the estimated EC_{50}, Hill exponent, E_{max}, depending on the structural model used. Other applications of the PK-PD-models could be the translational integration of pre-clinical and clinical study results, to further understand the implications of manipulation of the nicotinic cholinergic neuronal system.

A learning effect secondary to consecutive testing, measured as a slight improvement in performance after several repetitions during the course of the occasion, was identified for the reaction time of the N-back as has been previously described (Bartels *et al*, 2010; Collie *et al*, 2003; Goldberg *et al*, 2015). This learning effect was successfully incorporated in both models using time-dependent functions (Ito *et al*, 2010; Samtani *et al*, 2015). Mecamylamine also induced a reduction of the practice or learning curve resulting of repetition during the reaction time of the 2-back test. Even though mecamylamine 10 mg administration by itself did not produced a statistically significant effect in this test, modelling showed that corresponding low levels did decrease the ability of subjects to learn (or perform better after practicing) in a quantifiable way.

Scopolamine induces more sedative effects (Robbins *et al*, 1997) when compared to mecamylamine, and it is possible that scopolamine-induced cognitive deficits are at least partly related to sedation rather than direct disturbances of muscarinic brain cortical and basal areas involved in cognitive processing. The most sensitive tests to measure sedation (induced by sleep deprivation or pharmacological agents), namely the adaptive tracker and saccadic eyes movement tests (de Haas *et al*, 2008; van Steveninck *et al*, 1991, 1999) were less affected by mecamylamine when compared to scopolamine. The fact that scopolamine produced more sedative effects than mecamylamine is in accordance with the fact that muscarinic receptors populate more densely the brain stem (including the ascending reticular ascending system), which regulates arousal (Flynn *et al*, 1997).

The mecamylamine pharmacological challenge model is useful to investigate the role of nAChR in neuro-physiological functions and to support clinical research. The better understanding of the relationship between the plasma concentrations of mecamylamine and its pharmacodynamic effects that this model has yielded, will aid to quantify the more subtle differences in performance that with other statistical methods are not discovered. This is of particular importance when trying to show cognitive improvement due to drugs that are being developed, as detrimental effects of psychoactive compounds on cognition are already difficult to demonstrate, but reversal or improvement of cognitive functions has rarely been reported (Buccafusco, 2009). Using a pharmacokinetic and pharmacodynamic model we provide a better insight into the complexity of the mechanism of action of central nicotine receptor blockade in healthy subjects. Antagonism of the nicotinic cholinergic system using mecamylamine resulted mainly in impairment of cognitive functions such as acquisition, processing and execution. The mecamylamine model in humans could be useful as a proof of pharmacology tool in drug development of novel nicotinic agents.

FIGURE 7 Mecamylamine simulation of different doses. Plasma mecamylamine concentrations and resulting effects in the different physiologic and neurologic tests versus time. The simulations were performed using a normalized weight of 70 kg.



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CHAPTER 5

First in human study with a prodrug of galantamine: improved benefit-risk ratio?

Alzheimers Dement (NY). 2016 Jan 20;2(1):13-22

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ABSTRACT

INTRODUCTION Gln-1062 (Memogain®) is a pharmacologically inactive prodrug of galantamine. Due to its lipophilic nature, it preferentially enters the brain, where it's cleaved into active galantamine. Gln-1062 is expected to have fewer peripheral side effects than other ChEIs, with improved effectiveness.

METHODS This was a double-blind, comparator and placebo-controlled, sequential cohort, single ascending dose study in 58 healthy subjects with Gln-1062 in doses of 5.5 mg, 11 mg, 22 mg, 33 mg and 44 mg, compared to oral galantamine 16 mg and donepezil 10 mg. Safety, tolerability, pharmacokinetics and pharmacodynamics were assessed.

RESULTS Gln-1062 doses up to 33 mg were well tolerated and induced a dose-dependent increase in the plasma concentrations of Gln-1062 and galantamine. Gln-1062 had a dose dependent positive effect on verbal memory and attention, mainly in the first hours after drug administration.

DISCUSSION Gln-1062 was better tolerated than galantamine in doses with the same molarity and led to improved effects in cognitive tests. This is most likely caused by the more favourable distribution ratio between peripheral and central cholinesterase inhibition. These results give reason for further exploration of this compound.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia. Its pathogenesis involves the progressive development of amyloid plaques and tangles, loss of cholinergic neurons and cholinergic deficiency. Recent trials with disease modifying compounds, such as gamma secretase inhibitors and monoclonal antibodies against amyloid beta, have had negative results.[1-3] Post-hoc analysis of trial data of studies with solanezumab in patients with mild AD and the first results of trials with aducanumab in patient with mild or prodromal AD seem to underline the idea that disease modification might only be useful in earlier stages of the disease.[4;5] All trials in patients with moderate or severe AD with disease modifying compounds have been negative so far. The first registered treatment in line for the symptoms of mild to moderate AD are cholinesterase inhibitors (ChEIs). Although not curative, ChEIs can reduce symptoms for 6-36 months.[6] However, this positive effect is only seen in 14-36% of patients.[7-11] Administration of higher doses, for example 24 mg of galantamine or 23 mg of donepezil, leads to an increase in peripheral side effects, such as nausea, vomiting and diarrhoea, which overshadows a possible positive effect on cognition and functioning in daily life.[12;13] As disease modification has not yet been demonstrated for any drug in patients with AD, it is worthwhile to optimize the available symptomatic drugs. Therefore, Gln-1062 (Memogain®) was developed as a modification of galantamine having much higher lipophilicity and hence higher preference for the brain than the parent drug. Gln-1062 was designed as an inactive pro-drug (in casu a benzoic ester) of galantamine that, after entering the brain, is cleaved into active galantamine by a carboxy-esterase. Gln-1062 is administered intranasally to prevent cleavage to galantamine in the acidic environment of the stomach, and in the presence of carboxy-esterases known to be expressed in the intestines and the liver. In female Wistar rats, intravenous administration of 5.0 mg/kg Gln-1062 led to a maximum concentration (C_{max}) of 650 ng/ml in blood with an AUC_{last} of 528 ng·h/ml and a C_{max} of 13627 ng/mg in the brain with an AUClast of 9717 ng·h/g. The brain-to-blood AUC ratio of Gln-1062 was therefore 18.40. After intranasal administration of 5.0 mg/kg, this ratio was 8.1 and intranasal administration of 20.0 mg/kg resulted in a ratio of 10.2 (see supplementary material online).

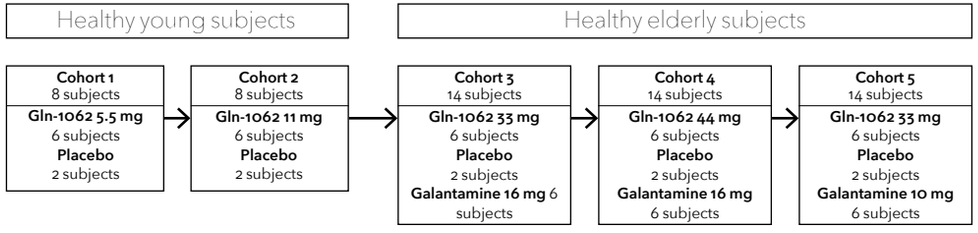
Due to its more favourable brain-to-blood ratio, Gln-1062 is expected to have fewer peripheral side effects than galantamine and other ChEIs and a comparable, or possibly an improved, effectiveness in cognition enhancement. In this study, safety, pharmacokinetic and pharmacodynamic effects of Gln-1062 were assessed and compared to orally administered galantamine and donepezil in healthy young and elderly male subjects.

METHODS

Trial design and subjects

This was a double blind, double dummy, double comparator and placebo controlled, sequential cohort single ascending dose study (ie each subject received Gln-1062 nasal spray or placebo and capsules of either dummy or active substance for both comparator drugs). Five dose levels of intranasal Gln-1062, one dose level of oral galantamine and one dose level of oral donepezil were tested in healthy, non-smoking, male subjects. Main exclusion criteria were a Mini Mental State Examination of 27 or lower, impaired renal or liver function, use of interfering concomitant medication and intranasal abnormalities. The first two cohorts each consisted of 8 healthy young male subjects. In each cohort, 6 subjects received a single dose of intranasal Gln-1062 5.5 mg (cohort 1) or 11 mg (cohort 2) and 2 subjects received placebo. The last three cohorts each consisted of 14 healthy elderly male subjects. In each cohort, 6 subjects received a single dose of Gln-1062 22mg (cohort 3), 33 mg (cohort 4) or 44 mg (cohort 5). Oral galantamine 16 mg was administered to 12 subjects in total (spread over cohort 3 and 4) and oral donepezil 10 mg was administered to 6 subjects (cohort 5). In each cohort, 2 subjects received double placebo (6 subjects in total; figure 1). In cohort 3 and 4, all drugs were administered at the same time. In cohort 5, donepezil or placebo was administered 3 hours before administration of Gln-1062 or placebo in order to have the expected T_{max} at approximately 3-4 hours after dosing at the same time point as the T_{max} of Gln-1062, which was expected to be approximately 0.5-1 hour after dosing. All subjects gave written informed consent for participation in the study. The study was approved by the ethics committee of the Leiden University Hospital, The Netherlands. The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. The trial was registered in the European Union Clinical Trials Register (2013-004354-25).

FIGURE 1 Schematic overview of cohorts



Dosing Rationale

GLN-1062 In a 28 day intranasal toxicity study in Wistar rats, a NOAEL for intranasal Gln-1062 was observed at a dose level of 5 mg/kg. The human equivalent dose was estimated to be 48 mg. With a 10-fold safety margin, a starting dose of 5.5 mg was chosen.

GALANTAMINE The recommended starting regimen for galantamine (slow release formulation) in patients with Alzheimer's Disease is a titration period of four weeks on 8 mg daily after which the dose can be increased to 16 mg daily, and, if necessary, to 24 mg daily. In previous clinical trials, immediate release formulations without preceding dose titration have been given to healthy subjects as a single dose up to 15 mg.[14;15] Three out of eight subjects not pre-treated with a peripheral anticholinergic drug as antidote experienced nausea at a dose of 15 mg, and one of eight patients vomited. Since the main advantage of Gln-1062 would be a reduction of side effects, we chose to give a single oral dose of galantamine 16 mg.

DONEPEZIL The recommended starting dose for donepezil (tablet formulation) is 5 mg/day, and is administered as a single daily dose, usually in the morning. The dose can be increased to 10 mg/day as needed. Donepezil 10 mg was chosen because it was the highest dose that was previously given as a single oral dose to healthy subjects without titration.[16]

Pharmacokinetic assessment

Venous blood samples were obtained via an indwelling catheter before administration of Gln-1062 or galantamine or placebo and at 0h15, 0h30, 1h00, 1h30, 2h00, 2h30, 3h00, 3h30, 4h00, 5h00, 7h00, 10h00 and 23h00 hours after administration. In cohort 4 and 5, the sample at 7h00 after drug administration was replaced by samples at 6h00 and 8h00 and an extra sample at 30h00 after drug administration was added. Plasma concentrations of Gln-1062 and galantamine were determined at WIL Research Europe (Den Bosch, The Netherlands) by a validated method using high performance liquid chromatography coupled to tandem-mass spectrometry (LC/MS-MS).

Pharmacodynamic assessments

The 'NeuroCart' is a battery of sensitive tests for a wide range of CNS domains that was developed to examine different kinds of CNS-active drugs. The N-back test

was used to evaluate working memory,[17-19] the Stroop test evaluated inhibition, interference and controlled versus automatic processing,[20] adaptive tracking measured attention and eye-hand coordination,[21-26] the visual analogue scale according to Bond & Lader was used to assess subjective states,[27;28] pharmaco-electroencephalography (p-EEG), eye movements and pupil size were used to monitor any drug effects, which can be interpreted as evidence of penetration and activity in the brain,[24;26;29-31] body movements were measured with the body sway meter,[32] the face encoding and recognition task evaluated visual memory[33] and the Visual Verbal Learning Test (VVLT) measured the whole scope of learning behaviour (i.e., acquisition, consolidation, storage and retrieval).[34]

All tests with this device were performed twice at baseline, and repeated at 1h00, 2h00, 3h00, 4h00, 5h00, 6h00, 8h00 and 10h00 hours after administration of Gln-1062 or galantamine or placebo. In cohort 5 an additional measurement was performed 2 hours after administration of donepezil or placebo (i.e. 1 hour before administration of Gln-1062 or placebo). The only exceptions were VVLT, which was only performed 1h30 after dosing of Gln-1062 or placebo, and face recognition, which was performed predose and 1h45 hours after administration of Gln-1062 or placebo. Measurements were performed in a quiet room with ambient illumination with only one subject per session in the same room.

Safety assessments

All subjects underwent medical screening, including medical history, physical examination, nasal examination, vital signs measurement in supine and standing position, 12-lead electrocardiogram (ECG), urinalysis, drug screen and safety chemistry and haematology blood sampling. During study periods, safety was assessed using monitoring of adverse events (AEs), nasal examination, vital signs, ECG and safety chemistry and haematology blood sampling.

Statistics

All pharmacodynamic endpoints are summarized (mean and standard deviation of the mean, median, minimum and maximum values) by treatment and time. To establish whether significant treatment effects could be detected, repeatedly measured variables were analysed with a mixed model analysis of variance with treatment, time and treatment by time as fixed factors and subject as random factor and the (average) baseline measurement as covariate.[35] Single measured variables were analysed by a mixed model analysis of variance with fixed factor treatment. The young subjects receiving active treatment were compared to the young subjects on placebo and the elderly subjects on active treatment were compared to the elderly on placebo.

RESULTS

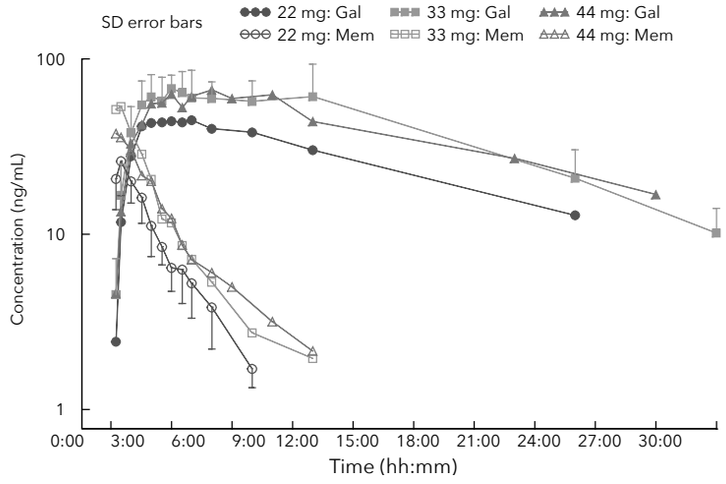
Subjects

The study was conducted between November 2013 and April 2014. A total of 16 healthy young and 42 healthy elderly male subjects participated in this study. The healthy young males had a mean age of 42.9 years (range 19 to 62), a mean body weight of 77.6 kg (range 58.4 to 92.9) and a mean BMI of 24.2 kg/m² (range 18.7 to 28.6). The healthy elderly males had a mean age of 71.2 years (range 66 to 89), a mean body weight of 81.8 kg (range 62.6 to 121.5) and a mean BMI of 25.9 kg/m² (range 20.4 to 32.4). There were no drop-outs after drug administration.

Pharmacokinetics

After administration of Gln-1062, concentrations of Gln-1062 and galantamine were measured. Based on the non-compartmental pharmacokinetic analysis of the plasma Gln-1062 concentrations, a dose dependent increase in exposure was observed up to a dose of 33 mg (table 2, figure 2).

FIGURE 2 Plasma concentrations of Gln-1062 and galantamine in cohort 3-5



Mem: Gln-1062; Gal: galantamine

TABLE 2 Pharmacokinetic parameters.

	Cohort 1: Gln-1062 5.5 mg		Cohort 2: Gln-1062 11 mg		Cohort 3: Gln-1062 22 mg		Cohort 4: Gln-1062 33 mg		Cohort 5: Gln-1062 44 mg	
	Mem	Gal	Mem	Gal	Mem	Gal	Mem	Gal	Mem	Gal
C_{max} (ng/ml)	15.2 (6.51-29.9)	17.6 (13.0-21.6)	19.4 (7.40-5.42)	27.7 (19.0-41.2)	26.5 (12.3-39.2)	46.5 (29.2-67.2)	58.5 (16.4-103)	76.1 (49.2-121)	43.2 (16.9-97.3)	74.7 (42.7-114)
T_{max} (h)	0.29 (0.25-0.50)	2.27 (1.00-3.53)	0.48 (0.25-1.00)	4.35 (1.53-10.0)	0.60 (0.50-1.00)	3.28 (1.57-4.02)	0.59 (0.25-1.53)	4.58 (2.00-9.89)	0.71 (0.25-1.67)	4.66 (2.82-8.00)
AUC$_{max}$ (ng*h/ml)	20.0 (11.6-35.8)	268 (166-445)	32.9 (16.2-71.8)	367 (273-489)	69.1 (40.8-95.4)	799 (629-954)	125 (49.3-221)	1190 (926-1800)	112 (69.6-177)	1530 (826-3270)
T_{max} (h)	1.07 (0.68-1.51)	9.85 (6.28-19.9)	1.37 (0.83-1.93)	7.94 (4.92-10.4)	2.64 (1.37-4.64)	8.71 (6.85-12.1)	2.34 (1.39-2.87)	9.24 (6.67-11.2)	2.80 (1.79-3.97)	11.1 (4.27-18.1)

Mean, range in parentheses; C_{max}: maximum concentration; T_{max}: time of maximum concentration; AUC: area under the curve; $T_{1/2}$: halflife

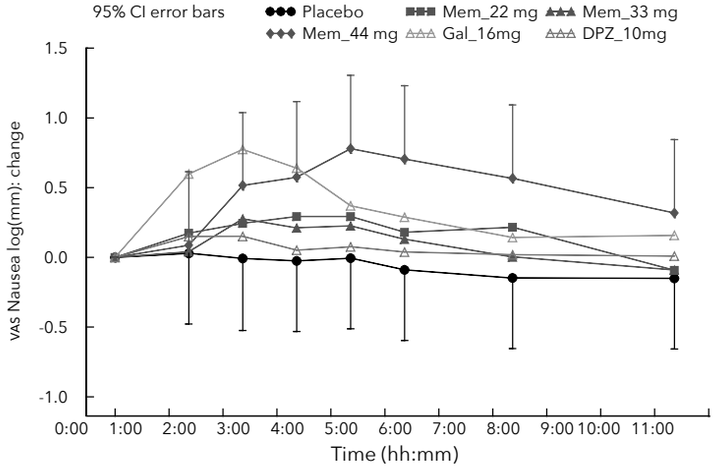
The 44 mg Gln-1062 dose led to a mean exposure that was comparable to the 33 mg dose, however, in view of the considerable inter-individual variability in exposure, the number of subjects per dose level (n=6) and the limited increase in dose from 33 mg to 44 mg (+33%), it cannot be concluded from these data that a dose dependent increase in exposure is not present beyond a dose of 33 mg. In general, subjects with a relatively high C_{max} for Gln-1062 also had a relatively high C_{max} for Gln-1062 derived galantamine. The T_{max} Gln-1062 was 15-45 minutes, while the T_{max} for galantamine after administration of Gln-1062 was 2-4.5 hours. The half-life of Gln-1062 increased with the administered dose form 1.07 to 2.08 hours. For galantamine derived from Gln-1062, the half-life was approximately 10 hours for all dose levels.

Safety

On each treatment, at least 50% of the subjects experienced one or more treatment emergent adverse event (table 1). Nasal symptoms, such as nasal discomfort, rhinorrhoea and sneezing, were reported most frequently and exclusively in the Gln-1062 dosing groups, except for one case of nasal discomfort in the donepezil group. Nasal symptoms subsided in most cases within half an hour (data not presented). No clear dose relationship was observed. Cholinergic symptoms (e.g., nausea, vomiting, diarrhoea and hyperhidrosis) were reported on all treatments, except for Gln-1062 5.5 mg and placebo. After administration of Gln-1062 11 mg and 22 mg, one subject in each cohort (16.7%) experienced nausea. Gln-1062 at the highest dose levels led to nausea in 50% of subjects (n=3), which was higher than the incidence of nausea in the galantamine 16 mg group (33.3%, n=4) and in the donepezil 10 mg group (16.7%, n=1). Although 33 mg of Gln-1062 led to a higher incidence of nausea compared to galantamine, the severity, measured with the VAS nausea, was on average lower for Gln-1062 33 mg (figure 3). The results on VAS nausea also indicated a difference in time profile. The peak of nausea occurred two hours after administration of galantamine, versus four hours after administration of Gln-1062.

Vomiting did not occur after administration of Gln-1062 5.5 mg in healthy young subjects or after administration of 22 mg in healthy elderly subjects. Gln-1062 11 mg led to vomiting in one healthy young subject (16.7%). Gln-1062 33 mg and 44 mg led to vomiting in two subjects in each cohort (33.3%), which was lower than the incidence of vomiting after administration of galantamine 16 mg, which led to vomiting in five of twelve subjects (42%). After administration of donepezil 10 mg one subject (16.7%) vomited. One subject (10%) who was administered placebo vomited. Diarrhoea did not occur after administration of Gln-1062 5.5 mg or 11 mg in healthy young subjects, and administration of Gln-1062 22 mg, 33 mg and 44 mg in healthy elderly subjects led to diarrhoea in one subject (16.7%) in each cohort. This incidence was higher than after administration of galantamine 16 mg (8.3%, n=1), but lower than after administration of donepezil 10 mg (33.3%, n=2).

FIGURE 3 Scores on VAS nausea in cohort 3-5



VAS: visual analogue scale; Mem: Gln-1062; Gal: galantamine; DPZ: donepezil

TABLE 1 **Most frequent occurring treatment emerging adverse events.**

	Gln-1062 5.5 mg	Gln-1062 11 mg	Gln-1062 22 mg	Gln-1062 33 mg	Gln-1062 44 mg	Galantamine 16 mg	Donepezil 10 mg	Placebo
Any event	6 (100%)	6 (100%)	5 (83.3%)	5 (83.3%)	5 (83.3%)	10 (83.3%)	3 (50%)	5 (50%)
Nasal discomfort	6 (100%)	6 (100%)	4 (66.7%)	4 (66.7%)	3 (50%)	-	1 (16.7%)	-
Rhinorrhoea	2 (33.3%)	4 (66.7%)	2 (33.3%)	1 (16.7%)	3 (50%)	-	-	-
Sneezing	1 (16.7%)	1 (16.7%)	1 (16.7%)	1 (16.7%)	3 (50%)	-	-	-
Nausea	-	1 (16.7%)	1 (16.7%)	3 (50%)	3 (50%)	4 (33.3%)	1 (16.7%)	-
Vomiting	-	1 (16.7%)	-	2 (33.3%)	2 (33.3%)	5 (41.7%)	1 (16.7%)	1 (10%)
Diarrhoea	-	-	1 (16.7%)	1 (16.7%)	1 (16.7%)	1 (8.3%)	2 (33.3%)	-
Cold sweat or hyperhidrosis	-	1 (16.7%)	-	-	3 (50%)	4 (33.3%)	-	-
Headache	2 (33.3%)	2 (33.3%)	3 (50%)	2 (33.3%)	-	1 (8.3%)	2 (33.3%)	3 (30%)

Number of subjects; percentage in parentheses.

Cold sweat or hyperhidrosis was seen in one subject (16.7%) on Gln-1062 11 mg, three subjects (50%) on Gln-1062 44 mg and four subjects (33.3%) on galantamine. Headache was frequently reported in all dose groups. All AEs were self-limiting and most AEs were mild in intensity, except for moderate nausea in one subject on 44 mg of Gln-1062, one subject on galantamine and one subject on donepezil, one subject with moderate vomiting on placebo and 2 subjects with moderate postural dizziness on galantamine. No severe AEs occurred.

On nasal examination, three subjects in de Gln-1062 44 mg group had dry white plaques in the nostrils, which were not seen at the follow-up visit approximately one week after dosing. Of these subjects, one had red and irritated nasal mucosa at follow-up. One subject in the donepezil group had red and irritated nasal mucosa at follow-up, while no nasal abnormalities were seen during the day of drug administration. There were no clinically relevant abnormalities in vital signs, ECG or chemistry and haematology values in any of the subjects.

Pharmacodynamics

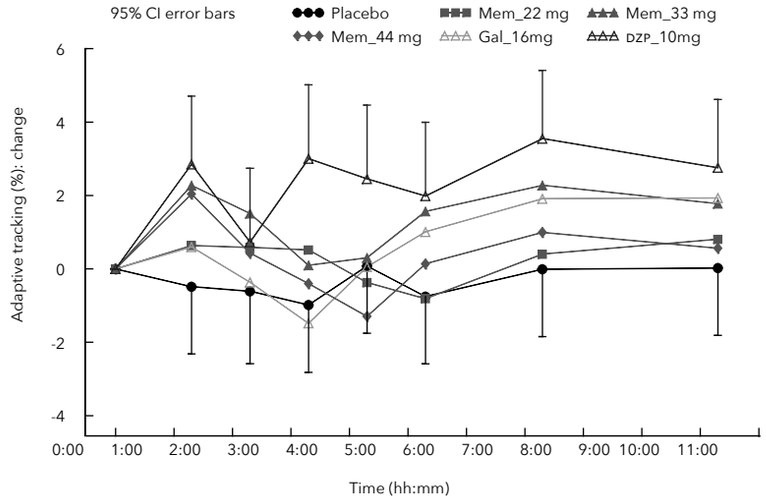
Pharmacodynamic effects of Gln-1062 compared to placebo are summarized in table 3. An improvement on the adaptive tracking performance was seen in the healthy young subjects receiving Gln-1062 11 mg and healthy elderly subjects receiving Gln-1062 33 mg or 44 mg (figure 4).

On the VVLT, an improved immediate recall of the words was seen for all doses of Gln-1062 in both young and elderly subjects, when compared to galantamine. In the healthy young subjects, the delayed word recall also improved for both the 5.5 mg and the 11 mg dose level. Word recognition did not improve on any of the Gln-1062 dose levels.

Pharmaco-EEG, face encoding and recognition test, pupil-to-iris ratio, eye movements, the VAS mood and calmness composite scores, the N-back test, body sway and Stroop Colour-Word Interference test did not show consistent differences compared with placebo for any of the Gln-1062 dose levels.

Administration of galantamine 16 mg did not induce any measurable pharmacodynamic effects compared to placebo. Administration of donepezil 10 mg only led to an improvement in adaptive tracking. The maximum effect on the adaptive tracker test performance of Gln-1062 33 and 44 mg was comparable to the maximum effect of donepezil 10 mg.

FIGURE 4 **Effect on adaptive tracking in cohort 3-5 (healthy elderly males).**



Mem: Gln-1062; Gal: galantamine; DPZ: donepezil

TABLE 3 **Pharmacodynamic effects compared to placebo.**

	Cohort 1: Gln-1062 5.5 mg	Cohort 2: Gln-1062 11 mg	Cohort 3: Gln-1062 22 mg	Cohort 4: Gln-1062 33 mg	Cohort 5: Gln-1062 44 mg
Adaptive tracking (%)	1.96 (-0.88, 4.80) p=0.1581	3.47 (0.52, 6.42) p=0.0247	0.64 (-1.06, 2.35) p=0.4474	1.79 (0.07, 3.52) p=0.0424	0.74 (-0.99, 2.48) p=0.3887
VVLT: immediate word recall trial 1	3.42 (-0.73, 7.56) p=0.0984	3.92 (-0.23, 8.06) p=0.0621	2.67 (0.17, 5.16) p=0.0367	1.97 (-0.65, 4.58) p=0.1355	2.57 (-0.05, 5.18) p=0.0541
VVLT: immediate word recall trial 2	3.17 (-1.93, 8.26) p=0.2025	1.83 (-3.26, 6.93) p=0.4510	3.50 (0.33, 6.67) p=0.0315	1.77 (-1.56, 5.09) p=0.2880	1.77 (-1.56, 5.09) p=0.2880
VVLT: immediate word recall trial 3	5.00 (-0.58, 10.58) p=0.0749	3.00 (-2.58, 8.58) p=0.2662	0.67 (-3.23, 4.56) p=0.7302	0.60 (-3.49, 4.69) p=0.7672	1.00 (-3.09, 5.09) p=0.6222
VVLT: delayed word recall	3.42 (-2.62, 9.45) p=0.2431	3.25 (-2.79, 9.29) p=0.2657	-0.33 (-3.58, 2.91) p=0.8354	-0.00 (-3.63, 3.63) p=1.0000	-1.00 (-4.63, 2.63) p=0.5780
VVLT: word recognition	-0.15 (-8.05, 7.75) p=0.9674	1.45 (-6.45, 9.35) p=0.6939	0.87 (-4.09, 5.82) p=0.7231	-0.67 (-6.45, 5.12) p=0.8153	2.17 (-3.11, 7.45) p=0.4083
N-back, 0-back (correct-incorrect/ total)	0.04, (-0.02, 0.10) p=0.1696	0.03 (-0.03, 0.09) p=0.3145	0.02 (-0.02, 0.05) p=0.3729	0.03 (-0.01, 0.06) p=0.1690	0.03 (-0.00, 0.07) p=0.0654
N-back, 1-back (correct-incorrect/ total)	-0.00 (-0.17, 0.16) p=0.9835	-0.05 (-0.21, 0.12) p=0.5597	0.02 (-0.02, 0.06) p=0.3402	-0.03 (-0.07, 0.02) p=0.2212	-0.01 (-0.06, 0.03) p=0.5016
N-back, 2-back (correct-incorrect/ total)	0.09 (-0.06, 0.25) p=0.2199	0.13 (-0.02, 0.28) p=0.0867	-0.02 (-0.09, 0.06) p=0.6835	-0.02 (-0.10, 0.06) p=0.5957	0.02 (-0.05, 0.10) p=0.5430
Face recognition number correct	-0.77 (-4.17, 2.64) p=0.6332	4.54 (1.22, 7.87) p=0.0116	-2.58 (-7.22, 2.06) p=0.2642	-0.54 (.5.15, 4.08) p=0.8135	-1.63 (-6.30, 3.05) p=0.4822
Stroop (correct congruent – correct incongruent)	-0.39 (-0.94, 0.15) p=0.1411	-0.60 (-1.21, 0.01) p=0.0535	0.10 (-1.05, 1.25) p=0.8606	0.58 (-0.52, 1.68) p=0.2909	0.11 (-0.98, 1.20) p=0.8406
EEG alpha Fz-Cz (uV)	-13.2% (-30.5%, 8.5%) p=0.1914	-0.8% (-19.5%, 22.1%) p=0.9310	7.8% (-7.0%, 25.1%) p=0.3088	21.5% (4.8%, 40.8%) p=0.0116	-10.1% (-24.2%, 6.6%) p=0.2121
EEG alpha Pz-Oz (uV)	-8.9% (-23.8%, 8.9%) p=0.2782	-13.1% (-27.2%, 3.7%) p=0.1086	13.8% (-5.4%, 36.9%) p=0.1633	19.7% (-0.2%, 43.6%) p=0.0530	2.1% (-16.3%, 24.5%) p=0.8306
Saccadic peak velocity (deg/sec)	9.90 (-17.50, 37.30) p=0.4465	8.32 (-18.32, 34.95) p=0.5097	26.92 (4.11, 49.73) p=0.0223	0.92 (-21.84, 23.68) p=0.9349	19.19 (-5.54, 43.92) p=0.1242
Saccadic inaccuracy (%)	-1.56 (-2.77, -0.35) p=0.0157	-1.78 (-3.03, -0.54) p=0.0088	0.24 (-1.01, 1.48) p=0.7005	0.08 (-1.14, 1.30) p=0.8970	-0.22 (-1.57, 1.12) p=0.7363

Mean, confidence interval in parentheses; VVLT: visual verbal learning test; EEG: electroencephalogram

DISCUSSION

In this study we examined the pharmacokinetics, side effect profile and pharmacodynamic effects of Gln-1062 and compared these to the pharmacodynamics effects and side effect profile of galantamine and donepezil in healthy male subjects.

Gln-1062 was rapidly absorbed into the systemic circulation with a C_{max} in plasma reached after approximately 15-45 minutes and a half-life of 1.1-2.8 hours, depending on the dose administered. The T_{max} of galantamine after administration of Gln-1062 was 2.3-4.7 hours in all except the third cohort, which is approximately two half-lifes of Gln-1062. This would be consistent with the hypothesis that Gln-1062 rapidly enters the brain, where it may be cleaved into active galantamine. It is established that the approved ChEIs all distribute into the brain according to their lipophilicity. The lipophilic nature of Gln-1062 and the avoidance of the first-pass effect due to the intranasal administration could increase the concentrations of Gln-1062 in the brain. A direct route from the nose to the brain has never been demonstrated in humans.[36]

As a pro-drug of galantamine, a low exposure of Gln-1062 generally resulted in a low formation of galantamine in most subjects. However, some individuals seemed to reach lower galantamine concentration than expected based on their measured Gln-1062 exposure. This may be indicative of differences between subjects in the rate of conversion of Gln-1062 to galantamine.

All doses of Gln-1062 were safe and reasonably well tolerated. The most frequently reported AEs were related to irritation of nasal mucosa to which Gln-1062 is dispositioned after intranasal administration. The reported irritation was rapidly reversible and will be further studied in the next clinical trial. The subjects generally considered the intranasal administration to be easy and well tolerable and compared it with the use of a nasal spray as is used in case of a common cold.

As Gln-1062 is expected to have fewer peripheral side effects than galantamine and other ChEIs, the comparison of AEs between the different treatments was an important aspect of this study. After administration of galantamine 16 mg, the most frequently reported treatment emergent adverse events were nausea, vomiting and cold sweat or hyperhidrosis, which is consistent with previous studies.[37;38] Gln-1062 22 mg has the same molarity as the 16 mg dose of galantamine and based on preclinical studies Gln-1062 22 mg is expected to lead to at least tenfold higher galantamine concentrations in the brain compared to orally administered galantamine 16 mg. At this dose of Gln-1062, nausea occurred in 16,7% of subjects, compared to 33,3% in the galantamine subjects, and vomiting did not occur at all, while this was present in 41,7% of subjects on galantamine. The Gln-1062 33 mg

dose led to a higher incidence of nausea, compared to galantamine, but a lower severity of nausea, as measured using a VAS for nausea. After administration of Gln-1062 44 mg, both incidence and severity of nausea were higher, compared to galantamine 16 mg. Compared to donepezil, Gln-1062 33 and 44 mg both had a higher incidence of nausea and vomiting, but a lower incidence of diarrhoea. It can be concluded that single doses of Gln-1062 up to 33 mg seem to be tolerated at least as well as a single dose of galantamine 16 mg, but are likely to lead to substantially higher galantamine concentrations in the brain in comparison to an oral dose of 16 mg galantamine. The single dose design of the study is a limitation with respect to the extrapolation of the results to clinical practice, because the treatment of symptoms of AD with one of the registered ChEIs will always imply daily dosing with a period of uptitration. The results of this study provide a good base for a multiple dose study to investigate this in more detail. Another way to reduce side effects with classic ChEIs is transdermal administration, which, at this stage, is only possible with rivastigmine. However, this does not alter the ratio of peripheral and central cholinesterase inhibition, while the preclinical data of Gln-1062 and the results of the presented study suggest that this ratio might be more favourable for Gln-1062 compared to the currently registered ChEIs.

The analysis of pharmacodynamic effects in this study was exploratory in nature, since the study was not powered to detect differences between treatments and there was no correction for multiple testing. This needs to be taken into account when interpreting the pharmacodynamic results. Previous research has shown that acetylcholine plays an important role in attentional processes and memory and cholinesterase inhibitors also primarily affect these domains in patients with Alzheimer's disease.[38;39] This is in line with the findings in our study, where administration of Gln-1062 led to consistent improvements on adaptive tracking, which is very sensitive to compounds that affect vigilance and arousal, and VVLT, a test of verbal memory. The improvements on the VVLT after administration of Gln-1062 in healthy elderly subjects were observed on the immediate recall trials, suggesting an effect on short term memory capacity or learning, but not on retrieval of previously stored information, which would be consistent with previous research.[39-42] The lack of effect of donepezil on VVLT might be explained by the fact that donepezil does not have a direct effect on nicotinic acetylcholine receptors, which galantamine also has.[43] Galantamine allosterically sensitizes neuronal nicotinic acetylcholine receptors, but when orally applied has limited brain penetration, which may explain the lack of acutely measurable pharmacodynamic effects of oral galantamine.

The time profiles of the adaptive tracking test in the healthy elderly subjects showed that the administration of Gln-1062 resulted in larger effects compared to oral administration of 16 mg galantamine and placebo mainly due to an improvement that occurred in the first hours after study drug administration. After approximately 4 hours, the adaptive tracker test curves of the Gln-1062 33 mg and 44 mg cohorts return to the same level as the galantamine 16 mg curve and continue to run in parallel. This is in line with the hypothesis that Gln-1062, as a pro-drug of galantamine, enters the CNS to a greater extent than (oral) galantamine in the initial hours following drug administration. The distribution of small molecules such as Gln-1062 and galantamine via the blood-brain barrier is extremely fast. It is the higher level of galantamine that is produced in the brain after enzymatic cleavage of Gln-1062 that causes the higher level of activation of nicotinic receptors and thus the higher pharmacodynamics effects compared to oral galantamine. Several hours post-dose (± 4 hours) Gln-1062 can be expected to be almost completely converted into galantamine, which is likely to be the reason why the Gln-1062 33 mg and 44 mg curves are no longer distinguishable from the galantamine 16 mg curve at 4 hours and beyond. Establishment of a pharmacokinetic-pharmacodynamic model may shed more light on the exact relationship between the pharmacodynamics effects observed and the estimated brain concentrations and measured plasma concentrations of Gln-1062 and galantamine. Donepezil showed an improvement in adaptive tracking performance that was similar in magnitude to Gln-1062 33 and 44 mg. However, the donepezil induced improvement lasted considerably longer. This is consistent with the pharmacokinetic profile of donepezil and its half-life of 70 hours.

In conclusion, this study demonstrates that Gln-1062 is safe and well tolerated at single dose levels up to 33 mg. The pharmacokinetic and pharmacodynamic profile of Gln-1062 as observed in this study are in accordance with the hypothesis that Gln-1062 enters the CNS very rapidly and is then enzymatically cleaved to the active ingredient galantamine, resulting in higher CNS concentrations than can be achieved by oral administration of galantamine. The observation that, in this study, the dose of 22 mg of Gln-1062 induces fewer cholinergic side effects than 16 mg of galantamine, which has the same molarity, supports this hypothesis. Based on these observations, Gln-1062 is expected to be better tolerated and to be more effective than oral galantamine in treating the symptoms of patients with AD and may be a promising compound for an improved symptomatic treatment.

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CHAPTER 6

Acute response to cholinergic challenge predicts long-term response to galantamine treatment in patients with Alzheimer's Disease

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ABSTRACT

BACKGROUND Cholinesterase inhibitors (CEIs) have been shown to improve cognitive functioning in Alzheimer's Disease (AD) patients, but are associated with multiple side effects and only 20-40% of the patients clinically improve. In this study, we aimed to investigate the acute pharmacodynamic (PD) effects of a single dose administration of galantamine on central nervous system (CNS) functioning in mild to moderate AD patients and its potential to predict long-term treatment response.

METHODS This study consisted of a challenge and treatment phase. In the challenge phase, a single dose of 16 mg galantamine was administered to 50 mild to moderate AD patients in a double-blind, placebo-controlled cross-over fashion. Acute PD effects were monitored up to 5 hours after administration with use of the NeuroCart CNS test battery and safety and pharmacokinetics were assessed. In the treatment phase, patients were treated with open-label galantamine according to regular clinical care. After 6 months of galantamine treatment, patients were categorized as either responder or as non-responder based on their MMSE, NPI and DAD scores. An analysis of covariance was performed to study the difference in acute PD effects during the challenge phase between responders and non-responders.

RESULTS A single dose of galantamine significantly reduced saccadic reaction time (-0.0099; 95%CI=-0.0195,-0.0003; p=.0430), absolute frontal EEG parameters in alpha (-14.9; 95%CI=-21.0,-8.3; p=.0002), beta (-12.6; 95%CI=-19.4,-5.3; p=.0019) and theta (-17.9; 95%CI=-25.0,-10.0; p=.0001) frequencies. Relative frontal (-1.669; 95%CI=-2.999,-0.339; p=.0156) and occipital (-1.856; 95%CI=-3.339,-0.372; p=.0166) EEG power in theta frequency and relative occipital EEG power in the gamma frequency (1.316; 95%CI=0.158,2.475; p=.0273), also increased significantly compared to placebo. Acute decreases of absolute frontal alpha (-20.4; 95%CI=-31.6,-7.47; p=.0046), beta (-15.7; 95% CI=-28.3,-0.93; p=.0390) and theta (-25.9; 95%CI=-38.4,-10.9; p=.0024) EEG parameters and of relative frontal theta power (-3.27%; 95%CI=-5.96,-0.58; p=.0187) on EEG significantly distinguished responders (n=11) from non-responders (n=32) after 6 months.

CONCLUSIONS This study demonstrates that acute PD effects after single dose of galantamine are correlated with long-term treatment effects and that patients who demonstrate a reduction in EEG power in the alpha and theta frequency after a single administration of galantamine 16 mg will most likely respond to treatment.

INTRODUCTION

Alzheimer's Disease (AD) is the major cause of dementia worldwide.[1] This neurodegenerative disorder is characterized by a profound loss of cholinergic innervation and cholinergic deficiency.[2-4] As the disease progresses, cognitive functions deteriorate in parallel with loss of cholinergic neurons, which correlates with disease severity.[5] Despite huge efforts, no curative therapy has been found yet, and current therapies mainly focus on the loss of cholinergic function. Cholinesterase inhibitors (CEIs) fall under the class of cholinergic treatments currently in use for the symptomatic treatment of dementia.[6-8] CEIs attempt to restore the loss of acetylcholine occurring after the neurodegeneration of the cholinergic system by increasing the acetylcholine (ACh) levels in the synaptic cleft of the remaining cholinergic neurons.[6-8] Galantamine is an example of a specific, competitive and reversible CEI, which, however, may also have a more direct modulating effect on the nicotinic acetylcholine receptor (AChR).[6] CEIs have shown to improve cognitive function in AD, Lewy Body Dementia and Parkinson's Disease Dementia.[7,8]

Unfortunately, CEIs lead to a clinical improvement in only 20-40% of the AD patients, depending on the definition of treatment response.[9,10] Since it is difficult to distinguish who will clinically improve in response to treatment and who will not at an early stage of disease[10,11] many patients are unnecessarily exposed to drug treatment and potentially experience adverse effects. It would be favourable to determine responsiveness to treatment before long-term drug exposure. In daily clinical practice, a favourable response to CEI treatment is defined by the postponement of progression of symptoms of AD. This can only be determined at a point in time when clinical progression is expected. Usually, patients are treated for at least 6 months before treatment response is assessed, using clinical scales for cognitive domains, functioning in daily life and behaviour. However, based on the mechanism of action, CEIs are expected to increase the level of ACh in the synaptic cleft immediately after dosing. We argue that acute pharmacodynamic (PD) effects of CEIs can be measured when sensitive methods are used at multiple time points in the hours after dosing, especially in comparison to placebo in a cross-over study design.

Acute PD effects of galantamine in AD patients have been reported previously,[12] but only in pharmacological magnetic resonance imaging studies at one timepoint after dosing.[12-14] One study showed an effect on paired associate learning after the administration of donepezil 5 mg,[15] however this study had no placebo-controlled cross-over design and measurements were performed at one fixed time point after

dosing. None of these studies reported a longer follow-up period or associated correlation parameters. Other studies attempted to link long term treatment effects of rivastigmine to the pharmacokinetics (PK) in plasma and cerebrospinal fluid at steady state[16] or measured electroencephalography (EEG) changes after one week of treatment.[17] However, neither performed PD measurements in the first hours after single dosing. Conceptually, acute PD effects, when accurately measured, are expected to be correlated with treatment response, if the clinical effect is related to the pharmacological activity of the compound. By inference, a single administration of a CEI could be used in clinical practice to decide which patient to treat and which patient not to expose to unnecessary side effects.

Based on the pharmacological properties of CEIs and evidence from previous studies, we hypothesized that reactivity to an acute cholinergic challenge will predict the long-term response to cholinergic treatment.[12,17] In the present study, we therefore aimed to investigate the acute PK and PD effects of a single dose administration of galantamine on central nervous system (CNS) functioning in mild to moderate AD patients in a placebo-controlled, cross-over fashion. Subsequently, patients were treated with galantamine for 6 months and clinical response to treatment was evaluated. Finally, the relationship between the reactivity to the acute cholinergic challenge and clinical response to long term cholinergic treatment was assessed.

METHODS

Study design and subjects

This was a multicentre, double-blind, placebo controlled, randomized cross-over study with galantamine compared to placebo, followed by a 6 months open label treatment phase in patients with AD. Fifty patients with mild to moderate AD were included in the study. Inclusion was based on a clinical diagnosis of AD, Mini Mental State Examination (MMSE) score ranging from 18 to 26 and a Clinical Dementia Rating (CDR)[18] score between 0.5 and 2.0. Main exclusion criteria were the previous or current use of CEIs, anti-cholinergic drugs or neuroleptics, contraindications for the use of CEIs, use of benzodiazepines 48 hours prior to the study days or any history of psychiatric disorders.

Before entering the study, all patients were screened for eligibility, including evaluation of diagnosis, use of medication, presence of contraindications for the use of galantamine, electrocardiogram (ECG) and laboratory investigations. Also, a training session for the pharmacodynamic measurements performed with the NeuroCart® CNS test battery was planned. This test battery includes 10 different computerized tasks and EEG on a wide range of CNS domains [19-22] and is also sensitive to cholinergic effects.[23,24] All eligible patients entered the challenge phase, consisting of two study days, during which the effects of galantamine or placebo were measured according to a predefined time schedule, with a one week wash-out period in between. Directly after the second challenge occasion, patients entered the open-label treatment phase. During this phase, patients were treated with galantamine according to standard care for 6 months and visited the clinic after two months and 6 months of treatment for the assessment of clinical outcome measures. This study was performed in collaboration with the VU University Medical Center (Amsterdam, The Netherlands), and the University Hospital of Bucharest (Romania). Subjects were also recruited via the memory clinic of the Spaarne Gasthuis Hospital (Haarlem, The Netherlands). All subjects gave written informed consent for participation in the study. The study was approved by the Medical Ethics Committee of the VU University Medical Center and the Medical Ethics Committee of the Clinicii de neurologie a Spitalului Universitar de Urgenta and it was carried out according to the ICH Good Clinical Practice.

Dosing rationale

CHALLENGE PHASE Previous studies have shown measurable changes in functional magnetic resonance imaging 3 hours post-administration, and no serious side effects as a consequence of the administration of a single dose of 8 mg galantamine.[12-14] Therefore, this study started with a challenge dose of 8 mg. An interim analysis was planned and performed when the first 11 patients completed the challenge phase to assess whether this dose induced any measurable acute PD effects compared to placebo. There were no significant differences in pharmacodynamic effects between galantamine 8 mg and placebo and side effects at this dose were minimal. A recently performed study by Klaassens and colleagues also found no pharmacodynamic effects after a single dose of galantamine 8 mg.[14] Based on this, it was decided to increase the challenge dose to 16 mg galantamine. Study drug was administered orally as one or two capsules, each containing 8 mg of galantamine hydrobromide or a placebo. During the challenge phase, an immediate release formulation of Reminyl® was used.

TREATMENT PHASE Directly after completing the challenge phase, patients entered the treatment phase. Patients were treated with extended release galantamine (Reminyl® or equivalent) capsules, according to the guidelines used in daily clinical practice: to prevent side effects caused by fast accumulation due to the long half-life of galantamine, the starting dose was 8 mg once daily for four weeks. The dose was then increased to 16 mg once daily for the remaining months.

Pharmacokinetic assessments

Venous blood samples were obtained via an indwelling catheter at baseline and at 0,25, 0,5, 1, 1,5, 2, 2,5, 3,5 and 5 hours following drug administration. Plasma galantamine concentrations were determined at the department of Clinical Pharmacy and Pharmacology at the VU University Medical Centre by a validated method using high-performance liquid chromatography coupled to a tandem-mass spectrometry.

Pharmacodynamic assessments

To evaluate the acute PD effects of galantamine, the NeuroCart® was used, including 10 different computerized tasks and EEG. The NeuroCart test battery has previously shown sensitivity to drug effects on a wide range of CNS domains [19-22] and is also sensitive to (anti)cholinergic effects.[23,24] The N-back tests evaluated working memory,[25,26,27] adaptive tracking measured sustained attention and eye-hand coordination,[28,29-32] and the Simple Reaction Time task measured the attention and speed of information processing.[29] The visual analogue scale according to Bond and Lader assessed changes in subjective states,[13] the facial encoding and recognition task episodic memory,[12,21] and the visual verbal learning test (VVLT) covered the scope of learning behaviour (i.e., acquisition, consolidation, storage and retrieval.[30] Pharmaco-electroencephalography, eye movements, and pupil size were used to determine drug effects on neurophysiological and autonomous system function.[10,31,34] Pupil size, eye movements, adaptive tracking, simple reaction time, visual analogue scales and N-back tests were performed twice at baseline, and at 1, 2, 4, and 5 hours following galantamine or placebo administration. The VVLT was executed 1.5 hours after drug-administration (immediate recall) and 3.0 hours following drug-administration (delayed recall and recognition). The facial recognition task was performed at baseline and 2.5 hours after dosage. Pharmaco-EEG measurements were performed at baseline and 0,5, 1, 1,5, 2, 4 and 5 hours post galantamine administration. Measurements were performed in a quiet room with ambient illumination with only one subject per session in the same room.

Clinical outcome assessments

The Alzheimer's Disease Assessment (ADAS)-cog subscale was used to evaluate the severity of cognitive and non-cognitive behavioral dysfunction characteristic for AD patients.[35] This subscale comprises 11 items that have been allocated to represent 3 key cognitive domains: language, memory, and praxis.[36-38] Positive changes on the ADAS-cog scale (0-70) imply worsening of cognition. Cognitive performance of subjects was assessed by the Clinical Dementia Rating Scale (CDR) in which statements related to the following 6 domains are scored: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care.[18] The global CDR score is derived from a synthesis of the individual ratings in each domain in accordance with established clinical scoring rules and represents a 5-point ordinal scale, where CDR 0 indicates no dementia, and CDR 0.5, 1, 2, and 3 indicate questionable, mild, moderate, and severe dementia. The Disability Assessment in Dementia (DAD) scale was used to evaluate basic and instrumental activities of daily living (ADL).[39] Items from this 46-item questionnaire can be divided into basic ADL and instrumental ADL. Higher scores represent fewer disabilities and lower scores indicate increased disabilities.[40] The Mini Mental State Examination (MMSE) is a brief 30-point questionnaire test which was used to screen for cognitive impairment.[41,42] With the Neuropsychiatric Inventory (NPI) diverse behavioural and psychological symptoms of dementia were measured.[43] The ADAS-cog, CDR, DAD, MMSE and NPI were carried out after two and 6 months of treatment.

Safety assessments

Before participation in the study, all subjects underwent medical screening, including medical history, physical examination, vital signs measurements, 12-lead ECG, urinalysis, urinary drug screen, haematology and biochemistry blood sampling. During study days, vital signs measurements, 12-lead ECG, urinalysis, urinary drug screen, haematology and biochemistry blood sampling were performed at baseline. ECG and vital signs were additionally performed at 0.5, 1.5 and 5.0 hours post- drug administration in order to monitor possible adverse effects of the drug and assess safety.

SAMPLE SIZE CALCULATION The study aimed to enrol 50 patients with mild to moderate AD. This number was based on a sample size calculation that hypothesized an effect size comparable to the reduction in theta power on EEG examination (-27.3%) after onset of treatment with rivastigmine in patients who clinically improved in another.[17] Of the 20 patients with mild to moderate AD who participated in that study, 8 patients (40%) clinically improved in response to treatment, defined as an improvement of short-term memory after 6 months. A logistic regression analysis revealed that 50% of the observed variance in clinical improvement as a result of treatment could be explained by the decrease in

theta power, one week after onset of treatment.[17] With an estimated correlation coefficient of r^2=0.50, a sample size calculation determined that with an alpha of 0.05 and a power (1-beta) of 0.8, at least 30 patients were needed to observe a significant correlation between the acute response to the galantamine challenge and clinical improvement after 26 weeks. With an estimated drop-out rate of 35%,[11] the total number of patients needed was calculated to be 46, which is why 50 patients were targeted.

INTERIM ANALYSIS After the challenge phase, data of the first 11 subjects were collected and a pre-defined interim analysis was performed. For the interim analysis, the PD variables were analysed by mixed model of analysis with treatment, time, and treatment by time as fixed factors, subject, subject by treatment, and subject by time as random factors and the average pre-value as covariate. The results were presented as a result table of the analysis with the p-value of the contrast between placebo and galantamine, the least square means of the treatments, the estimate of the difference and the 95% confidence interval around the difference. No individual data were reported to avoid unblinding.

PHARMACODYNAMIC ANALYSIS Acute effects on different PD variables were analysed as described for the interim analysis. Log transformation was used to correct for log-normal distribution of the data. Calculation of time and treatment by time effects were for graphical presentation purposes only; only contrasts within the overall treatment effect were estimated and reported, along with 95% confidence intervals. Log-transformed parameters were back-transformed after analysis where the results may be interpreted as percentage change. Due to the exploratory nature of this study, no formal adjustment for multiple testing was used.

CORRELATION ANALYSIS To investigate whether the acute PD effects were correlated with the MMSE, NPI and DAD scores at 6 months independently, change from baseline AUC for galantamine and placebo were calculated and Pearson (or Spearman) correlation coefficients were calculated. According to Chan et al., correlation was defined as poor (0.1 – 0.2), fair (0.3 – 0.5), moderate (0.6 - 0.7), very strong (0.8 – 0.9) or perfect (1).[44]

The group of patients was subsequently divided in responders and non-responders. If MMSE and NPI and DAD at month 6 were ≥ MMSE and NPI and DAD at baseline, a patient was a responder. If not all three measurements improved or at least stayed the same, the patient was a non-responder. The challenge effects of the PD variables were analysed comparing the responders with the non-responders. The challenge variables were analysed with a mixed model analysis of variance with fixed factor group (responder/non-responder), treatment, period, time, treatment by time, treatment by group and treatment by group by time as fixed factor, subject, subject by time and subject by treatment as random factor and the average pre-value as covariate. The contrast of interest was responders (galantamine-placebo) versus non-responders (galantamine-placebo). The difference of the change from baseline galantamine AUC and the placebo AUC was graphically analysed for the responders and the non-responders. The percentage of responders and non-responders outside the range of the non-responders and responders respectively, was calculated.

PHARMACOKINETIC ANALYSIS The following PK parameters were estimated using compartmental analysis: maximum plasma concentrations (C_{max}), time of maximum plasma concentrations (T_{max}), area under the concentration versus time curve from time zero to the time of the last quantifiable concentration and to infinity (AUC_{∞}), terminal elimination rate constant (λ_z), terminal elimination half-life ($T_{½}$), and clearance (CL/F).

RESULTS

In total, 50 patients with mild to moderate AD were included in our study. Of these patients, 39 were enrolled via the Centre for Human Drug Research and the VU medical center in the Netherlands (of whom 5 were recruited via the Spaarne Gasthuis in Haarlem) and 11 patients were enrolled at the Tangent data research unit at University hospital of Bucharest in Romania. Patients had a mean age of 66.8 years (range 49 - 90) and a mean weight of 75.8 kg (range 50 - 122). The first 11 patients (all tested in the Netherlands) received 8 mg of galantamine. Following the predefined interim analysis, it was decided to escalate the dose to 16 mg of galantamine for the remaining 39 patients. Two patients prematurely dropped out of the study during the challenge phase due to practical issues (lack of time or hospitalization for unrelated reasons). Therefore, 48 patients could be analyzed in the challenge phase of the study. During the treatment phase, three additional patients cancelled study appointments (one patient experienced side effects, two patients lacked time or were hospitalized for other reasons). Two patients had incomplete follow-up data. A total of 43 patients could therefore be analyzed in the treatment phase.

Challenge phase

INTERIM ANALYSIS An interim analysis after the first 11 subjects revealed no clear differences between 8 mg galantamine treatment and placebo on most of the PD measurements. Differences were observed between galantamine and placebo treatment for the second immediate recall of the VVLT (-1.8; 95% CI=-2.7,-0.9; *p*= 0.0084). However, since no differences were found for all other parameters (see supplementary material online), the measured PD effects of 8 mg galantamine were considered insufficient and it was decided to increase the dose of galantamine to 16 mg for the remaining 39 subjects. No interim analysis could be performed for the pupil size, N-back average reaction time 2 back and recognition of the VVLT, since too few subjects were able to perform these tests, due to the complexity of the computer interface. The computer interface was subsequently simplified based on this observation.

PHARMACODYNAMICS Acute PD effects of a single dose of galantamine in comparison to placebo in mild to moderate AD patients are displayed in table 1. A single dose of galantamine significantly reduced saccadic reaction time (-0.0099; 95%CI=-0.0195,-0.0003; *p*=.0430) when compared to placebo condition. Peak effects on saccadic eye movements were observed around the T_{max} of galantamine. An improvement in performance on the adaptive tracker was observed after administration of galantamine, but the difference was not significant. Notably, galantamine appeared to increase performance on adaptive tracking at 1, 4 and 5 hours post drug administration, but not around 2 hours following administration (Table 1).

In addition, galantamine administration acutely reduced absolute alpha (-14.9; 95%CI=-21.0,-8.3; *p*=.0002), beta (-12.6; 95%CI=-19.4,-5.3; *p*=.0019) and theta power (-17.9; 95%CI=-25.0,-10.0; *p*=.0001) and relative frontal (-1.669; 95%CI=-2.999,-0.339; *p*=.0156) and occipital (-1.856; 95%CI=-3.339,-0.372; *p*=.0166) EEG power in theta frequency and increased relative occipital EEG power in the gamma frequency (1.316; 95%CI=0.158,2.475; *p*=.0273) on the pharmaco-electroencephalography in comparison to placebo. For all EEG spectra, except for the delta range, a significant decrease in power was observed compared to placebo, with strongest reductions around the T_{max} of galantamine. For the delta range a reduction of absolute power was observed following galantamine administration, but the difference was not significant. Reductions in delta power were strongest around 2 hours post-drug administration and continued to be equally reduced over time. VAS scores on nausea significantly increased after galantamine compared to placebo (0.2908 log mm; 95%CI=0.0968,0.4848; *p*=.0043). All other PD parameters were not significantly affected by galantamine.

PHARMACOKINETICS Mean T_{max} was 2.42 h (range 1.00 – 4.58) for 8 mg and 1.38 h (range 0.45 – 4.60) for 16 mg of galantamine with a C_{max} of respectively 43.17 ng mL^{-1} (range 23.90 – 57.30) and 79.00 (range 43.30 – 139.00). Graphs and other PK parameters can be found in the supplemental material.

Treatment Phase

After 6 months, 11 (26%) patients were defined as responder to galantamine treatment and 32 (74%) patients were defined as non-responder, based on the a priori definition of response of no decline on MMSE, DAD and NPI. Table 2 describes the differences between responders and non-responders in their reactivity to the acute cholinergic challenge compared to placebo. Differences between responders and non-responders in their reactivity to the cholinergic challenge compared to placebo were statistically significant for absolute frontal alpha (-20.4; 95%CI=-31.6,-7.47; *p*=.0046), beta (-15.7; 95% CI=-28.3,-0.93; *p*=.0390) and theta power (-25.9; 95%CI=-38.4,-10.9; *p*=.0024) and for relative frontal theta power (-3.27%; 95%CI=-5.96,-0.58; *p*=.0187) on EEG. It is interesting to note that on visual inspection, long-term responders showed an acute increase after placebo on absolute frontal EEG parameters and on relative frontal theta power compared to baseline on the placebo occasion and a decrease compared to baseline on the galantamine occasion, whereas non-responders hardly showed any change from baseline on either the placebo nor galantamine occasion (figure 1). On the scatter plots, both absolute frontal alpha and frontal theta power distinguished responders from non-responders well, with minimal overlap between responders and non-responders (figure 2). For frontal alpha power, no responders were in the overlapping range. For frontal theta power, 2 responders (22,2%) and 3 non-responders (12,5%) were in the overlapping range. For relative frontal theta power on the EEG, 4 responders (80%) and 9 non-responders (64,3 %) were in the overlapping range. Acute improvements in saccadic eye movements that were observed after single dose galantamine, did not clearly predict long-term clinical improvement: saccadic peak velocity increased on average in responders but not in non-responders, but this failed to reach statistical significance (Table 2).

Correlations between the acute PD effects and MMSE, NPI and DAD scores at 6 months independently, are shown in the supplementary material (online available). Supplemental Table 2 shows that the majority of the coefficients of correlation reached a value under (-)0.50, which can be considered as fair.[44] Coefficients reaching levels over (-)0.50 showed a moderate correlation between acute effects on smooth pursuit (r=0.58), alertness (r=0.54), N-back (r=0.63) and relative frontal alpha power on EEG (r=-0.59) and treatment response according to the DAD only.

TABLE 1 **Acute pharmacodynamic effects of a single dose of galantamine in mild to moderate Alzheimer patients.**

	LS Means		LS Means change from baseline		Contrasts	
Parameter	**Placebo**	**Galantamine**	**Placebo**	**Galantamine**	**Galantamine- Placebo**	
					Treatment effect (95% CI)	**Treatment p-value**
Smooth Pursuit (%)	27.9	28.1	-1.95	-1.78	0.16 (-1.26,1.59)	0.8147
Saccadic Inaccuracy (%)	7.3	6.6	-0.27	-0.95	-0.69 (-1.38,0.01)	0.0516
Saccadic Peak Velocity (deg/s)	489.6	496.8	-12.28	-5.08	7.20 (-4.62,19.02)	0.2173
Saccadic Reaction Time (sec)	0.253	0.243	0.0061	-0.0038	-0.0099 (-0.0195,-0.0003)	0.0430
Simple reaction time task (sec)	392.99	393.15	1.4%	1.5%	0.0% (-6.8%,7.4%)	0.9911
Adaptive tracking (%)	17.76	18.54	0.084	0.863	0.779 (-0.247,1.805)	0.1320
VAS Alertness (mm)	61.5	56.7	-0.98	-5.83	-4.85 (-9.83,0.13)	0.0560
VAS Calmness (mm)	63.0	59.5	1.89	-1.62	-3.51 (-9.71,2.70)	0.2541
VAS Mood (mm)	64.4	62.5	0.17	-1.75	-1.92 (-6.37,2.53)	0.3813
VAS Nausea log(mm)	0.633	0.924	-0.0341	0.2567	0.2908 (0.0968,0.4848)	0.0043
N-back mean RT 0 back (msec)	512	524	5.7	17.9	12.2 (-21.6,46.1)	0.4631
N-back mean RT 1 back (msec)	651	627	-13.0	-37.1	-24.1 (-80.8,32.5)	0.3754
N-back mean RT 2 back (msec)	743	726	-26.6	-43.8	-17.2 (-102.9,68.4)	0.6797
N-back corr-incorr/total 0	5.93	5.97	-0.054	-0.007	0.047 (-0.070,0.163)	0.4081
N-back corr-incorr/total 1	5.28	5.33	0.202	0.248	0.046 (-0.357,0.448)	0.8158
N-back corr-incorr/total 2	3.43	3.37	-0.449	-0.513	-0.064 (-0.584,0.456)	0.8014
EEG Alpha Fz-Cz (uV)	2.17	1.86	10.8%	-5.3%	-14.9% (-21.0%,-8.3%)	0.0002
EEG Alpha Pz-Oz (uV)	3.26	3.22	-0.7%	-2.0%	-1.3% (-10.8%,9.2%)	0.7953
EEG Beta Fz-Cz (uV)	1.88	1.66	10.2%	-3.0%	-12,0% (-18,7%,-4,7%)	0.0026
EEG Beta Pz-Oz (uV)	1.87	1.92	1.2%	3.9%	2.6% (-6.0%,12.0%)	0.5505
EEG Delta Fz-Cz (uV)	1.48	1.35	12.5%	2.9%	-8,3% (-19,9%,4.9%)	0.2033
EEG Delta Pz-Oz (uV)	1.60	1.49	2.2%	-4.6%	-6.7% (-18.6%,7.0%)	0.3111
EEG Gamma Fz-Cz (uV)	0.56	0.53	7.7%	2.2%	-5,10% (-13,8%,4,5%)	0.2763
EEG Gamma Pz-Oz (uV)	0.63	0.72	-1.4%	12.3%	14.0% (-2.3%,33.0%)	0.0923
EEG Theta Fz-Cz (uV)	2.03	1.67	16,9%	-3,9%	-17.9% (-25.0%,-10,1%)	0.0001
EEG Theta Pz-Oz (uV)	2.26	2.05	2.2%	-7.5%	-9.5% (-20.9%,3.5%)	0.1403
EEG Relative Alpha Fz-Cz (%)	26.48	25.93	-0,398	-0.950	-0,552 (-1,497, 0,393)	0.2427
EEG Relative Alpha Pz-Oz (%)	33.43	33.87	-0,745	-0.307	0.438 (-1.442, 2.318)	0.633
EEG Relative Beta Fz-Cz (%)	23.12	23.01	-0,447	-0.552	-0.106 (-0.814, 0.603)	0.7628
EEG Relative Beta Pz-Oz (%)	19.09	19.9	-0,082	0.727	0.809 (-0.162, 1.781)	0.099
EEG Relative Delta Fz-Cz (%)	18.26	19.5	0,121	1.359	1238 (-0.347, 2.823)	0.1213
EEG Relative Delta Pz-Oz (%)	17.09	16.21	0,717	-0.168	-0.885 (-2.207, 0.437)	0.1811
EEG Relative Gamma Fz-Cz (%)	7.22	8.08	-0,225	0.643	0.868 (-0.018, 1.753)	0.0544
EEG Relative Gamma Pz-Oz (%)	7.04	8.35	-0,226	1.091	1.316 (0.158, 2.475)	0.0273
EEG Relative Theta Fz-Cz (%)	25.07	23.4	1,098	-0.571	-1.669 (-2.999,-0.339)	0.0156
EEG Relative Theta Pz-Oz (%)	23.54	21.68	0,524	-1.332	-1.856 (-3.339,-0.372)	0.0166
Left Pupil/Iris ratio	0.3486	0.3537	-0.01204	-0.00690	0.00513 (-0.01380,0.02406)	0.5846
Right Pupil/Iris ratio	0.3485	0.3557	-0.00350	0.00367	0.00717 (-0.01071,0.02506)	0.4219
Face: number correct	14.8	14.7	-0.46	-0.60	-0.14 (-1.68,1.39)	0.8506
Face: avg RT correct (msec)	1807	1733	117.6	44.2	-73.4 (-332.9,186.1)	0.5574
Word recall 1 correct	2.4	2.5			0.14 (-0.31,0.60)	0.5242
Word recall 2 correct	4.1	4.1			-0.00 (-0.68,0.67)	0.9946
Word recall 3 correct	4.7	5.0			0.30 (-0.46,1.05)	0.4319
Delayed word recall correct	0.9	0.7			-0.21 (-0.63,0.21)	0.3138
Delayed word recognition correct	11.2	10.4			-0.89 (-2.72,0.94)	0.3301
Delayed word recog RT correct (msec)	5285.3	4111.3			-1174.07 (-2602.93,254.80)	0.1038
IGF_BP3 serum (mg/L)	2.54	2.62	1.1%	4.6%	3.5% (-1.0%,8.2%)	0.1297
IGF_I serum (nmol/L)	19.06	19.42	2.2%	4.1%	1.9% (-1.4%,5.2%)	0.2502

SAFETY Of all patients in the challenge phase, 39 reported at least one treatment emergent adverse event. Nausea was the most frequent reported adverse event, with 6 (54.5%) patients receiving 8 mg and 25 (64.1%) patients receiving 16 mg of galantamine and 2 (4%) patients receiving placebo. Diarrhoea was reported in 5 (12.8%) patients on galantamine 16 mg and 1 (2.6%) patient on placebo. Vomiting was reported in 2 (18.2%) patients on galantamine 8 mg and 14 (35.9%) patients on galantamine 16 mg. Dizziness was reported in 2 (18.2%) patients on galantamine 8 mg, 15 (38.5%) patients on galantamine 16 mg and 2 (4%) patients on placebo. Malaise and somnolence were reported in 4 (10.3%) patients on galantamine 16 mg and somnolence was reported in 1 (2.6%) patient on placebo. None of the

TABLE 2 Differences between responders and non-responders in their reactivity to the cholinergic challenge compared to placebo. PD variables were analysed by mixed model of analysis with treatment, time, and treatment by time as fixed factors, subject, subject by treatment, and subject by time as random factors and the average pre-value as covariate. Subjects were responders if MMSE, NPI and DAD at 6 months ≥ MMSE, NPI and DAD at baseline.

	LS Means		Contrast	
Parameter	**Resp. (Gal-Plac)**	**Non-Resp. (Gal-Plac)**	**Resp. (Gal-Plac) vs Non-Resp. (Gal-Plac)**	
			Treatment effect (95% CI)	**P-value**
Smooth Pursuit (%)	0.80	-0.40	1.21 (-1.63, 4.05)	0.3882
Saccadic Inaccuracy (%)	-0.90	-0.50	-0.43 (-1.80, 0.94)	0.5218
Saccadic Peak Velocity (deg/s)	18.20	-3.80	22.09 (-1.38, 45.57)	0.0636
Saccadic Reaction Time (sec)	-0.008	-0.012	0.0043 (-0.01, 0.02)	0.6498
Simple reaction time task (sec)	1.04%	0.96%	7.80% (-6.40%, 24.10%)	0.2841
Adaptive tracking (%)	0.71	0.85	-0.14 (-2.19, 1.92)	0.8948
VAS Alertness (mm)	-6.50	-3.20	-3.35 (-13.31, 6.61)	0.4968
VAS Calmness (mm)	-2.80	-4.20	1.43 (-10.9, 13.85)	0.8135
VAS Mood (mm)	-2.90	-0.90	-2.03 (-10.87, 6.82)	0.6398
VAS Nausea log(mm)	0.20	0.379	-0,17 (-0.56, 0.21)	0.3595
N-back mean RT 0 back (msec)	9	15	-6.10 (-73.40, 61.10)	0.8518
N-back mean RT 1 back (msec)	-21	-27	5.40 (-106.80, 117.60)	0.9187
N-back mean RT 2 back (msec)	0	-34	33.60 (-142.30, 209.50)	0.6948
N-back corr-incorr/total 0	-0.05	0.14	-0.19 (-0.42, 0.04)	0.1028
N-back corr-incorr/total 1	-0.11	0.21	-0.32 (-1.12, 0.48)	0.4126
N-back corr-incorr/total 2	0.00	-0.13	0.14 (-0.90, 1.17)	0.7873
EEG Alpha Fz-Cz (uV)	0.77%	0.95%	-18.4% (-29.6%, -5.5%)	0.0086
EEG Alpha Pz-Oz (uV)	0.93%	1.05%	-11.2% (-27.5%, 8.9%)	0.2440
EEG Beta Fz-Cz (uV)	0.82%	0.95%	-14.0% (-26.6%, 0.9%)	0.0629
EEG Beta Pz-Oz (uV)	0.99%	1.07%	-7.7% (-22.6%, 10.1%)	0.3605
EEG Delta Fz-Cz (uV)	0.86%	0.98%	-11.6% (-32.8%, 16.2%)	0.3644
EEG Delta Pz-Oz (uV)	0.91%	0.96%	-5.3% (-32.8%, 16.2%)	0.6889
EEG Gamma Fz-Cz (uV)	0.93%	0.97%	-3.7% (-20.7%, 16.9%)	0.6924
EEG Gamma Pz-Oz (uV)	1.13%	1.15%	-2.0% (-20.7%, 16.9%)	0.8970
EEG Theta Fz-Cz (uV)	0.71%	0.95%	-25.3% (-37.8%, -10.4%)	0.0027
EEG Theta Pz-Oz (uV)	0.81%	1.02%	-20.7% (-39.5%, 4.0%)	0.0903
EEG Relative Alpha Fz-Cz (%)	-0.82	-0.28	-0.538 (-2.441, 1.364)	0.5679
EEG Relative Alpha Pz-Oz (%)	0.73	0.14	0.590 (-3.184, 4.365)	0.7481
EEG Relative Beta Fz-Cz (%)	0.04	-0.25	0.282 (-1.147, 1.711)	0.6898
EEG Relative Beta Pz-Oz (%)	1.19	0.43	0.767 (-1.178, 2.711)	0.4258
EEG Relative Delta Fz-Cz (%)	2.06	0.42	1.644 (-1.556, 4.845)	0.3029
EEG Relative Delta Pz-Oz (%)	-0.50	-1.27	0.771 (-1.874, 3.415)	0.5548
EEG Relative Gamma Fz-Cz (%)	1.54	0.20	1.341 (-0.456, 3.137)	0.1375
EEG Relative Gamma Pz-Oz (%)	1.60	1.04	0.561 (-1765, 2.886)	0.6256
EEG Relative Theta Fz-Cz (%)	-3.30	-0.03	-3.271 (-5.958, -0.584)	0.0187
EEG Relative Theta Pz-Oz (%)	-3.18	-0.53	-2.651 (-5.631, 0.328)	0.0785
Left Pupil/Iris ratio	0.0037	0.0065	-0.00282 (-0.04087, 0.03524)	0.8811
Right Pupil/Iris ratio	0.0083	0.0060	0.00232 (-0.03353, 0.03817)	0.8966
Face: number correct	-1.1	0.8	-1.86 (-4.90, 1.19)	0.2226
Face: avg RT correct (msec)	-72	-75	2.3 (-513.3, 518.0)	0.9924
Word recall 1 correct	0.1	0.2	-0.06 (-0.97, 0.85)	0.8962
Word recall 2 correct	-0.7	0.7	-1.34 (-2.68, 0.01)	0.0517
Word recall 3 correct	0.3	0.3	-0.08 (-1.59, 1.43)	0.9129
Delayed word recall correct	-0.3	-0.1	-0.21 (-1.05, 0.62)	0.6072
Delayed word recognition correct	-0.7	-1.1	0.41 (-3.23, 4.05)	0.8207
Delayed word recog RT correct (msec)	-1885.8	-462.4	-1423.38 (-4257.69, 1410.93)	0.3135
IGF_BP3 serum (mg/L)	1.04%	1.03%	1.0% (-7.6%, 10.4%)	0.8265
IGF_I serum (nmol/L)	1.01%	1.03%	-1.8% (-8.0%, 4.8%)	0.5649

FIGURE 1 Changes in relative frontal EEG alpha and theta parameters of responders and non-responders. Figure 1 shows the changes in relative frontal EEG alpha (A) and theta (B) parameters of responders and non-responders compared to baseline on either the placebo or galantamine occasion. Long-term responders showed an acute increase after placebo on absolute frontal EEG parameters and on relative frontal theta power compared to baseline on the placebo occasion and a decrease compared to baseline on the galantamine occasion, whereas non-responders hardly showed any change from baseline on either the placebo nor galantamine occasion.

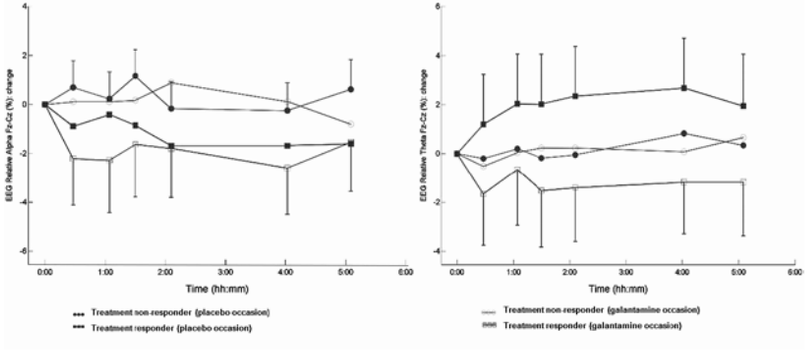
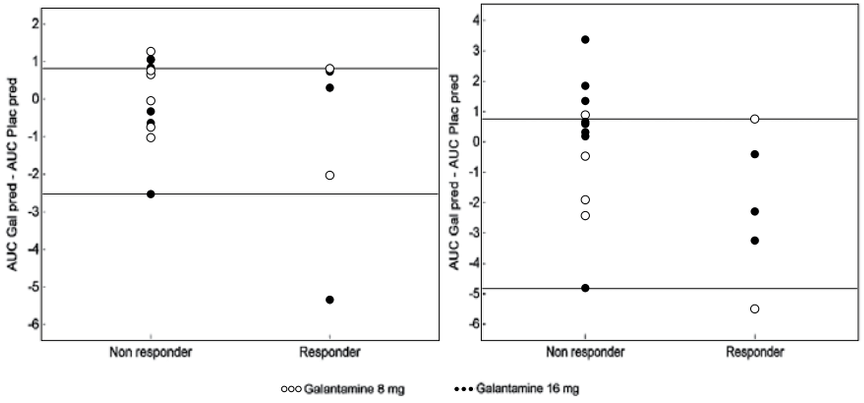


FIGURE 2 Delta AUC in relative frontal EEG alpha and theta parameters of responders and non-responders. Figure 2 shows a plot of delta AUC in relative frontal EEG alpha (A) and theta (B) power parameters of responders and non-responders. On the scatter plots, both absolute frontal alpha and frontal theta power distinguished responders from non-responders, with minimal overlap between responders and non-responders. For frontal alpha power, no responders were in the overlapping range. For frontal theta power, 2 responders (22,2%) and 3 non-responders (12,5%) were in the overlapping range.



other reported AEs occurred in more than 10% of patients. All adverse events were considered mild or moderate and spontaneously disappeared after a few hours.

During the treatment phase, one patient experienced moderate nausea during the first week of treatment and decided to discontinue the study and stop using galantamine. Two patients experienced mild nausea in the first two months of treatment. This subsided spontaneously and patients continued the use of galantamine. One patient reported moderate hyperhidrosis at the 6 month visit. In hindsight, this has been present the whole period. This patient decided to stop using galantamine.

DISCUSSION

In this study, we investigated the acute pharmacodynamic effects of a single dose administration of the galantamine on CNS functioning in mild to moderate AD patients and its role as a potential predictor of long-term treatment response. The results show improvements of saccadic eye movements and reductions of frontal EEG parameters in alpha, beta and theta frequencies after the challenge phase. Acute decreases of absolute frontal alpha, beta and theta power on EEG and an acute decrease in relative theta power significantly correlated with long-term response to galantamine treatment. In addition, a highly significant effect on the nausea VAS score was found, which may have particularly had an impact on tests that required sustained attention or active participation.

Reductions in saccadic inaccuracy and reaction time during the challenge phase might reflect an improvement in visual attentional function.[45] The cholinergic neuronal system plays a well-known role in the maintenance of attention through projections of neurons in the basal forebrain complex to broad areas of the neocortex. Moreover, slowing of saccadic eye movements is considered as a biomarker of declining alertness, particularly caused by benzodiazepines,[46-50] and eye movements are also sensitive to anticholinergic drugs. In this context it is interesting to note that patients demonstrated a clear and anticipated improvement in attentional function, without a statistically significant improvement in mean adaptive tracker performance. The adaptive tracker is known for its sensitivity to disturbances and enhancement of central cholinergic neuronal functioning and can be regarded as a test of sustained attention.[21,23,47] It might be that a reduced eye-hand coordination in this population of elderly patients has played a role in this discrepancy. The occurrence of adverse events (e.g. nausea) during the challenge phase of the study, as well as the highly significant effect on the nausea VAS score may also have played a role in obscuring

some of the beneficial effects of galantamine on CNS test performance, as some patients were not able to perform all tests, and particularly the adaptive tracking test which requires sustained attention.

In addition to the acute improvement in attentional function, the results show decreases of frontal alpha, beta and theta EEG parameters after dosing in the challenge phase. Slow wave activity, such as theta and delta waves, are associated with a lower cognitive function in AD patients.[51,52] Previous studies have already reported reductions in theta power following chronic CEI treatment.[17,53] In this study we demonstrate that galantamine administration also acutely reduces theta power in AD patients. Previous, an increase in frontal theta power was observed in a condition of mental exhaustion.[54] This might explain the observed increase in theta power during the day on the placebo occasion among patients classified as responders. This might also explain the increase of theta power in responders after the administration of placebo in the challenge phase. Interestingly, our results indicate that a single dose of galantamine is already able to reduce theta power. It is surprising that galantamine administration also reduced alpha and beta power in our study, while faster wave lengths are associated with improved cognition.[51,52,55,56] However, the absolute values for alpha and beta power reduction were relatively small and there was no reduction in relative alpha or beta power. Also, studies involving the anti-cholinergic and cognitive impairing drug scopolamine have reported conflicting results regarding the effects on alpha and beta power.[21,57]

Overall, there is a serious need for predictive markers of treatment response following CEI treatment in AD patients. So far it has been impossible to predict who will respond to CEI treatment and only 20-40% of the patients clinically improve. Most of the attempts to predict clinical response to long term treatment included pre-dose characteristics, for example sex,[58-61] age,[62,63] severity of cognitive impairment and impaired performance on baseline neuropsychological test scores at baseline,[11,64-67] pre-treatment progression rate,[68-71] cerebrospinal fluid levels of Aβ42, T-tau and P-tau at baseline,[68,72] carotid intima media thickness,[73] regional cerebral blood flow of the lateral and medial frontal lobes,[74] substantia innominata atrophy,[75,76] performance on baseline alertness tests,[9] baseline behavioural[77] and SPECT profile,[78] pre-treatment blood pressure drop,[62,79] and APOE genotype.[58-61,80-83] Some of these factors showed a positive correlation with treatment response. Our findings suggest that patients demonstrating a reduction in EEG alpha and theta power and saccadic eye movements after a single administration of galantamine 16 mg are more likely to respond to treatment. Nevertheless, it remains to be investigated how the addition of a galantamine challenge adds value on top of the above-mentioned correlations found in previous studies in predicting treatment response.

Lanctot and colleagues reviewed studies focusing on methods to predict the response to anticholinesterase therapy and markers for treatment response.[84] They were able to demonstrate the predictive value of qEEG profile after a test dose of the CEI tacrine, based on four clinical trials. Alhainen et al[85] firstly demonstrated that an increased alpha-theta ratio 90 minutes after a 50 mg test dose of tacrine led to higher MMSE scores four weeks post-treatment in 14 patients with probable AD. Alhainen and Riekkinen confirmed these findings on a longer term and showed that responders after seven weeks demonstrated a significant increase in mean absolute alpha power and alpha/theta ratio 90 minutes after a 50 mg test dose of tacrine.[86] Knott et al observed an increase of relative alpha and delta power waves in responders at 12 weeks, only 2 hours after an oral dose of 30 mg tacrine.[87] Almkvist et al suggested the validity of baseline EEG profiles as predictors of response to CEI therapy in 24 mildly to very mildly demented AD patients.[88] Except for the trial of Almkvist, these trials had an open-label design and all of them included only small numbers of patients, thus replication of these findings under double-blind conditions with larger patient samples was in our view necessary before conclusions can be drawn. Adler et al. further showed that treatment with rivastigmine 3 mg/day for one week led to a significant decrease in theta power on EEG which significantly correlated with responder status. When theta power and a baseline score for short term memory were both included as independent variables in a logistic regression model, treatment response could be accurately predicted.[17] Interestingly, the decrease in absolute alpha and theta power on EEG also predicted treatment response in our study. If we would have selected patients to be treated with galantamine based on either absolute frontal alpha power or absolute frontal theta power, *and* would also treat patients in the overlapping range, all patients defined as treatment responder would receive treatment. If this selection would be based on absolute alpha power, no non-responders would be treated. A selection based on absolute frontal theta power would result in the treatment of 3 non-responders. When selecting based on relative frontal theta power, 9 non-responders would be treated. Several combinations of these parameters have been investigated, but do not lead to a better prediction of treatment response. Moreover, all these explorations were *post hoc* and they would obviously require prospective validation.

While the studies of Adler, the Lanctot trials and our study show some inconsistencies, i.e. none of the other studies investigated the effects of galantamine and all of them used different definitions for 'acute response' (ranging from 90 minutes to one week), the predictive role of theta power on EEG seems consistent and is also confirmed in the current study. The Lanctot trials interestingly report on the increased alpha/theta ratio as a discriminator between

responders and non-responders, and not on absolute power EEG bands. Previous studies have shown that high/low band frequency ratios, e.g. alpha/theta ratios, can easily differentiate between AD patients and controls.[89-91] In our study, alpha/theta ratio was not a pre-defined outcome measure.

The sizeable group, the placebo controlled cross-over design and frequently repeated measures after dosing in the challenge phase and the combination with a follow-up study are strong aspects of the current study. Although the predefined response criteria of improvement on all three clinical scales may seem strict, this definition is based on not only improvement in cognition, but also activities of daily living and behavioural aspects, and it is closer therefore to a true clinical improvement than a responder criterion based on only one of these tests. If a patient declines in one dimension, e.g. ADL functioning, but not in another, e.g. cognitive functioning, both patient and doctor are likely to still regard this as an unsatisfactory non-response to treatment. Also, the correlations between the individual challenge tests and clinical follow-up measures did not show any consistent correlations and the number of responders (11 (25%)), which was consistent with expectations based on previous studies.[10,17,83] The difference between responders and non-responders could not be attributed to differences in levels of drug exposure, since there was no difference in average plasma concentrations of galantamine after two months of treatment between responders and non-responders.

It should be noted that sample size calculations were based on the observed variance in clinical improvement correlated with the decrease in theta power in a comparable study,[17] while we mainly draw conclusions about dichotomized treatment response (responder and non-responder) at 6 months in relation with acute challenge effects of PD variables. As data from that study was most comparable to data in the current study at that time, we believe this as the most appropriate method. Also, a responder score based on MMSE, NPI *and* DAD instead of independent scores, seemed more representative for real-world clinical improvement in AD patients. Other weaknesses of this study include the occurrence of side effects due to a pharmacological challenge, which were such that in the challenge phase some patients were not able to perform all tests due to nausea or had to decline the last round of tests due to fatigue. Also, especially the 2-back condition of the N-back turned out to be too difficult for AD patients.

This study is the first placebo controlled study with cross-over design that links typical PD effects in an early phase clinical drug trial to the clinically relevant outcome measures used for phase III registration studies in the field of AD. Furthermore, this study generates a well-defined time-profile of the effects of galantamine in the target population of patients with mild to moderate AD, with an observed T_{max} of galantamine around 2 hours after administration, which is consistent with previously reported findings of a T_{max} of approximately 1.5 hours after a single oral dose of 10 mg galantamine with immediate release formulation.[92] Reductions in both absolute and relative theta power were obviously most pronounced around 2 hours after the administration of galantamine and continued to be equally reduced over time. Cut-off criteria seem arbitrary, however we believe that cut-off criteria based on multiple tests are more representative for the actual patient condition, compared to cut-off criteria based on one test.

Conclusion and future perspectives

This study demonstrates that acute PD effects after single dose of galantamine are correlated with long-term treatment effects and that patients demonstrating a reduction in EEG alpha and theta power and saccadic eye movements after a single administration of galantamine 16 mg are more likely to respond to treatment. Further confirmation of these findings is needed from prospective trials. This study takes a first step towards finding predictive biomarkers of treatment response to CEIs. In the future, these biomarkers might prevent the redundant exposure of AD patients to drug treatment and its related side effects.

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CHAPTER 7

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

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ABSTRACT

INTRODUCTION In previous studies, the H_3R antagonist CEP-26401 had a subtle effect on spatial working memory, with the best effect seen at the lowest dose tested (20µg), and a dose-dependent disruption of sleep. In the current study, three low dose levels of CEP-26401 were compared with modafinil and donepezil.

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

RESULTS The main endpoint between-errors of the SWM-10-boxes task only improved for the 125 µg dose of CEP-26401 with a difference of 2.92 (CI -1.21 – 7.05), 3.24 (CI -1.57 – 8.04) and 7.45 (CI 2.72 – 12.19) for respectively the 5, 25 and 125 µg dose of CEP-2640, -1.65 (CI -.572 – 1.96) for modafinil and -3.55 (CI -7.13 – 0.03) for donepezil. CEP-26401 induced an improvement of adaptive tracking, saccadic peak velocity and reaction time during N-back, but a dose-related inhibition of sleep and slight worsening of several cognitive parameters at the highest dose. CEP-26401 significantly changed several subjective VAS scales, which was strongest at 25 µg, causing the same energizing and happy feeling as modafinil, but with a more relaxed undertone.

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

INTRODUCTION

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

In preclinical studies, mainly in mice and rats, positive effects of H_3R antagonists were found on working memory, memory consolidation, social memory, spatial orientation and attention and impulsivity.[11,12] These positive effects were also seen in models for negative symptoms of schizophrenia, but not in Alzheimer's disease models.[11,12] Human studies have mainly focused on treatment of ADHD and excessive daytime sleepiness (EDS). Pitolisant, an H_3R inverse agonist, has been shown to improve ADHD symptoms and reduce EDS in patients with narcolepsy and obstructive sleep apnoea syndrome.[13,14] The effects of H_3R antagonists on cognitive disturbances in Alzheimer's disease and schizophrenia were not consistent.[15-17]

CEP-26401 ([6-[4-[3-[(2R)-2-methyl-1-pyrrolidinyl]propoxy]phenyl]-3-(2H)-pyridazinone hydrochloride]) is an H_3R antagonist/inverse agonist that displays high-affinity H_3R binding and potent functional antagonism in both rat and human recombinant cell and native rat brain cortical systems.[18-22] Two clinical studies with orally administered single and multiple doses of CEP-26401 in healthy volunteers have been performed prior to this study with interesting results on the spatial working memory (SWM) task.[23] In this task several boxes are presented on the screen, in one of which a token is to be found. The token never appears in the same box more than once and the test continues until a token had been found in all of the boxes once. Each click on an empty box is counted as an error.

Applying a population pharmacokinetic/pharmacodynamic (PK/PD) model, an effect on spatial working memory (SWM) was found with a maximal decrease of 10.8 errors (clinically relevant improvement of cognitive function) at plasma levels ≤0.01 ng/mL (dose ≤ 20 µg), but with a maximal increase of 17.6 errors (worsening of cognitive function) at plasma levels ≥0.1 ng/mL (dose ≥ 80 µg). Sleep was affected in a dose-related fashion with an increase in time awake after sleep onset to about a 2.4-fold increase at plasma levels ≥16 ng·h/mL (dose ≥ 50 µg) after single dose. Although data were derived from two different studies with parallel-group designs, where differences between groups and study design may have played a role (i.e. studies were not powered nor specifically designed to detect differences in cognition enhancement), the PK/PD-model based on these studies consistently indicated the largest cognitive effects at the lowest dose.[23] It was therefore of interest to investigate the dose-response relationship of CEP-26401 on cognition at a dose range below as well as above 20 µg.

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

METHODS

Study design

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

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

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

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

Subjects

A total of 40 healthy male and female subjects in an approximate 1:1 ratio, aged 18-50 years (inclusive) with a body mass index (BMI) of 18.0-30.0 kg/m² (inclusive), were recruited for this study. Main exclusion criteria were smoking or use of nicotinic products within 3 months before inclusion, alcohol or drug abuse, excessive daily use of caffeine (>800 mg per day), use of medication with CNS effects or PK interactions and irregular diurnal rhythm. Because CEP-26401 could bind to melanin containing tissues (data on file, Teva Pharmaceuticals), subjects with a dark skin (Fitzpatrick scale 5 or 6[25]) were excluded. Also, subjects had to have a performance score on the spatial working memory test within normal range in order to reduce ceiling effects on cognitive testing.

Randomization

In this partial 4-period- 6-treatment cross-over study, subjects were first randomized to one of the three cohorts, each with a different combination of treatments (Table 1). Within each cohort, subjects were randomly assigned to a treatment sequence using a Williams design. Each of the cohorts was comprised of 13 or 14 subjects. A total of 40 subjects were enrolled, with the intention of at least 36 subjects

completing the entire study, 12 from each cohort. All treatments were administered as a single dose, with 14 days separating each treatment administration. Each subject underwent 4 study periods and received placebo on one of these periods. As this was a double dummy design, each subject received on each occasion CEP-26401 or placebo, modafinil or placebo and donepezil or placebo. Modafinil and donepezil and its matching placebos were over-encapsulated.

TABLE 1 **Treatments per cohort.**

	Placebo	CEP-26401 5 mcg	CEP-26401 25 mcg	CEP-26401 125 mcg	Donepezil HCL 10 mg	Modafinil 200mg
Cohort 1	+	+	+	+	-	-
Cohort 2	+	+	+	-	+	-
Cohort 3	+	+	-	+	-	+

Study medication and dosing rationale

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

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

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

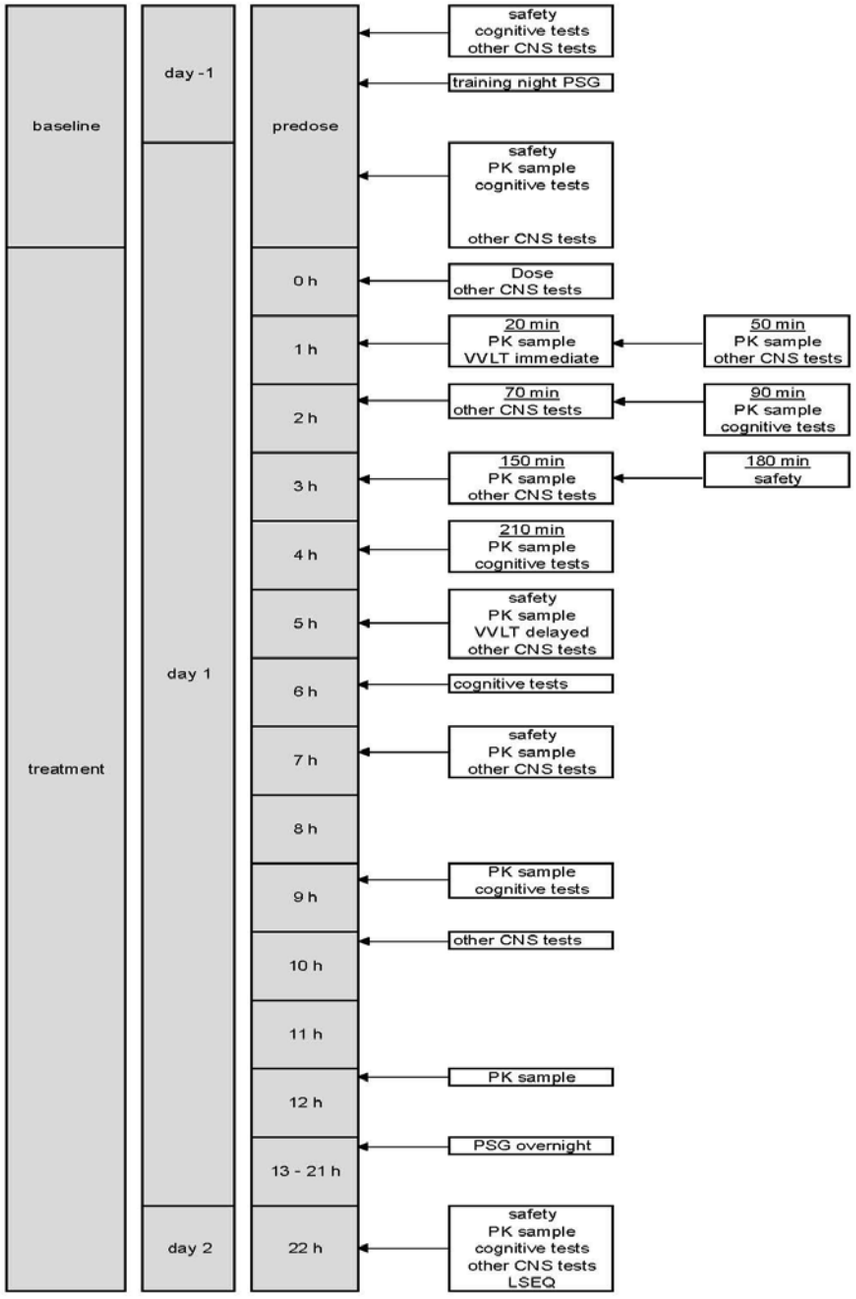
Pharmacodynamic methods

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

Cognitive tests

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

FIGURE 1 **Schedule of assessments.**



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

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

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

VISUAL VERBAL LEARNING TASK (VVLT) The Visual Verbal Learning Test contains three different subtests that cover basically the whole scope of learning behaviour (i.e., acquisition, consolidation, storage and retrieval). Volunteers performing the VVLT were presented 30 words in three consecutive word trials. Each trial ended with a free recall of the presented words (Immediate Recall). Approximately thirty minutes after start of the first trial, the volunteers were asked to recall as many words as possible (Delayed Recall). Immediately thereafter, the volunteers underwent memory recognition test, which consisted of 15 presented words and 15 new 'distractors' (Recognition).[35]

MAZE LEARNING Subjects had to complete a maze by using trial and error learning to locate a 28-step pathway (from upper-left to bottom right) that was hidden beneath a 10×10 grid of tiles. Individuals had to find the same pathway on five successive trials. Approximately 30 minutes after start of the first trial, the volunteers were asked to identify the same maze again (delayed test, one trial). Immediately thereafter, the volunteers underwent the reversed test, which consists of one trial of the same maze backwards (from bottom-right to upper-left).[36]

N-BACK This test evaluates working memory and requires buffering and updating consonants, matching, encoding and responding. The N-Back test consists of three conditions, with increasing working memory load. Letters were presented consecutively on the screen with a speed of 30 letters per minute. In the first condition subjects had to indicate whether the letter on the screen was an 'X'. In

the second condition, subjects indicated whether the letter seen was identical to the previous letter. In the third condition, subjects were asked to indicate whether the letter was identical to two letters before the letter seen.[37-39]

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

Subjective measurements: VAS Bowdle, VAS Bond & Lader, VAS task enjoyment

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

Other CNS tests

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

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

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

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

Measurement of Sleep

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

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

Assessment of safety

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

Pharmacokinetic methods

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

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

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

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

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

RESULTS

Demographics and disposition

A total of 80 subjects were screened for enrolment into this study. Of the 80 subjects screened, 40 subjects met inclusion criteria and were considered to be eligible for enrolment into the study. Of the 40 subjects who were not enrolled, 29 were excluded based on enrolment criteria and 11 subjects did not participate for other reasons. Of the 40 subjects enrolled, all received at least 1 dose of study drug and were evaluated for safety. Four subjects withdrew from the study for personal reasons (10%). Of these subjects, 1 subject completed 3 occasions, 1

subject completed 2 occasions and 2 subjects completed 1 occasion. All 4 missed their placebo occasion. Consequently, 36 subjects completed a placebo occasion. The cohorts were similar with regard to age, weight, and BMI (Table 2).

TABLE 2 Demographics.

	Cohort 1 (n=13)	Cohort 2 (n=14)	Cohort 3 (n=13)	Total (n=40)
Age (years)	29.0 (18 – 48)	25.4 (19 – 48)	26.2 (18 – 48)	26.8 (18 – 48)
Sex (n male)	5 (38%)	10 (71%)	7 (54%)	22 (55%)
Weight (kg)	70.8 (55.5 – 92.3)	76.1 (53.8 – 95.2)	71.9 (47.6 – 86.3)	73.0 (47.6 – 95.2)
BMI (kg/m2)	23.4 (19.3 – 29.4)	23.7 (18.3 – 28.2)	23.2 (18.2 – 28.9)	23.4 (18.2 – 29.4)

For age, weight and BMI: mean, range in parenthesis. For sex: number of male subjects, percentage in parenthesis.

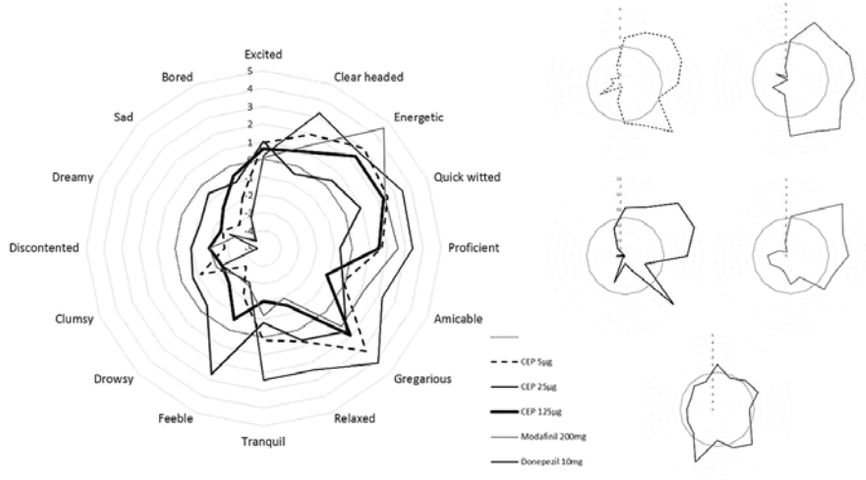
Pharmacodynamics

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

SUBJECTIVE EFFECTS The two lowest doses of CEP-26401 induced significant improvements on several subscales of the VAS Bond & Lader, which were strongest at the 25 µg dose (Figure 2). Administration of CEP-26401 5 µg led to feelings of alertness, energy, contentedness, quick-wittedness, attention, happiness and gregariousness ($p<0.05$). Administration of CEP-26401 25 µg induced feelings of strength, clear-headedness, coordination, contentedness, quick-wittedness, attention, proficiency, happiness, interest and gregariousness. The increase in alertness and energy almost reached significance at this dose of CEP-26401. Administration of CEP-26401 125 µg did not lead to any statistically significant changes on the VAS Bond & Lader. All doses of CEP-26401 induced a significant improvement on the VAS score for task enjoyment. Task enjoyment was also improved by modafinil, which additionally only increased VAS Bond & Lader scores for energy and happiness. Administration of donepezil did not lead to any changes on the VAS Bond & Lader, or task enjoyment, but there was an increase on VAS Bowdle scores of feeling high, change in surroundings and feeling of unreality. These effects in VAS Bowdle were not seen with CEP-26401 or modafinil.

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

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



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

Pharmacokinetics

CEP-26401 was absorbed with median t_{max} values of approximately 3.5 to 5.0 hr (Figure 3, table 5). After reaching peak plasma levels, CEP-26401 slowly declined with mean concentrations at the 22-hr time point representing approximately 60% of C_{max} Systemic exposure to CEP-26401, (C_{max}, AUC_{0-T}) increased in an approximately dose-proportional manner across the dose range evaluated. The mean C_{max} values for the 5-, 25-, and 125-µg doses were 9.1, 45.4, and 245.4 pg/mL, respectively, and the corresponding mean AUC_{0-T} values were 152, 743, and 3925 pg·hr/mL. The coefficient of variation associated with these parameters was between 15 and 20%. Despite 14-day washout periods, low but quantifiable levels of CEP-26401 were observed in some of the pre-dose samples from all treatments. This finding was not completely unexpected given the long terminal elimination half-life observed for CEP-26401 in previous PK studies [23] and in consideration of the sensitivity of the bioanalytical method.

TABLE 3 Cognitive, subjective and general CNS effects compared to placebo, using a mixed-model analysis o covariance.

	CEP-26401 5 µg (n=38)	CEP-26401 25 µg (n=26)	CEP-26401 125 µg (n=25)	Modafinil 200 mg (n=13)	Donepezil 10 mg (n=13)
Spatial Working Memory – Between errors 10 boxes	2.92 (-1.21 – 7.05) P=0.1630	3.24 (-1.57 – 8.04) P=0.1837	7.45 (2.72 – 12.19) P=0.0024	2.30 (-3.84 – 8.45) P=0.4583	-0.71 (-7.12 – 5.71) P=0.8276
Rapid Visual Information Processing – A Prime	0.00 (-0.00 – 0.01)	0.00 (-0.00 – 0.01)	0.00 (-0.00 – 0.01)	0.01 (0.00 – 0.02)	-0.01 (-0.02 - -0.00)
Stop Signal Task – Reaction Time	-11.57 (-26.28 – 3.15)	-8.79 (-25.46 – 7.89)	-11.39 (-28.07 – 5.28)	-1.79 (-23.12 – 19.55)	19.77 (-2.32 – 41.86)
Paired Associate Learning – Total Errors Adjusted	1.78 (-0.47 – 4.02)	2.69 (0.12 – 5.26)	2.97 (0.42 – 5.53)	-2.41 (-5.47 – 0.91)	4.97 (1.51 – 8.42)
N–back – 0-back Reaction Time (msec)	-3.37 (-16.43 – 9.68)	10.52 (-4.78 – 25.82)	2.58 (-12.52 – 17.67)	3.51 (-15.76 – 22.78)	0.48 (-19.81 – 20.76)
N-back – 2-back Accuracy	0.0 (-0.03 – 0.03)	-0.01 (-0.04 – 0.03)	-0.04 (-0.08 - -0.00)	-0.02 (-0.07 – 0.03)	0.00 (-0.05 – 0.05)
N–back – 2-back Reaction Time (msec)	-17.65 (-36.66 – 1.36)	-25.04 (-47.34 – -2.73)	-39.25 (-61.10 – -17.40)	-5.97 (-34.32 – 22.38)	-27.72 (-57.26 – 1.82)
VAS Task Enjoyment	3.21 (0.86 – 5.55)	3.87 (1.16 – 6.58)	3.19 (0.49 – 5.89)	4.64 (1.17 – 8.12)	0.52 (-3.10 – 4.13)
Adaptive tracking (%)	0.74 (0.06 – 1.43)	1.08 (0.28 – 1.88)	1.20 (0.42 – 1.98)	1.80 (0.80 – 2.81)	0.49 (-0.57 – 1.54)
Saccadic Peak Velocity (degree/sec)	4.00 (-2.29 – 10.28)	6.75 (-0.50 – 13.99)	16.99 (9.73 – 24.24)	24.62 (15.32 – 33.92)	3.06 (-6.92 – 13.04)
Body Sway (mm)	-16.89 (-46.08 – 12.30)	3.43 (-30.08 – 36.93)	-28.72 (-61.94 – 4.51)	-54.44 (-97.29 - -11.60)	24.22 (-20.54 – 68.88)
EEG Frontal Gamma Frequency	0.06 (0.01 – 0.12)	0.07 (0.01 – 0.14)	0.05 (-0.01 – 0.12)	0.10 (0.01 – 0.18)	0.06 (-0.03 – 0.14)

Mean, confidence interval in parentheses. Statistically significant differences in bold.

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

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

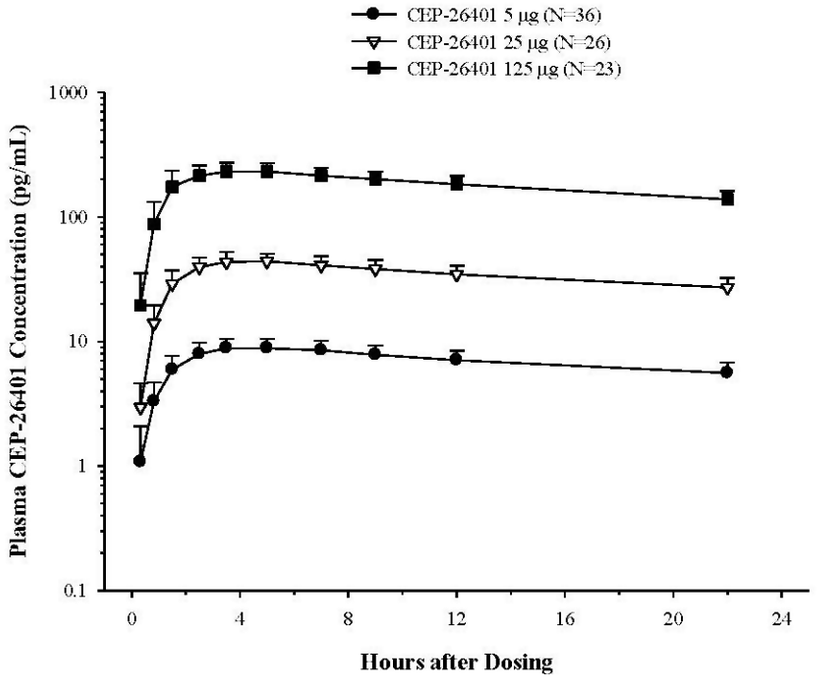
Mean, confidence interval in parentheses. Statistically significant differences in bold. Sleep efficiency is the percentage of time in bed while the subject is asleep. LSEQ: Leeds Sleep Evauation Questionairre

TABLE 5 Pharmacokinetic parameters of CEP-26401 in healthy subjects administered single oral doses of CEP-26401 at 5, 25, and 125 µg

Parameter	CEP-26401 5 µg (N=36)	CEP-26401 25 µg (N=26)	CEP-26401 125 µg (N=23)
C_{max}, pg/mL	8.97 (1.770)	44.83 (7.344)	242.56 (38.818)
t_{max}, hr	4.205 (1,300)	4.453 (1,243)	3.685 (1,756)
AUC_{0-t}, pg·hr/mL	149.63 (29.168)	732.93 (126.472)	3882.36 (612.045)

Geometric mean, standard deviation in parenthesis

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



Safety

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

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

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

MedDRA System Organ Class MedDRA Preferred Term	Number (%) of subjects					
	Placebo (N=36)	CEP-26401 5 µg (N=38)	CEP-26401 25 µg (N=26)	CEP-26401 125µg (N=25)	Modafinil 200mg (N=13)	Donepezil 10 mg (N=13)
Number of subjects with at least 1 adverse event	27 (75)	25 (66)	16 (62)	16 (64)	10 (77)	13 (100)
Gastrointestinal disorders						
Abdominal Pain	1 (3)	2 (5)	1 (4)	2 (8)	1 (8)	2 (15)
Nausea	1 (3)	2 (5)	3 (12)	5 (20)	1 (8)	12 (92)
Vomiting	0	1 (3)	0	0	0	7 (54)
General disorders and administration site conditions						
Fatigue	7 (19)	9 (24)	4 (15)	3 (12)	1 (8)	2 (15)
Malaise	1 (3)	1 (3)	0	0	1 (8)	
Feeling Hot	0	0	0	1 (4)	1 (8)	4 (31)
Infections and infestations						
Nasopharynigits	3 (8)	2 (5)	2 (8)	2 (8)	2 (15)	0
Injury, poisoning and procedural complications						
Procedural dizziness	0	0	0	0	1 (8)	2 (15)
Nervous system disorders						
Headache	6 (17)	7 (18)	6 (23)	6 (24)	5 (38)	3 (23)
Somnolence	10 (28)	7 (18)	3 (12)	1 (4)	0	2 (15)
Dizziness	3 (8)	3 (8)	2 (8)	3 (12)	0	6 (46)
Tremor	0	0	0	0	0	2 (15)
Psychiatric disorders						
Hypervigilance	1 (3)	0	0	1 (4)	3 (23)	0
Respiratory, thoracic and mediastinal disorders						
Oropharyngeal Pain	0	3 (8)	0	0	2 (15)	0
Skin and subcutaneous tissue disorders						
Hyperhidrosis	0	1 (3)	1 (4)	3 (12)	0	3 (23)
Vascular disorders						
Hot Flush	0	0	0	0	0	2 (15)

Number of subjects, percentage in parentheses; MedDRA= Medical Dictionary for Regulatory Activities.

and tremor all occurred at least in at least 10% of subjects and more frequently than after placebo. In contrast, somnolence and fatigue were reported less often after donepezil than under placebo.

All AEs were mild or moderate, except for one subject with severe headache and vomiting after administration of CEP-26401 5 µg. One subject had an asymptomatic increased intraocular pressure of 23 mmHg on the right eye at 5 hours after administration of CEP-26401 125 µg, which was normalized at the next measurement at 22 hours after drug administration. Three subjects had an increase in eosinophils during the study. One experienced a progressive rise throughout the study. Two others had eosinophilia at baseline and experienced fluctuations during the study with one reaching 23.41% eosinophils (absolute eosinophil count of $1730x10^6$/L) before returning to near baseline. A relationship between the eosinophilia and the study drug could not be excluded, but the AEs for these subjects did not seem to point to clinical significance for the eosinophil elevation. There were no clinically significant changes in other laboratory values, vital signs, ECG and physical examination.

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

DISCUSSION

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

Despite the significant CNS-stimulating effects that were demonstrated with the NeuroCart, CEP-26401 did not have any beneficial effect on cognitive testing, even though this was expected based on previous studies with CEP-26401 in healthy volunteers.[23] At the highest dose of 125 µg there was even some decline at the accuracy of the N-back task and an increase in total errors on SWM and

PAL. As administration of modafinil led to an improvement on RVIP, it is unlikely that the lack of effect of CEP-26401 on this test is due to inadequate study design or test conditions. As the cholinesterase inhibitor donepezil did not induce any measurable effects on SWM, PAL and SST either, it is also possible that this was precluded by ceiling effects in this healthy population or that the tests used were not sensitive enough. The improvement on RVIP after administation of modafinil argues against this explanation, although it is possible that SWM, PAL and SST have a ceiling effect, while RVIP has not. Another possibility is that cognitive enhancement was obscured by AEs of the 10 mg dose in these young subjects. Previous studies at CHDR have shown positive effects of donepezil 10 mg on N-back and adaptive tracking in healthy elderly volunteers.[60] It is possible that a slight, age related cholinergic deficiency in elderly subjects has contributed to the measurability of these effects, and that they tolerate the drug better.[61] The current study however provides no indication that CEP-26401 might have cognitive enhancing effects, and does not provide reasons to assume efficacy in cognitive disorders such as AD. This is in contrast with results from several preclinical studies with other H_3 antagonists, which demonstrated an effect on working memory, memory consolidation, spatial orientation and attention.[11] Also, two clinical trials in patients with mild to moderate Alzheimer's disease reported small improvements in attention and memory with the H_3R antagonist GSK239512.[15,62] On the other hand, it is consistent with a large phase 2 trial with two doses of an H_3 antagonist in patients with AD, which was aborted prematurely, because futility criteria were met.[17] Other trials aimed to improve cognitive impairment associated with schizophrenia with an H_3R antagonist, also failed to demonstrate efficacy.[16,63] This study seems to add to the evidence against beneficial cognitive effects of H_3R antagonists.

Although not immediately expected, CEP-26401 had extensive positive effects on several subjective VAS scales, which were significant in 8/16 scales at 5 µg, in 12/16 scales at the 25 µg dose, but in none of the scales at the 125 µg dose. The positive effects were not limited to feelings of energy, happiness and task enjoyment, as was observed after administration of modafinil, but also included feelings of contentedness, proficiency, interest and gregariousness. It is of interest that the two lowest doses of CEP-26401 also produced the lowest number of cognitive AE reports (31-32%) - lower even than placebo (56%) and much lower than modafinil (62%) or donepezil (100%). The subjective energetic and alert feeling is also reflected in the dose dependent improvements on adaptive tracking and saccadic peak velocity, as these indicate an increase in vigilance and motivation. Thus, CEP-26401 seems to induce the same, subjectively and objectively measured, energizing and happy feeling as modafinil, but with a more relaxed undertone – at least in the low doses used in this study. It is known that modafinil acts on dopaminergic neurons.[28] Since CEP-26401 affects basically the same parameters as modafinil, it could be suggested that it has – at least – indirect influence on this neurotransmitter system. However, since CEP-26401 has more extensive effects than modafinil, most likely other neurotransmitter systems are also involved. This would be consistent with a microdialysis study in rats, where administration of CEP-26401 led to an increase of both dopamine and acetylcholine.[64]

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

Although CEP-26401 did not have the expected positive effect on cognition and cannot be typified as a cognitive enhancer, it may be a useful drug for certain indications that are characterized by a lack of internal drive and energy. The stimulant effects of CEP-26401 on objective CNS tests (PSG, adaptive tracking, saccadic peak velocity) were generally dose-dependent, whereas subjective effects were most favourable at a dose of 25 µg, but virtually disappeared after 125 µg. Except for a dose dependent inhibitory effect on sleep, CEP-26401 was well-tolerated by most study subjects with only one patient experiencing severe adverse events (an episode of headache and nausea). Based on these observations, the 25 µg dose of CEP-26401 has the optimal balance between favourable subjective and stimulatory effects, and inhibitory effects on sleep. The more strong, clear-headed, well-coordinated, interested and quick-witted feeling in combination with a more contented, attentive, proficient, happy and gregarious feeling might give benefit to patients suffering from certain types of mood disorders, such as major depression or dysthymia, negative symptoms of schizophrenia or anxiety disorders, especially social anxiety. The energizing aspects of CEP-26401 might give extra benefit to elderly patients with mood disorders, because they usually have more apathy, compared to younger patients.[65]

However, there are also possible challenges with the use of CEP-26401 in a clinical setting. There appears to be a bell-shaped response curve which

implies a relatively narrow therapeutic window. It remains to be established whether the pleasurable effect might generate abuse potential, especially in already vulnerable, psychiatric patient populations. Stimulant effects may also be undesirable in (unrecognized) bipolar disorder, and the effects may differ in elderly subjects, particularly with cognitive impairment. Also, the effects on sleep cannot be ignored and might constitute a clinically relevant adverse reaction.

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

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

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CHAPTER 8

Summary and discussion

SUMMARY AND DISCUSSION

This thesis describes studies of the effects on cognition of drugs that stimulate or inhibit the cholinergic system by direct or indirect mechanisms. All study designs included extensive pharmacodynamic testing in various phases of drug development. Consequently, the study designs and study populations differed, depending on the aim of the study. In general we performed extensive studies of pharmacodynamics effects in subjects with normal or impaired cognition, or in healthy volunteers with a previously pharmacologically impaired cognitive system (challenge studies).

Chapter 2 describes a study of the effects of several doses of the α7 nAChR partial agonist EVP-6124, alone and in combination with two doses of the cholinesterase inhibitor donepezil in healthy elderly subjects, receiving a scopolamine challenge prior to administration of EVP-6124 and/or donepezil. As preclinical studies showed a complete reversal of the scopolamine effect, the expectations were high. However, efficacy of EVP-6124, alone or in combination with donepezil, could not be shown. Despite these negative results, the compound was tested in patients with AD and provided some indications for therapeutic efficacy.[1] Subsequent clinical trials were less positive, and the compound was withdrawn from further development. The lack of effect reversal of scopolamine effects by EVP-6124 in Chapter 2 is therefore in line with the equivocal clinical effects of the compound. However, there are other possible explanations for the negative results of the scopolamine study. First, the results may have been obscured by the strong sedative effect of scopolamine in the healthy elderly subjects. Another explanation is that scopolamine is a muscarinic antagonist, which may not be antagonized by an α7 nAChR partial agonist. This highlights some problems of pharmacological challenge studies. The validation of such an intervention may require more work to get an optimal representation of the precise pharmacological effect or the real clinical condition that is mimicked.

The limitations of scopolamine as an anticholinergic challenge model were addressed in **Chapter 3.** This describes an extensive exploration of another anticholinergic challenge model with the nAChR specific antagonist mecamylamine. Although this challenge model has been used before, its PD and PK characteristics were largely unknown and a detailed comparison with scopolamine was never done. The pharmacokinetics of mecamylamine are described in **Chapter 4**. In contrast to scopolamine, mecamylamine did not have any sedative effects, but it did cause measurable cognitive decline. From a pharmacological point of view, it seems more logical to use this nicotinic challenge model for proof of concept studies with nAChR agonists. Clearly more work needs to be done to fully validate this model a challenge for drug development, but this study demonstrates the need for a systematic approach for such challenge models.

In **Chapter 5,** Gln-1062, a prodrug of the cholinesterase inhibitor galantamine was tested in an adaptive design. The reason of this was that although the active moiety was well known and safe, the prodrug was designed to have an improved brain penetration and hence increased cholinesterase inhibition in the brain. Consequently, equimolar dosage could not *a priori* be considered safe. The trial started with the standard starting dose of 10% of the level of no adverse effects (NOAEL) in animal studies. Since no PD effects were expected at the two lowest doses to be administered, these were given to healthy young male volunteers and PK, PD and safety were measured. Unexpectedly, even these low doses induced some measurable effects on attention and memory. The study continued in three cohorts of healthy elderly male volunteers, as they are expected to have a slight, age-related cholinergic deficiency, which was expected to increase the possibility of finding any pharmacodynamic effects, without the necessity of administration of an anticholinergic challenge. MMSE score was included in the exclusion criteria to prevent inclusion of demented patients in the study. The increasing doses of Gln-1062 in these cohorts were compared to donepezil and galantamine. Galantamine did not induce any measurable effects, while donepezil improved the performance on the adaptive tracking, comparable with the effect of the 33 mg and 44 mg dose of Gln-1062. While improvements were demonstrated on the adaptive tracking test and the VVLT, ceiling effects may well have limited the extent to which positive effects could be demonstrated in healthy younger subjects. Therefore in this study we introduced a novel approach to the evaluation of compounds for AD, namely to perform the study in 'physiologically impaired subjects', elderly without overt cognitive symptoms but with likely some incipient cholinergic neuronal dysfunction in contrast to the pharmacologically challenged subject.

In Chapter 5 we showed that the effects of galantamine can be measured acutely in healthy young and elderly subjects, even after a single dose. In **Chapter 6** we proceeded to a study in AD patients. Here we asked the question if the acute effects of a single dose of an anticholinergic medicine, galantamine, could predict a response to chronic treatment at 6 months. In this study, there was no significant effect on the adaptive tracking (primary endpoint), but the effect on EEG parameters after a single dose predicted the treatment response to galantamine at 6 months. Such an approach could lead to a more personalized approach to treatment. In case of CEI treatment, this could spare +/- 70% of the current medication prescriptions. Rather than directly affecting the cholinergic system, other approaches that affect cholinergic and cognitive systems indirectly are studied

and we applied our system of development to a histamine 3 receptor (H_3R) inverse agonist (CEP-26401) in **Chapter 7**. As histamine has an indirect effect on several neurotransmitter systems, including the cholinergic system, this was considered a target for procognitive medication. Based on previous studies with this compound, low doses were administered and its effects were compared to placebo, donepezil and modafinil. The population of healthy volunteers was chosen as initial target, because a comparison with positive controls was incorporated in the design, which included two very extensive test battery with sensitive tests (CANTAB and NeuroCart). In this study, the primary (cognitive) endpoint was not met, but there was a positive effect on subjective feelings, which was strongest at the 25 µg dose. There was no improvement on cognitive testing and even some worsening on the spatial working memory test (SWM 10 boxes; primary endpoint of the study) and paired associate learning test after administration of the highest dose of CEP-26401. As donepezil did not induce any improvement either, ceiling effects might still have influenced the outcome of this study, despite the chosen test battery. This remains a problem of the study of such drugs on a healthy population with optimal cognitive functioning that is difficult to improve. In our approach, but outside the scope of this thesis, the compound also requires testing in physiologically impaired or pharmacologically challenged subjects before any definite conclusions can be drawn about its clinical value. The positive effects of CEP-26401 on subjective feelings may also indicate that its mechanism of action (histamine 3 receptor (H_3R) inverse agonism) renders this class of compounds more suitable for the treatment of mood disorders than for the treatment of cognitive disorders.

Even though the primary endpoints were not met in some of the studies, the profile of cholinergic intervention on the used biomarkers seems to be quite consistent. Based on the function and localisation of acetylcholine receptors in the brain, cholinergic drugs are expected to influence mainly memory and attention. In all studies in this thesis, both with procholinergic and anticholinergic compounds, effects were mostly observed on the adaptive tracker (attention), N-back (working memory), visual verbal learning test (working memory and recall) and EEG parameters (table 1).

With regard to the effects on memory, it is remarkable that the direct recall is more often influenced than the delayed recall and recognition. This is inherent to the function of acetylcholine in learning. Memorising is a complicated process, consisting of an encoding phase, when information is received and comprehended, a consolidation phase to 'store' memories for a longer time and a retrieval phase to reproduce the previously learned information. Both preclinical and clinical research suggest that the encoding phase requires high acetylcholine levels in the brain, while for the consolidation phase lower levels of acetylcholine are sufficient.

TABLE 1 **Effects of pro- and anti-cholinergic compounds on N-back, VVLT, EEG and adaptive tracking**

		N-back	VVLT	EEG	Tracker
PRO-CHLINERGIC COMPOUNDS	donepezil	improvement RT 0-back (5 mg); deterioration RT 2-back (5 mg)			improvement (2,5 mg and 10 mg)
	galantamine			decrease in relative frontal theta power; decrease in absolute alpha, beta and theta power (16 mg)	trend to improvement (16 mg)
	EVP-6124	dose dependent improvement ACC 0-back (all doses); improvement ACC 1-back (2 mg)		increase in absolute alpha power (16 mg)	
	GLN-1062		improvement IR (all doses compared to GAL 22 mg compared to placebo); improvement DR (5,5 and 11 mg compared to placebo)		improvement (11, 33 and 44 mg)
	CEP-26401	deterioration ACC 2-back (125 mcg)[1]			dose dependent improvement for all doses
ANTI-CHOLINERGIC COMPOUNDS	scopolamine	deterioration RT and ACC of all paradigms (0,5 mg)	deterioration on all parameters (0,5 mg)	decrease in absolute alpha and beta power (0,5 mg)	severe deterioration (0,5 mg)
	mecamylamine		deterioration on IR and DR (20 mg)		deterioration, but less severe than for scopolamine (20 mg)

VVLT: visual verbal learning test; EEG: electroencephalography; RT: reaction time; ACC: accuracy; IR: immediate recall; GAL: galantamine; DR: delayed recall.

1. Based on previous studies, the lower doses were expected to be more effective

This is illustrated by a study by Miranda et al., where acetylcholine was measured in the rat brain and only in the encoding phase, a peak was registered.[2] In an fMRI study described by Kukolja et al., the cholinesterase inhibitor physostigmine was administrated during a memory task and was demonstrated to enhance activity related to neuronal encoding in the hippocampus, while it could not be shown to influence activity associated with memory consolidation or retrieval.[3] Gais et al. taught healthy subjects a paired associated learning task before the night and administered physostigmine during deep sleep.[4] They found that memory

retrieval was significantly worse after administration of physostigmine compared to placebo, and concluded that low cholinergic levels during deep sleep is essential for memory consolidation. Our results demonstrate that a relevant set of biomarkers used in a systematic approach, can be successfully applied during the development of new cholinergic interventions.

The EEG results of the study in patients with AD are especially interesting. At first glance, the increase in relative frontal theta frequency in responders to long-term galantamine treatment on the placebo occasion and the decrease on galantamine occasion seems to be counterintuitive, as theta activity is usually associated with worse cognitive functioning. However, the study of Wascher et al. reported an increase in frontal alpha and theta power over time in young healthy subjects, during a day filled with cognitive tasks.[5] They reasoned that this is a reflection of mental fatigue. Possibly, the cognitive tasks during the pharmacological challenge days result in less electroencephalographic signs of cognitive fatigue after administration of a single dose of galantamine in patients with AD, who are responders to long-term treatment with galantamine. One could even argue that the fact that there is no change in EEG activity during the pharmacological challenge study days in non-responders to long-term galantamine treatment is actually abnormal. This approach requires more attention, because the response rate of all cholinesterase inhibitors for AD is little higher than 30% and if a more personalized approach could be used considerable cost (and unnecessary side effects) could be saved.

The central question of this thesis is if the integration of pharmacokinetics (PK), CNS pharmacodynamics (PD) and clinical assessments in early phase drug development is feasible for drugs for Alzheimer's disease. At this moment, the only registered procholinergic drugs for Alzheimer's disease or Lewy Body dementia are cholinesterase inhibitors (galantamine, rivastigmine and donepezil). During the development trajectories of these drugs, the classical process of drug development was followed:

- Disease with a known or assumed pathophysiology, in this case AD.
- Possibility for pharmacological interference in the (assumed) cascade of pathophysiology, either in a curative or in a symptomatic manner. In this case the depletion of acetylcholine was targeted by acetylcholinesterase inhibitors and largely tested in animal models.
- This preclinical research leads to selection of a compound that is considered to be safe and effective.
- The compound is tested in healthy volunteers to evaluate safety, tolerability and PK.

In classic drug development, the possibility of physiologically or pharmacologically challenged subjects is skipped. Studies in large groups of patients are initiated, with largely questionnaire based outcomes. Such simple outcome measurements are inevitable due to the multinational, multicentre approach that is necessary in these classical Phase IIa and Phase III studies.

In the studies in this thesis, a different approach was chosen. In all studies, safety, tolerability, pharmacokinetic and pharmacodynamic measurements were included, regardless of the phase of drug development. This gave at least an impression of pharmacodynamics effects in an early stage of drug development. Our approach with quantitative and objective measurement of a central impaired function, memory and cognitive functioning in normal, physiologically impaired and pharmacologically challenged individuals before proceeding to large, risky and expensive trials may assist in a more economically and faster development.[6] This approach is clearly easier for interventions that have an immediate effect like cholinesterase inhibitors, but we also demonstrated that these short time effects predict long term clinical improvement to a certain extent. Additionally, a thorough systematic approach to drug development may demonstrate potential other beneficial effects that otherwise would not be detected as we demonstrated with the effects on mood of the H_3-antagonist in chapter 7 of this thesis.

Disease modifying treatments for Alzheimer's disease – if ever found – may require a different approach, but well validated quantitative measurements of clinical importance are still in short supply. Also, a wide variety of tests is used in clinical trials, which complicates mutual comparison.[7] Intermediate biomarkers of pathology like amyloid imaging or other functional imaging studies have value but do not always correlate with (lack of) clinical improvement. Clinical outcome measures are partly based on questionnaire evaluations by caregivers with a high interrater variability and often designed to diagnose dementia instead of measuring a relevant (reduction of) progression of dementia over time.[8] New biomarkers may be found in more specific, validated questionnaires, for example the Amsterdam iADL scale, or continuous wearable measurements.[9–12] The use of more specific and sensitive biomarkers in a carefully selected population will lead to a more efficient drug development process and probably faster availability of either disease modifying or more effective symptomatic treatment. This could eventually lead to an enormous reduction of drug development costs, and, even more important, health care costs.[6]

CONCLUSION

Clearly the work presented in this thesis does not produce the final answer to all problems associated with the development of treatments for cognitive decline, which is until now largely unresolved, despite an enormous burden on healthcare. Cognitive decline is a complex process with many potential pathophysiological mechanism that allow many approaches, and we have only studied the cholinergic system. However for all interventions it would be ideal if there were good biomarkers of the severity of the disease that were shown to respond to interventions. Finding useful and disease modifying treatments for cognitive decline does not appear to be in close reach, but assuming that this will eventually occur, it is obvious that more efficient development paradigms are necessary to keep the pharmacological development trajectories economically feasible. Rapid evaluation of the most promising treatments in the right dose requires preclinical and early development, already directed towards the final clinical value based endpoint. Rapid elimination of interventions that do not work will of course help to focus limited resources on the more hopeful ones.

Thus, the road between 'working' (on a particular mechanism) and 'helping' (the patient) needs to be paved by improved selection and composition of subject populations to be maximally informative. Necessarily this may involve challenges to induce cognitive dysfunction. The cement between the paving stones is a set of biomarkers that are clinically practical, physiologically relevant and –not to forget– well-validated in a systematic manner. This road is by no means finished but this thesis has hopefully produced some building material.

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Nederlandse samenvatting

SAMENVATTING

Dit proefschrift beschrijft verschillende studies, die het effect op cognitie onderzoeken van medicamenten die het cholinerge neuronale systeem via directe of indirecte mechanismen beïnvloeden. De precieze studieopzet en studiepopulatie is steeds anders gekozen, afhankelijk van het doel van de studie. De studies vonden allen plaats bij mensen, maar in verschillende fasen van het medicatieontwikkelingstraject. In alle studies is gekozen voor uitgebreide farmacodynamische profilering van de middelen. De studies werden uitgevoerd in proefpersonen met een normale of een in enige mate aangedane cognitie of in gezonde proefpersonen met een farmacologisch geïnduceerde cognitieve vermindering ('farmacologische challenge studies').

Hoofdstuk 1 beschrijft het ontwikkelingsproces van de cholinesteraseremmers galantamine, rivastigmine en donepezil, die worden voorgeschreven bij patiënten met de ziekte van Alzheimer. Bij de ontwikkeling van deze middelen is gekozen voor een klassieke aanpak met studies naar veiligheid en verdraagbaarheid in gezonde proefpersonenen, zonder uitkomstmaten gerelateerd aan farmacodynamiek of effectiviteit, gevolgd door grote studies in patiënten met de ziekte van Alzheimer, waarbij het effect van de medicatie in het algemeen pas na weken tot maanden werd gemeten. In de volgende hoofdstukken worden methoden beschreven, waarmee al in een vroegere fase van geneesmiddelonderzoek een indruk kan worden verkregen van de effectiviteit van een nieuw middel.

Hoofdstuk 2 beschrijft een onderzoek naar het effect van verschillende doses van de partiele α7 nicotinerge acteylcholine receptor (nAChR) agonist EVP-6124, alleen en in combinatie met twee doses van de cholinesteraseremmer donepezil, in gezonde oudere proefpersonenen, die een scopolamine challenge ondergingen voorafgaand aan de toediening van EVP-6124 en donepezil. Omdat in dierexperimentele studies EVP-6124 en donepezil het scopolamine-effect volledig teniet deden, waren de verwachtingen hooggespannen. Desondanks kon geen effectiviteit worden aangetoond van EVP-6124 alleen, noch in combinatie met donepezil. Hiervoor zijn verschillende mogelijke verklaringen. Het zou verklaard kunnen worden door het te sterke effect van scopolamine op de gezonde oudere proefpersonen. Een andere mogelijke verklaring is dat scopolamine een muscarinerge antagonist is, die mogelijk niet geantagoneerd wordt door een nicotinerge agonist. Dit illustreert een van de problemen van farmacologische challenge studies. Het optimaliseren en valideren van een farmacologische challenge voor een representatie van het precieze farmacologische effect is een grote uitdaging.

De beperkingen van scopolamine als anticholinerg challenge model werden besproken in **hoofdstuk 3**. Dit hoofdstuk beschrijft een uitgebreid onderzoek van een ander cholinerg challenge model met de nAChR specifieke antagonist mecamylamine. Hoewel dit model al eerder is gebruikt, zijn de precieze farmacodynamische en farmacokinetische eigenschappen grotendeels onbekend en is nooit een gedetailleerde vergelijking met scopolamine gemaakt. Uit deze studie bleek dat scopolamine, zoals verwacht, een groot effect heeft op alle gemeten cognitieve domeinen en daarnaast ook een sederend effect heeft. Mecamylamine heeft weliswaar een kleiner, maar wel een meetbaar effect op aandacht en geheugen en werkt niet sederend. De farmacokinetische eigenschappen van mecamylamine worden beschreven in **hoofdstuk 4**. Vanuit een farmacologisch perspectief lijkt het logsicher om een nicotinerg anticholinerg challenge model te gebruiken voor studies met nAChR agonisten. Om het mecamylamine model te valideren, is vervolgonderzoek nodig. Deze studie benadrukt de noodzaak van een systematisch onderzoek van dergelijke modellen.

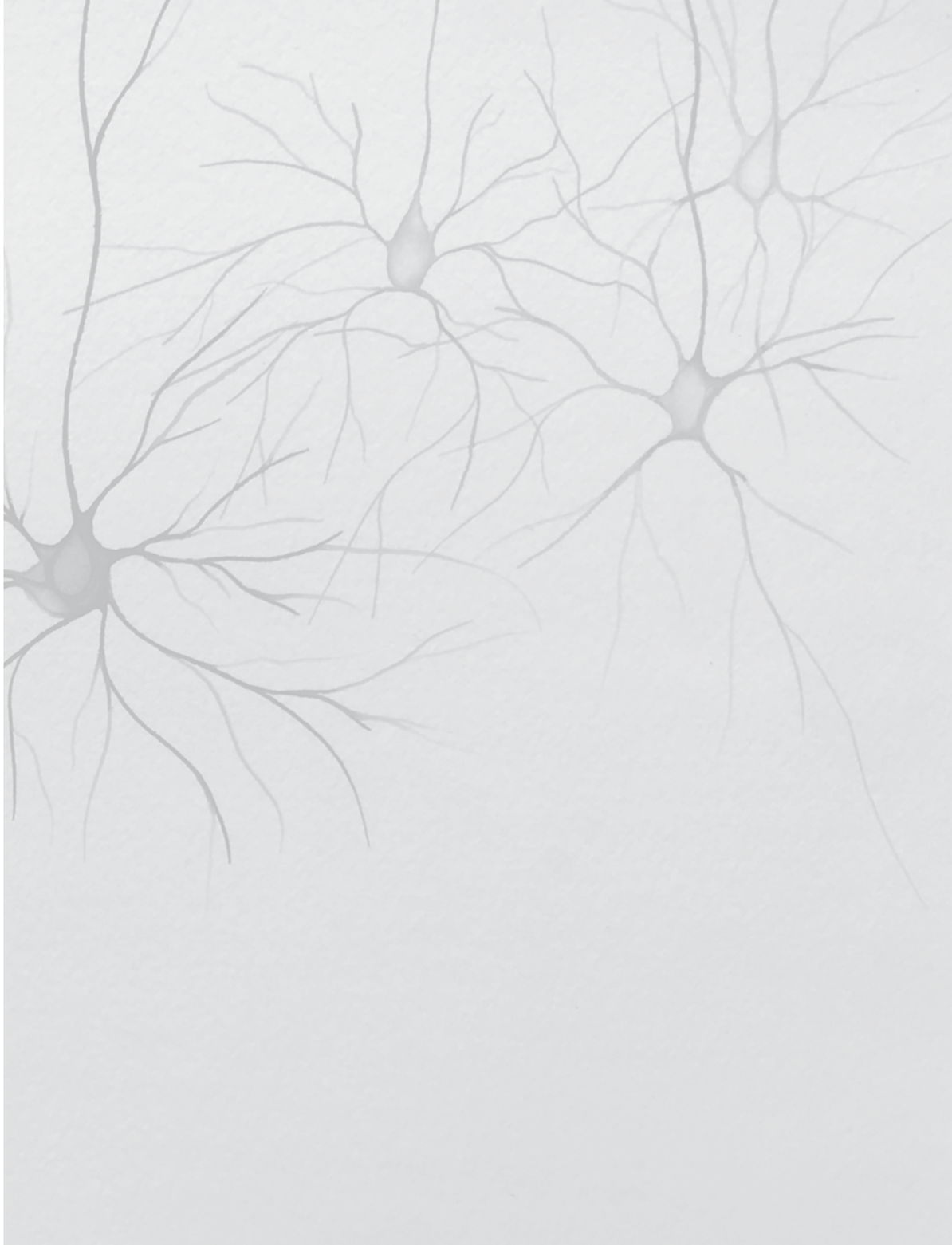
In **hoofdstuk 5** wordt de adaptief opgezette studie naar Gln-1062, een prodrug van de cholinesteraseremmer galantamine, beschreven. Hoewel al bekend was dat de actieve vorm van galantamine veilig en in enige mate effectief is, werd deze prodrug ontworpen met als doel een betere penetratie door de bloed-hersenbarriere en daarmee een betere remming van acetylcholinesterase in het centraal zenuwstelsel. Om deze reden kon een equimolaire dosis dan ook niet a priori als veilig worden beschouwd. De studie begon daarom met een standaard startdosering van 10% van de dosering zonder bijwerkingen (level of no adverse effects, NOAEL) in proefdieronderzoek. Omdat nog geen farmacodynamische effecten werden verwacht bij de laagste twee doseringen, werden deze toegediend aan gezonde jonge mannen. Er werden farmacokinetische, farmacodynamische en veiligheidsmetingen gedaan. Zelfs deze lage doseringen gaven onverwacht enige meetbare verbeteringen van aandacht en geheugen. Deze effecten waren gering, waarschijnlijk door een plafondeffect bij deze jonge gezonde mannen. Daarom werd een nieuwe benadering geïntroduceerd, waarbij de studie werd vervolgd met drie cohorten van gezonde oudere mannen met de gedachte dat zij enige leeftijdsgerelateerde cholinerge deficiëntie zouden hebben, wat de kans op meetbare farmacodynamische effecten groter maakt, zonder dat er een anticholinerge challenge wordt toegediend. In de inclusiecriteria was gedefinieerd dat de cognitieve functies in tact moesten zijn, om te voorkomen dat er demente mensen aan de studie zouden deelnemen. De oplopende doseringen van Gln-1062 werden vergeleken met donepezil en galantamine. Galantamine induceerde geen aantoonbare effecten, terwijl donepezil de prestaties op de

adaptive tracking test verbeterde, vergelijkbaar met het effect van de Gln-1062 doseringen van 33 mg en 44 mg.

In hoofdstuk 5 toonden we aan dat prochlinerge medicatie ook na één dosis al tot meetbare effecten kan leiden in gezonde jonge en oudere proefpersonen. In **hoofdstuk 6** stapten we over naar patiënten met de ziekte van Alzheimer. In dit hoofdstuk vroegen we ons af of het acute effect van de eerste dosis van de cholinesteraseremmer galantamine voorspellend was voor de respons op langdurige behandeling met hetzelfde middel gedurende 6 maanden. In deze studie was er geen significant effect op de adaptive tracker (primaire uitkomstmaat), maar een effect op het EEG na de eerste dosis voorspelde de respons op behandeling gedurende 6 maanden. Een benadering waarbij het effect van de eerste dosis wordt gebruikt als indicator voor succesvolle langdurige behandeling zou kunnen leiden tot een meer gepersonaliseerde behandeling van patiënten met de ziekte van Alzheimer. In het geval van cholineseraseremmers zou dit tot een besparing van ongeveer 70% van de voorschriften kunnen leiden.

Naast directe beïnvloeding van het cholinerge systeem zijn er ook middelen die het cholinerge systeem indirect beïnvloeden. In **hoofdstuk 7** pasten we onze systematiek toe op het onderzoek naar een histamine 3 receptor (H_3R) inverse agonist (CEP-26401). Aangezien histamine een indirect effect heeft op verschillende neurotransmittersystemen, inclusief het cholinerge systeem, werd deze receptor gezien als een mogelijk aangrijpingspunt voor cognitie verbeterende medicatie. Op basis van eerder onderzoek werd gekozen voor lage doseringen van CEP-26401 en werden de effecten vergeleken met placebo, donepezil en modafinil. Deze vergelijkingen werden gemaakt gebruik makend van zeer uitgebreide testbatterijen met sensitieve testen (CANTAB en NeuroCart), en het onderzoek werd uitgevoerd in gezonde jonge proefpersonen. In deze studie werd het primaire (cognitieve) eindpunt niet behaald, maar er was wel een positief effect op subjectieve gevoelens van onder andere alertheid, energie, tevredenheid, met het sterkste effect bij de dosering van 25 µg. Er was geen verbetering op de cognitieve testen en na toediening van de hoogste dosering CEP-26401 zelfs enige verslechtering op de spatial working memory test (SWM 10 boxes; primaire uitkomstmaat van de studie) en de paired associate learning test. Aangezien ook donepezil geen effect op cognitieve testen liet zien, zou de uitkomst van de studie, ondanks het gebruik van zeer sensitieve testbatterijen, beïnvloed kunnen zijn door een plafondeffect van de testen in gezonde jonge proefpersonen. Dit blijft een lastig probleem bij het onderzoeken van procognitieve medicatie in gezonde proefpersonen met optimale cognitieve functies, waarbij het aantonen van verbetering moeilijk is. De positieve effecten van CEP-26401 op subjectieve gevoelens zou ook kunnen duiden op een werkingsmechanisme dat meer geschikt is voor de behandeling van stemmingsstoornissen dan voor de behandeling van cognitieve stoornissen. In de door ons voorgestelde benadering, maar buiten het bestek van dit proefschrift, zou deze compound ook getest moeten worden in fysiologisch cognitief beperkte proefpersonen of na het toedienen van een farmacologische challenge voordat er conclusies kunnen worden getrokken over de werkzaamheid van dit middel.

Hoewel in sommige van de beschreven studies het primaire eindpunt niet werd behaald, lijkt het profiel van cholinerge interventie op de verschillende uitkomstmaten vrij consistent. Dit wordt beschreven in **hoofdstuk 8**. Hierin wordt ook het belang van farmacodynamische testen en een efficiënte methodiek in de vroege fase van geneesmiddelontwikkeling toegelicht. Hierbij zijn goede biomarkers, die klinisch en fysiologisch relevant en goed gevalideerd zijn, essentieel.



CURRICULUM VITAE

Anne Catrien Baakman was born on January 11th 1983 in Vollenhove. She completed secondary school (gymnasium) at the Revius Lyceum in Doorn in 2001 and commenced medical school at University Utrecht in the same year. As a third-year student, she represented the students' interests as member of the management team of the Faculty of Medicine. She was also actively involved in workgroup Gamma, which organised lectures on medical topics that were not covered as part of the core curriculum. For her internships in gynaecology and ophthalmology, she travelled to South Africa and lived in Pretoria for 3 months. During her studies, Anne Catrien held part-time jobs as a teaching assistant in statistics and epidemiology and at the clinical pharmacology unit of Kendle, a contract research organisation for early phase clinical pharmacology trials in healthy volunteers. After graduating as medical doctor in June 2009, Anne Catrien worked as a physician at the neurology departments of the University Medical Centre in Utrecht and the Elisabeth-TweeSteden Hospital in Tilburg. In December 2010, she was appointed as a project leader and research physician at the neurology group of the Centre for Human Drug Research in Leiden. She contributed to several clinical trials, which were supervised by professor Groeneveld and professor van Gerven, focussing on procognitive compounds and anticholinergic pharmacological challenges. For the galantamine trial, there was a close collaboration with the Alzheimer Center of the Amsterdam UMC. She presented her work at several neurological and pharmacological conferences, including the Alzheimer's Association International Conference and the conference of the British Pharmacological Society. Anne Catrien began her resident training in neurology, supervised by professor Berendse, at the VU Medical Centre in Amsterdam in July 2016. She lives in Laren with her husband Jarmil and their three children: Stijn, Julia and Olivier.

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