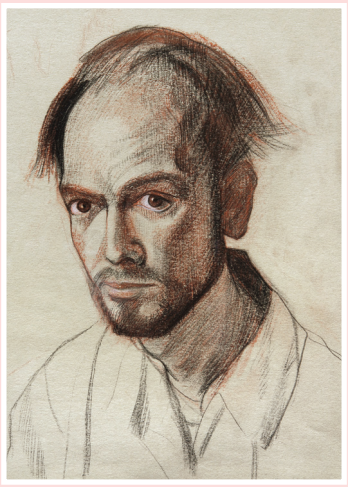
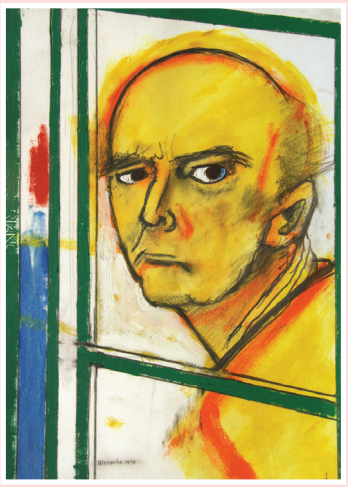
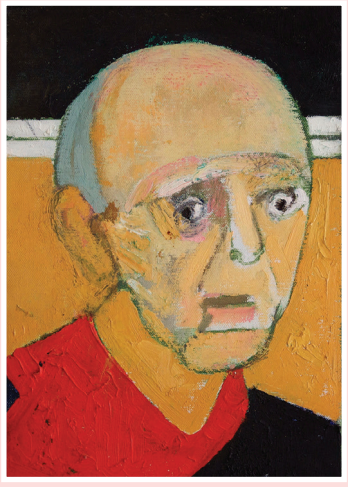
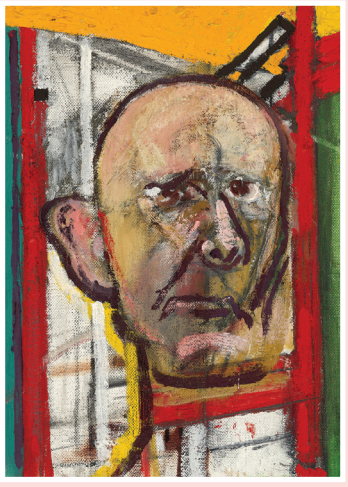
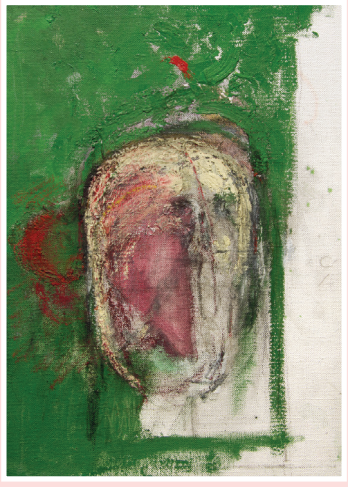
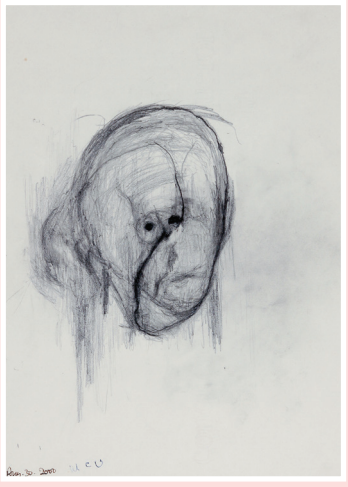



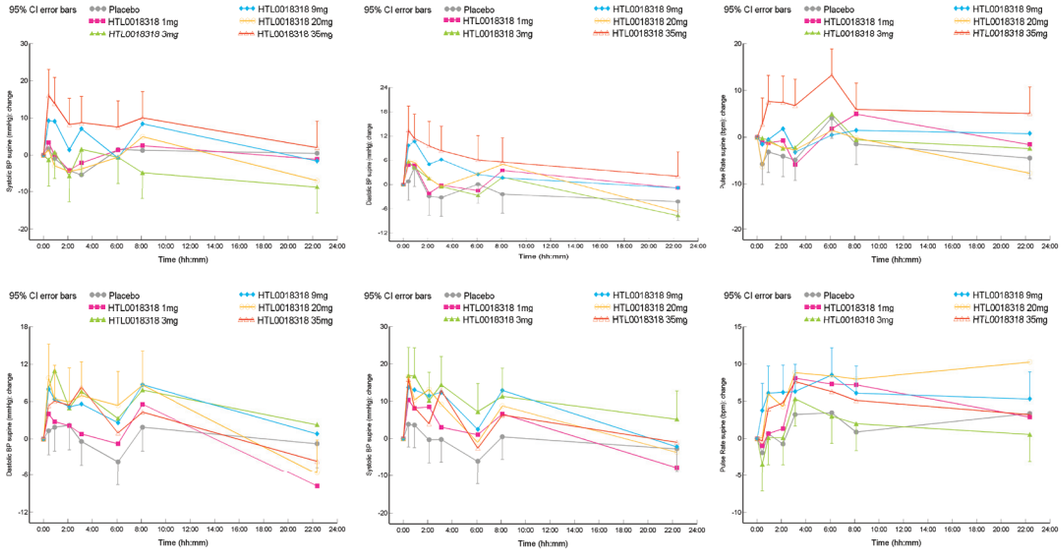


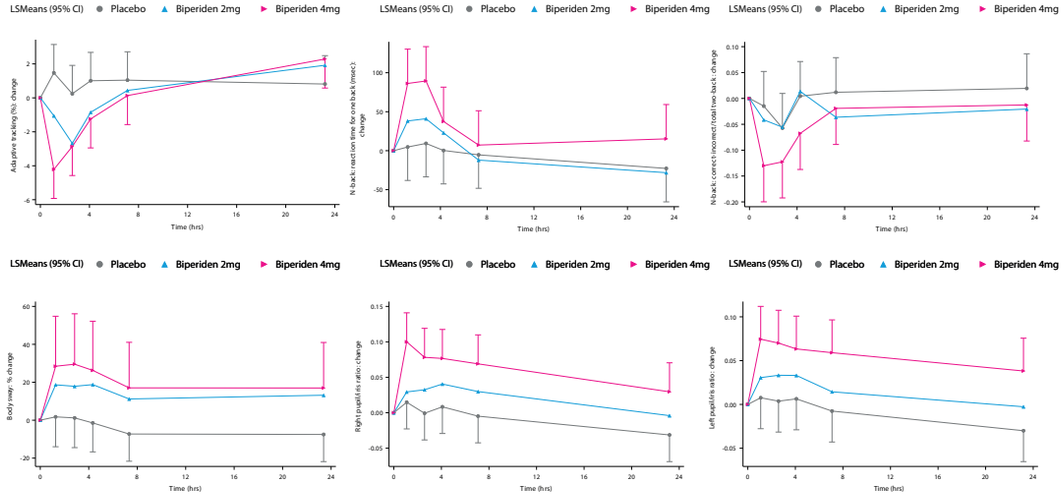
# INNOVATIVE CHOLINERGIC COMPOUNDS FOR THE TREATMENT OF COGNITIVE DYSFUNCTION

Charlotte Bakker

PAGE 61 CHAPTER III – FIGURE 1 Vital signs in adult subjects (A,B,C) and elderly subjects (D,E,F) presented as change from baseline (mean, 95% CI error bars).




PAGE 153 CHAPTER VII - FIGURE 2 Pharmacodynamic effects on adaptive tracking, n-back test, body sway and pupil size presented as change from baseline (mean, 95% CI error bars).



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# INNOVATIVE CHOLINERGIC COMPOUNDS FOR THE TREATMENT OF COGNITIVE DYSFUNCTION

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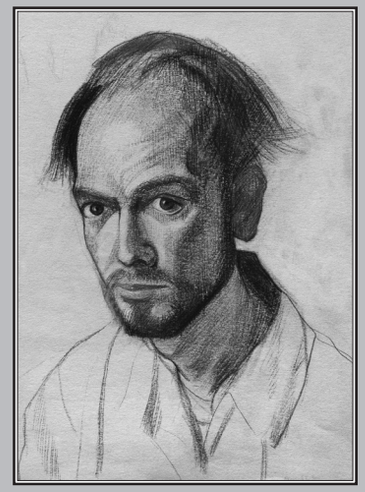
CHAPTER I



# INTRODUCTION

## CHOLINERGIC SYSTEM

The human nervous system contains approximately 86 billion neurons[1]. Transmission of the signals between neurons occurs either by direct flow of the impulse from neuron into the other neuron when the neurons are very close to each other or by neurotransmitters. There are well over 100 different neurotransmitters, including glutamate, serotonin, acetylcholine, gamma amino butyric acid (GABA), dopamine and norepinephrine[2]. Each neurotransmitter targets specific receptors resulting in specific effects.

The neurons that are activated by or contain and release the neurotransmitter acetylcholine form the cholinergic system. Acetylcholine is an endogenous chemical and derives its name from its chemical structure: an ester of acetic acid and choline. It is synthetized by choline acetyltransferase at the end of the cell in the synapse bud and is released from synaptic vesicles. Acetylcholine is removed from the synaptic cleft in less than a millisecond through diffusion and degradation by the enzyme acetylcholinesterase[3,4]. The cholinergic system is active in both the central nervous system and in the peripheral nervous system. There is often an interaction between the different neurotransmitters so that each neurotransmitter system cannot be seen as completely independent. For example, acetylcholine can lead to an increase in concentration of the excitatory neurotransmitter glutamate but also to an increase of the inhibitory neurotransmitter GABA[5]. The neurotransmitter dopamine, in turn, influences the concentration of acetylcholine[6]. In addition, a neuron can contain different neurotransmitter receptors, making the neuron sensitive to different types of neurotransmitters.

**MUSCARINIC RECEPTORS** There are two classes of acetylcholine receptors: the muscarinic receptors and the nicotinic receptors.

The muscarinic receptors are named after the molecule muscarine that is present in the poisonous mushroom *Amanita muscaria*, which is an agonist of all muscarinic (but not nicotinic) receptors. There are five subtypes of muscarinic receptors, designated $M_1$-$M_5$. The level of expression of each subtype differs by area. All these receptors have seven transmembrane regions and are coupled to intracellular G proteins[7]. Activation of the G proteins coupled to the $M_1$, $M_3$ and $M_5$ receptors result in dissociation of the $G_q\alpha$-subunit, which in turn results in activation of phospholipase C. Phospholipase C cleaves phosphoinositol into the second messengers $IP_3$ and diacylglycerol. $IP_3$ binds to the calcium channel located in the endoplasmatic reticulum, which results in release of calcium and consequently various calcium regulated intracellular signals. The $M_2$ and $M_4$ receptors interact mainly with $G_i$ and $G_o\alpha$-subunits.



Activation of the $G_i\alpha$subunit inhibits adenylate cyclase resulting in a decrease of second messenger cAMP and consequently inhibition of the calcium channels[7].

The $M_1$ receptor is the most abundant receptor of all muscarinic receptors in the brain and is mainly expressed post-synaptically. It is predominant in the hippocampus (47-60%) and in the cortex (34-55%)[8-10]. These areas are involved in memory, learning and executive functioning[11]. In the peripheral nervous system, this receptor is widely present on end-organs of the autonomic nervous system, in the endothelium, arteries and pulmonary veins mediating vasodilation and vasoconstriction (review[12]). In the salivary glands the $M_1$ receptor is involved in the control of high-viscosity lubrication[13].

The $M_2$ receptor is also highly expressed in the central nervous system[14]. It is mainly present in the brain stem (parabrachial nuclei and facial, and trigeminal motor nuclei), occipital region of the cortex, dorsal region of the caudate, putamen and the superficial layers of superior and inferior colliculi[8,14]. This mainly presynaptically expressed receptor subtype might play a role in cognitive function. This is suggested by studies in $M_2$ and $M_2$/$M_4$ knockout mice that showed deficits in behavioural flexibility, working memory and hippocampal plasticity[15,16]. In the peripheral nervous system, the $M_2$ receptor is highly expressed in smooth muscle which can be found in the heart, vessels, intestines, and bladder. In the heart, the $M_2$ receptor mediates the parasympathetic control of the heart rate and force of contraction; stimulation of the $M_2$ receptor results in a decrease in heart rate and force of contraction[17,12]. Stimulation of the $M_2$ receptor in the intestines leads to contraction. Although the $M_2$ receptor is also present in the bladder, its role there is unclear.

$M_3$ receptors have been detected in the hippocampus and striatum although their level of expression is low. Their role in cognitive function is limited[18,19]. In the bladder the $M_3$ receptor mediates bladder contraction[20] and in the salivary glands it controls both high- and low viscosity[13]). In the sphincter pupillae, stimulation of the $M_3$ receptor causes pupillary dilation.

The $M_4$ receptor is primarily found in the brain and is highly expressed in the striatum where it modulates dopaminergic neurotransmission[21]. To a lower extent this receptor has been demonstrated in many brain regions, including the cerebral cortex, the hippocampus[14] and the brainstem (pons, facial, and trigeminal motor nuclei)[8]. In the peripheral nervous system, the $M_4$ receptors are mainly present in the lungs.

Like the $M_1$ and $M_4$ receptors, the $M_5$ receptor has been primarily found in the central nervous system. It is expressed in the outermost layer of the cortex, hippocampus, striatum, superior and inferior colliculi, basal forebrain and substantia nigra[14]. It has been shown that this receptor is involved in memory[22]. $M_5$ receptors on the dopami-

nergic neurons of the ventral tegmental area mediate a key role in the mesolimbic reward pathway[23]. Additionally they mediate vasodilation of cerebral blood vessels[22].

**NICOTINIC RECEPTORS** The name of the nicotinic receptors is derived from the ability of nicotine to selectively bind nicotinic receptor but not muscarinic acetylcholine receptors. Like the muscarinic receptors, the nicotinic receptors are widely distributed throughout the central and the peripheral nervous system. In the brain, they are generally expressed in a lower density than muscarinic receptors[24]. The nicotinic receptor are cationic channels which consists of five subunits that can be classified as α (α2- α7, α9 and α 10) or β (β2- β4), δ, ε, and γ. These subunits can be combined in a heteromeric and homomeric way. Neuronal nicotinic receptors are only assembled from α and/or β subunits. Within the brain, nicotinic receptors are expressed in a variety of brain structures, in particular in the thalamus, cortex and striatum[24], and mainly pre-synaptically and on cell bodies or dendrites[25]. The nicotinic receptors that are most present in the brain are the heteromeric α4β2 subtypes and the homomeric α7 subunit combinations[26]. These are predominant in the hippocampus and cortical neurons and are known to play a role in memory and learning[27,28].

**ACETYLCHOLINESTERASE** Acetylcholinesterase hydrolyzes acetylcholine to acetate and choline with the speed of approximately 25.000 molecules of acetylcholine per second[29,30]. This enzyme is localized on the neurons at both the pre- and postsynaptic sides. The activity of cholinesterase controls the availability of the neurotransmitter acetylcholine in the synaptic cleft and consequently the duration of transmitter action. After the break down of acetylcholine, the remaining choline molecules are absorbed into the synaptic cell and recycled.

## Cholinergic system and diseases of the central nervous systems

Altered levels of acetylcholine or damaged cholinergic receptors have been described in several neurodegenerative diseases, including Alzheimer's disease, schizophrenia, and Lewy bodies disease.

**ALZHEIMER'S DISEASE** Alzheimer's disease is the most common form of dementia, mainly affecting the elderly. It is clinically characterized by progressive impairment of cognitive functions such as memory, executive function and problem solving. In the middle and late course of the disease, neuropsychiatric symptoms such as agitation, aggression and psychosis are common.



The main pathological characteristics of Alzheimer's disease are neuritic plaques consisting of extracellular deposits of amyloid β, and neurofibrillary tangles comprising p-tau proteins[31,32]. Accumulation of these proteins leads to massive loss of synapses, dendrites and eventually the whole neurons. The loss of cholinergic neurons starts in the basal forebrain and its axons projecting to the cerebral cortex, hippocampus and amygdala[33]. As a result, there is less acetylcholine released from the presynaptic neurons and consequently disturbed control of the projection areas. In addition, damage to the nicotinic receptors on the post-synaptic neurons is observed. In advanced Alzheimer's disease, 50% of α4β2 receptors are lost compared with healthy elderly whereas the α7 receptors remain relatively stable[34,35]. Therefore, α7 receptors are considered to be a useful therapeutic target. Remarkably, the post-synaptically expressed $M_1$ receptors) are also relatively well preserved making it also a target for the treatment of cognitive dysfunction[36]. Investigation of two different partial agonists selective for the $M_1$ receptor are described in **Chapters II-V**.

The current treatment of Alzheimer's disease consists of acetylcholinesterase inhibitors galantamine, donepezil and rivastigmine. These symptomatic treatments are prescribed to patients with mild to moderate Alzheimer's disease. Acetylcholinesterase inhibitors inhibit the breakdown of the neurotransmitter acetylcholine in the synaptic cleft resulting in a higher availability of acetylcholine at the post synaptic receptors. Subsequently, the duration of activation of the muscarinic and nicotinic receptors in the neocortex and hippocampus, which are involved in cognitive function, is prolonged. The benefit of this treatment is modest, and many patients experience dose limiting side effects[37-39]. As suggested before, treatments that target the nicotinic α7 receptor or $M_1$ muscarinic receptor might offer an alternative. Compared with acetylcholinesterase inhibitors, these selective treatments might ameliorate the symptoms of cognitive decline and may be associated with more a favorable side effect profile. If a more selective drug causes fewer side effects, higher dose levels can be administered before dose limiting side effects occur. Additionally, muscarinic and nicotinic receptor agonists are not dependent on the acetylcholine level, which is increasingly reduced over the course of the disease due to degeneration of the neurons. Therefore these selective agonists have the potential to be effective in more advanced stages of Alzheimer's disease. As development of selective nicotinic and muscarinic receptor agonists is not as advanced as the status of acetylcholinesterase inhibitors, another possibility for treatment is improving the approved acetylcholinesterase inhibitors. In **Chapter VI** we describe the investigation of Gln-1062, a pro-drug of galantamine.

**SCHIZOPHRENIA** Schizophrenia is a disease clinically characterized by positive symptoms (hallucinations, delusions, disorganized speech), negative symptoms (reduced social drive, apathy) and cognitive symptoms (impaired memory, executive function and attention). The onset of schizophrenia is typically during adolescence.

The cause of schizophrenia is hypothesized to be a consequence of a complex interplay between genetic and environmental risk factors[40]. Multiple neurotransmitter systems including the dopaminergic, glutamatergic, GABAergic and cholinergic systems are altered[41]. Current therapy targets the hyperactive dopaminergic system resulting in antipsychotic effects. These antipsychotics have no beneficial effect on cognitive deficits[42], and therefore these symptoms continue to hamper social functioning[43]. As the cholinergic system, which is involved in cognitive functioning, is also altered in schizophrenia, this may be a potential target to pharmacologically improve cognition. Multiple α7 receptor binding drugs have shown promising effects in preclinical studies and therefore support the α7 nicotinic receptor as a potential therapeutic target[44-46]. Early phase studies in humans showed limited effects so far[47,48]. Postmortem studies using a radioligand demonstrated a reduced binding to $M_1$ and $M_4$ receptors in the prefrontal cortex, anterior cingulate, striatum, superior temporal gyrus, and hippocampus[49-52]. In a study with a larger sample size it appeared the expression of $M_1$ and $M_4$ receptors was reduced in 25% of the schizophrenic population[53]. The level of $M_1$ receptor binding is inversely related to cognitive functioning and correlated with the severity of negative symptoms[54]. A pilot study with xanomeline, an agonist selective for $M_1$ and $M_4$ receptors, showed improvement of both cognitive performance and of the positive and negative symptoms[55]. However, due to its side effects, xanomeline was not further developed as treatment for schizophrenia[56,57]. These findings support that targeting the $M_1$ and/or $M_4$ receptor could be beneficial in patients with schizophrenia.

Treatment with cholinesterase inhibitors donepezil and rivastigmine showed no significant improvement in cognition[58,59]. Galantamine treatment resulted in temporary improvement of social memory[60]. The better effect of galantamine compared to donepezil and rivastigmine might be explained by the allosteric binding the α7 nicotinic receptor[61].

**LEWY BODY DISEASE** Lewy body disease is the collective term for diseases that are pathologically characterized by an abundant amount of Lewy bodies, an aggregation of alpha-synuclein. Diseases that present with this characteristic are Parkinson's disease (PD), Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB).



The clinical feature of Parkinson's disease includes motor symptomatology (tremor, bradykinesia and rigidity), non-motor symptoms (fatigue, low blood pressure) and neuropsychiatric symptoms. The latter comprises hallucinations, mood disorders, and cognitive dysfunction (executive functioning, memory and visuospatial misperception) which can evolve into Parkinson's disease dementia. Eventually 48-80% of the patients with Parkinson's disease develop dementia as their disease progresses[62]. The clinical features of Dementia with Lewy bodies have many similarities with Parkinson's disease dementia. These can be distinguished from each other by the clinical course and severity of clinical symptoms[63]. Roughly speaking, dementia is the presenting feature of dementia with Lewy bodies, whereas Parkinson's dementia usually develops on average within 8-10 years of the Parkinson's disease diagnosis.

The most critical pathological abnormality in Parkinson's disease is the loss of neurons in the substantia nigra pars compacta leading to dopamine depletion and consequently motor symptoms[64]. Cognitive deficits in Parkinson's disease are thought to be caused by multiple mechanisms, including neuronal loss in the frontal and parietal cortices, hippocampus, nucleus basalis of Meynert and the pedunculpontine nucleus[65-68]. The underlying mechanism of neuronal loss is not well understood and is currently considered multifactorial. However, Lewy bodies are observed in all the affected brain areas and lead to cell-to-cell transmission of the pathology with progressive loss of neurons[69]. In Parkinson's disease dementia there is a more widespread Lewy body pathology throughout the brain than in Parkinson's disease[70-72]. The severity of this Lewy body pathology is for example more pronounced in the basal forebrain and the hippocampus in patients with Parkinson's disease dementia than in Parkinson's disease[72]. Both these brain areas are involved in cognitive functioning[11]. There are many similarities between the pathology of Parkinson's disease dementia and dementia with Lewy bodies. However, a specific characteristic of dementia with Lewy bodies is the occurrence of Lewy bodies in the cortex and/or in the brainstem of the patients at early stage of the disease[73]. Limited information is available about the muscarinic receptor changes in Parkinson's disease dementia and dementia with Lewy bodies. In patients with dementia with Lewy bodies, ligand binding to $M_1$ receptors was reduced in the temporal and parietal cortex[74], although an increased $M_1$ receptor binding in the temporal cortex has also been observed[75]. In the striatum of patients with dementia with Lewy bodies and Parkinson's disease dementia, ligand binding to $M_1$ receptors was lower than in healthy controls[76]. The level of $M_4$ receptors was decreased in the temporal cortex of patients with dementia with Lewy bodies compared with control subjects[75], while it was increased in the insula, cingulate and claustrum[76]. In Parkinson's disease dementia, binding of a $M_1$/

M4 receptors ligand was changed in several networks arising from the nuclear basalis of Meynert related to attention- and default mode networks[77].

The current treatment for Parkinson's disease is L-DOPA, which restores the balance between striatal dopamine and acetylcholine, resulting in antidyskinetic effects. Cognitive symptoms of dementia that evolve from Parkinson's disease and dementia with Lewy bodies are symptomatically treated with cholinesterase inhibitors[78]. However, these have demonstrated only mild to moderate benefits[79]. As also discussed for Alzheimer's disease and schizophrenia, the $M_1$ receptor is considered a promising target to improve cognition due to cholinergic neuronal dysfunction. In an animal model of dementia with Lewy bodies and Parkinson's disease dementia, an improvement of the maze task performance (orientation and working memory) was observed after $M_1$ receptor positive allosteric modulator T-495[80]. The $M_4$ receptor is involved in modulation of dopaminergic activity and could therefore be a selective target to restore the striatal dopamine imbalance.

## Drug Development

**(PRE-)CLINICAL TRIALS** Development of a new compound is a challenging and costly programme with high attrition rates. The duration from patent filing to marketing authorization takes on average between 10 and 15 year. It starts with identifying a potential beneficial compound based on its chemical characteristics. This is followed by investigating toxicity, pharmacokinetics and pharmacological effects in cell cultures and animal studies. When there are no safety-related concerns and the data support pharmacological effects the compound proceeds to the clinical phase. From all compounds studied in animals, 35 % will not reach this phase[81]. The clinical phase consists of four phases in conventional drug development. The trials in each phase contribute to increasing knowledge about safety, efficacy and pharmacokinetics.

- Phase 1: Studies in this phase usually include 20-100 healthy subjects or patients. The success rate of approximately 70%[82,83]
- Phase 2: Up to several hundred patients are included in phase 2 studies. Approximately 35% of the compounds studied in this phase will move to the next phase[82,83]
- Phase 3: In general 300-3000 patients are included in phase 3 studies. The success rate varies between 25%[82] and 55%[83]
- Phase 4: These studies are conducted after approval and wide-scale introduction of the compound as treatment. Patients are monitored for additional (low-frequency) side-effects.



Only 8% of the compounds targeting the CNS make it through development and will be approved for marketing authorization[83].

**COUNTERING THE HIGH ATTRITION RATE** The main reasons for the high attrition rate in compounds targeting the CNS is the (apparent) lack of efficacy[83-85]. The inability to demonstrate pharmacodynamics effects/efficacy may be caused by the inability of the compound to reach the target, inadequate dose selection, selection of inadequate biomarkers and lack of intended pharmacological effect. These issues can be addressed in time by evaluating the compound using the question-based development method[86]. This is an approach in which the following generic questions are generally answered:

- Does the biologically active compound/active metabolites reach the site of action?
- Does the compound cause its (un)intended pharmacological/functional effect(s)?
- Does the compound have beneficial effects on the disease or its clinical pathophysiology?
- What is the therapeutic window of the new drug?
- How do the sources of variability in drug response in the target population affect the development of the product?

An inability of the compound to **bind to the targeted receptor** (related to question 2) on a cell may prevent the compound from having its intended pharmacological effects. In preclinical studies, binding can be investigated in cells expressing the specific (human) receptor and animal models that express the same receptor with the same related messenger system.

The issue of **dose selection** is related to the therapeutic window (related to question 4). The planned dose levels in a first-in-human study are based on pre-clinical data. The observed effects from a range of toxicology and pharmacological pre-clinical studies can be related to plasma drug concentrations. This way the therapeutic index can be determined with on one side the dose limitations due to safety issues and on the other side the required dose level to induce therapeutic effects. To determine the starting dose in the first-in-human trial both the marker 'no observed adverse effect level' (NOAEL), which represents a safety marker, and the marker 'minimum anticipated biological effect level' (MABEL), which represents a pharmacological marker, should be taken into account. Using only the NOAEL, as was done for a long time, could lead to overdosing or underdosing.

**Reaching the site of action** (related to question 1) can be challenged by physical barriers, such as the blood-brain barrier which is a highly selective semipermeable barrier between the blood vessels and the brain that prevents solutes in the circulating blood from crossing into the central nervous system. Whether the drug reaches the site of action can be investigated in multiple ways. Including pharmacodynamic biomarkers in a clinical study is one of them. A clear dose- (or better: concentration-) related pharmacodynamic effect would suggest that the drug is able to reach its target site of action. If pharmacodynamic effects exerted in the CNS are not clear, alternative or additional methods can be used, such as measuring drug concentrations in the cerebral spinal fluid (CSF) as a proxy for the concentration in the brain or visualizing the distribution of labeled drugs competing with a tracer in a PET-MRI scan.

Biomarkers play a crucial role in answering the generic questions of the question-based development methods and therefore **relevant biomarkers are required**. The biomarkers should be related to the mechanism of action of the drugs and ideally also to the pathophysiology of the relevant disease. The ideal biomarker shows a clear dose-related response in the therapeutic dose range across all studies. Seen in this light, for a $M_1$ receptor agonist used for the symptomatic treatment of patients with Alzheimer's disease, the biomarker 'n-back task' seems a suitable biomarker because this task investigates working memory (a cognitive function impaired by Alzheimer's disease). In addition, working memory is mediated by the prefrontal cortex (a brain area damaged in patients with Alzheimer's disease), and also the $M_1$ receptor is highly expressed in this brain area and relatively well preserved (mechanism of action and pathogenesis of the disease). The n-back test has demonstrated clear dose related effects related to $M_1$ targeting drugs[87] and working memory related task showed dose related effects of muscarinic receptor targeting drugs in general (dose-relationship)[88].

Our search for useful biomarkers that can detect drug-induced effects on the cholinergic system is described in **Chapter VIII**.

Development of pro-cognitive drugs is challenging. Early phase clinical development is often performed in healthy subjects (the so called phase 1 and phase 2a clinical studies) and cognitive tests often have ceiling effects in healthy subjects[89,90]. These ceiling effects hinder detection of cognitive improvements. Investigating the pharmacological mechanism and pharmacodynamics of a compound in healthy subjects can be done by means of a pharmacological challenge model. In a challenge model, a subject is administered a drug acting on a specific pharmacological target to induce temporary effects (deficits if related to antagonism of the relevant receptor). Following drug administration, pharmacokinetics and pharmacodynamics are measured repeatedly to quantify these effects. If the newly developed compound



can restore the challenged function, this indicates that the pharmacological mechanism is successfully targeted. Preferably, the model challenges a selective system relevant for the drug to be investigated. In case of drug targeting the cholinergic system, mecamylamine and scopolamine challenge models are commonly used[91-94]. Mecamylamine is a non-selective nicotinic receptor antagonist, useful for investigating nicotinic receptor agonists. In previous studies, attenuation or reversal of mecamylamine induced effects was observed after nicotine and galantamine[91]. Scopolamine is a muscarinic receptor antagonist, binding to all five subtypes of the muscarinic receptors. The effects of scopolamine mimic cholinergic neuronal dysfunction, e.g. due to dementia or those observed in age related cognitive impairments in healthy subjects[95]. Reversal of the induced effects is observed after administration of cholinesterase inhibitors galantamine and donepezil[96,97]. Currently, development of new cholinergic drugs is focused on $M_1$ selective or $M_1$/$M_4$ selective drugs. The non-selectivity of scopolamine could hamper demonstration of effects of these new drug, because the effects induced by antagonizing the $M_2$, $M_3$ and $M_5$ receptors will not be reversed by the new drugs and can potentially blur attenuating effects. The drug biperiden is selective for the $M_1$ receptor and mildly affecting the $M_4$ receptor. A more selective challenge model was developed as described in **Chapter VII**.

This thesis focuses on early phase clinical drug studies related to new cholinergic drugs that are being developed for cognitive dysfunction. Furthermore biomarkers to be used to demonstrate pharmacodynamic effects and the improved characterization of selective pharmacological challenge models will be discussed.

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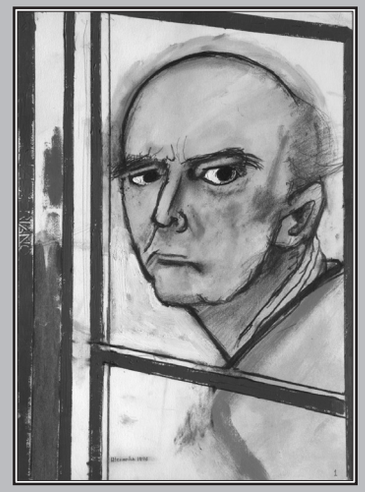
CHAPTER II



# HTL0009936, A SELECTIVE MUSCARINIC M1-ACETYLCHOLINE RECEPTOR AGONIST: A RANDOMIZED CROSS-OVER TRIAL

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## ABSTRACT

**AIMS** HTL0009936 is a selective $M_1$ muscarinic receptor agonist in development for cognitive dysfunction in Alzheimer's disease. Safety, tolerability and pharmacokinetics and exploratory pharmacodynamic effects of HTL0009936 administered by continuous IV infusion at steady state were investigated in elderly subjects with below average cognitive functioning (BACF).

**METHODS** Part A was a four-treatment open label sequential study in healthy elderly investigating 10-83 mg HTL0009936 (IV) and a 24 mg HTL0009936 single oral dose. Part B was a five-treatment randomized, double-blind, placebo and physostigmine controlled cross-over study with IV HTL0009936 in elderly subjects with BACF. Pharmacodynamic assessments were performed using neurocognitive and electrophysiological tests.

**RESULTS** Pharmacokinetics of HTL0009936 showed dose-proportional increases in exposure with a mean half-life of 2.4 h. HTL0009936 was well-tolerated with transient dose-related AEs. Small increases in mean systolic blood pressure of 7.12 mmHg (95% CI [3.99–10.24]) and in diastolic of 5.32 mmHg (95% CI [3.18–7.47]) were noted at the highest dose in part B. Overall, there was suggestive but no definitive positive or negative pharmacodynamic effects. Statistically significant effects were observed on P300 with HTL0009936 and adaptive tracking with physostigmine.

**CONCLUSIONS** HTL0009936 showed well-characterized pharmacokinetics and single doses were safe and generally well-tolerated in healthy elderly subjects. Due to physostigmine tolerability issues and subject burden, the study design was changed and some pharmacodynamic assessments (neurocognitive) were performed at suboptimal drug exposures. Therefore no clear conclusions can be made on pharmacodynamic effects of HTL0009936, although an effect on P300 is suggestive of central target engagement.




## INTRODUCTION

Alzheimer's Disease (AD) and Dementia with Lewy Body (DLB) are the most common cause of dementia[1]. Clinically, AD and DLB are characterized by the progressive decline of cognitive functions. Research has shown that AD is characterized by a significant and progressive loss of cholinergic neurons, especially in the nucleus basalis of Meynert, along with their cortically projecting axons[2], and this cholinergic degeneration is correlated with cognitive decline[3,4]. To date, no curative treatment is available and patients can only benefit from symptomatic treatments, such as the acetylcholinesterase inhibitors (AChEIs) galantamine, donepezil and rivastigmine[5]. However, the efficacy of treatment with AChEIs is moderate[6-8] due to only partial central inhibition of AChEIs[9,10] and it often leads to gastrointestinal side effects (e.g. nausea, vomiting and diarrhoea) associated with increased activation of peripherally located muscarinic receptors, causing dose limitations and a significant burden for patients[6-8].

The cholinergic receptors comprise two broad classes; the ionotropic nicotinic receptors and metabotropic muscarinic receptors. The muscarinic receptors are a group of Class I G-protein-coupled receptors (GPCRs) comprising five distinct subtypes, termed $M_1$, $M_2$, $M_3$, $M_4$ and $M_5$[11]. Drugs that selectively target specific muscarinic receptor type(s) may enhance cognitive and behavioural function in AD and DLB patients while minimizing the negative side-effects associated with non-selective activation of all muscarinic receptor types, in particular $M_2$ and $M_3$ receptors that have been predominantly linked to the gastrointestinal and cardiovascular side effects[12]. The muscarinic $M_1$ receptor ($M_1$ AChR) is predominant in the central nervous system (CNS) and found to be expressed in the prefrontal cortex, striatum and hippocampus. These brain areas are known to be associated with cognitive processes[13,14]. The $M_1$ AChR is relatively well preserved in AD and DLB patients[15,16]. Drugs that selectively target $M_1$ AChR could be potential treatment for cognitive and behavioural dysfunction in AD and DLB[17,12]. Additionally, the effects of selective $M_1$ AChR agonists are independent of the existence of cholinergic tone in the CNS and their benefit may be sustained further into disease progression than the benefit of cholinesterase inhibitors or $M_1$ receptor positive allosteric modulators which rely pre-synaptic cholinergic tone.

HTL0009936 ((S)-Ethyl 4-(4-(1-methylcyclobutylcarbamoyl)piperidin-1-yl) azepane-1-carboxylate)[18] is a potent and selective $M_1$ AChR agonist that is currently under development for the symptomatic treatment of the cognitive symptoms of dementias including AD and DLB. HTL0009936 has no detectable activity at $M_2$ and $M_3$ AChRs, and a seven-fold margin of functional selectivity over $M_4$ AChR in vitro. It

has been investigated in an oral solution formulation, dosed at 1-175 mg in a phase I trial in young adults and elderly subjects (in preparation). Pharmacokinetics (PK) of oral HTL0009936 showed a low oral bioavailability and a significant degree of variability between subjects. In order to reduce this variability and to ensure sustained exposure within the central nervous system (CNS) over the period of cognitive testing, HTL0009936 was given as an intravenous infusion in the current study.

This study was conducted in two parts. The aim of part A was to evaluate the safety, tolerability and PK in elderly subjects in order to identify a well-tolerated dosing regimen to take forward into part B, and to determine the absolute oral bioavailability of HTL0009936. In part B safety, tolerability, PK, and exploratory PD of IV HTL0009936 were investigated in elderly subjects with below average cognitive functioning (BACF). These subjects had no evidence of progressive cognitive deterioration.

## METHODS

This study was approved by the medical ethics review board Stichting Beoordeling Ethiek Biomedisch Onderzoek (BEBO, Assen, The Netherlands) and 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[19].

**TRIAL DESIGN AND SUBJECTS** This study consisted of part A and B. Part A was an initial pilot phase administering 0.1 and 1 mg HTL0009936 given as a 30 min infusion followed by a four-treatment open label sequential study with IV and oral administration of HTL0009936 in elderly subjects (n=10). The objectives of part A were to evaluate the safety, tolerability and the PK profile of HTL0009936, to identify a well-tolerated dosing regimen for part B and to determine the absolute oral bioavailability of HTL0009936. Part B was a five-treatment randomized, double-blind, placebo and positive comparator-controlled crossover study with IV HTL0009936 in elderly subjects with BACF (n=33). The objectives of part B were to evaluate safety, tolerability and PK of HTL0009936 and to evaluate PD in comparison to placebo and a positive comparator.

In both part A and B, subjects were healthy male and female elderly (65+ years) with a maximum blood pressure of 140/90 mm Hg and a heart rate between 45-100 bpm at screening. Use of antihypertensive drugs was not allowed. Consumption of alcohol and caffeine containing products, use of nicotine-containing products and drugs influencing CYP3A4 and CYP2D6 activity were not allowed prior to and during the study. Subjects were defined as intermediate (IM) or extensive (EM) CYP2D6 metabolizer based on their genotype and were excluded if they were poor or ultra-rapid metabolisers in order to minimize variability in the steady state plasma concentrations in part B.

Subjects in part B functioned below average on tests of cognitive functioning based on one of their scores on three tests: the auditory verbal learning test (AVLT) (memory), the word fluency test category (executive function), and the adaptive tracking test (attention). Below average cognitive functioning was defined as a score of ≤-1 SD on at least one of the tests. The reference value for the AVLT and word fluency test were based on available norms[20]. The mean score of the adaptive tracking test was calculated from data from previously performed studies in healthy elderly. Age and education level were taken into account in the calculation of the score. Per cognitive domain, a minimum of 8 subjects showed below average functioning. Subjects were excluded if they had a Clinical Dementia Rating scale (CDR) score of > 0, a mini-mental state examination (MMSE) score of <24 or a Becks Depression Index-II (BDI-II) score of >13. Thus, subjects did not have MCI (mild cognitive impairment) and did not have evidence of progressive cognitive deterioration and it was therefore unknown whether they were cholinergically deficient.

**MATERIALS** In part A, HTL0009936 was administered as an IV solution and as an oral solution. In the first treatment session, two subjects were dosed 0.1 mg HTL0009936 IV according to a sentinel procedure, followed by two subjects dosed 1 mg HTL0009936 IV, followed by six subjects dosed 10 mg HTL0009936 IV. The latter six subjects were administered 49.2 mg HTL0009936 IV during the second treatment session, 83 mg HTL0009936 IV during the third treatment session, and 24 mg HTL0009936 orally during the fourth treatment session to determine the absolute oral bioavailability. The IV administration lasted up to 5 hrs including the loading phase that varied per dose from 30 minutes to 2 hrs. Safety, tolerability and PK data of part A was used to find a well-tolerated dosing regimen for part B.

In part B, subjects received the following IV treatments in random sequence (30 sequences were used): 13.5 mg HTL0009936 in order to target an average concentration of HTL0009936 in plasma during infusion of the maintenance dose ($C_{mean}$) of 25 ng/mL, 40 mg HTL0009936 in order to target a $C_{mean}$ of 75 ng/mL, 79.5 mg HTL0009936 in order to target a $C_{mean}$ of 150 ng/mL, placebo (saline solution (sodium chloride 0.9%)), and physostigmine salicylate at a rate of 1 mg/hr for 50 minutes as positive comparator in combination with an IV bolus administration of 0.2 mg glycopyrrolate bromide (a peripheral muscarinic antagonist) administered immediately prior to physostigmine administration[21]. Physostigmine salicylate has reversed temporary cognitive impairment in cognitively normal subjects that was

induced by administration of the anticholinergic drug scopolamine[22,23]. The dual infusion of HTL0009936 in part B consisted of a 1 hr loading dose in order to reach the $C_{mean}$ followed by a 4 hr maintenance dose designed to maintain the target $C_{mean}$. As the infusion regimens for the study drug and the positive comparator were different, this study comprised a double-dummy condition.

**SAFETY AND TOLERABILITY ASSESSMENTS** For part A and B, all subjects underwent medical screening, including assessment of medical history, physical examination, urine drug screen, vital signs, ECG, and safety laboratory measurements. During treatment periods, safety was assessed by monitoring of adverse events (AEs), vital signs, ECG, 5-hour Holter monitoring, and safety chemistry and haematology blood sampling. Following a protocol amendment, subjects were to be withdrawn when a rise of >40% in systolic or diastolic blood pressure was measured as compared to the mean of three pre-dose vital signs measurements and blood pressure >150/90 mm Hg or when the blood pressure was >180/115 mm Hg regardless of the change from baseline.

**PHARMACOKINETIC ASSESSMENTS** In part A, venous blood samples were collected pre-dose and post-dose at different times during the different treatment sessions because of varying loading times. During all treatment sessions in part B, PK was sampled according to the same schedule pre dose, 9-15 times within the first 8 hrs after starting the administration and at 12 and 24 hrs post dose. Urine was collected continuously for PK determination of HTL0009936 (supplementary table S1).

All HTL0009936 plasma and urine concentrations were analysed using an achiral liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) assay validated according to current guidelines. The detection range was 0.5 to 1000 ng/mL. Physostigmine plasma concentrations were determined using a validated LC-MS/MS assay with a quantification range of 0.10–10 ng/mL.

PK non-compartmental analysis was performed to determine the maximum plasma concentration ($C_{max}$), time to reach $C_{max}$ ($T_{max}$), area under the concentration-time curve from time of dosing to the last quantifiable concentration measurement ($AUC_0$-last), apparent terminal elimination rate constant (lambda-z), AUC from time of dosing to infinity ($AUC_0$-inf), apparent terminal half-life ($t_{½}$), total plasma clearance (CLp), volume of distribution (Vd), absolute bioavailability (F), amount unchanged in urine (Ae), fraction excreted in urine (fe) and renal clearance (CLr). The AUC was calculated using the linear-logarithmic trapezoidal method. Dose-proportionality was evaluated by making pair-wise comparisons of the increase in dose and the corresponding increase in exposure between dose levels. However, in part A, the loading dose was not a constant fraction of the total dose. Therefore dose-exposure proportionality of $C_{max}$ was determined by relating the $C_{max}$ to the loading dose only. The software used for non-compartmental analysis was R version 2.14.1[24].

**PHARMACODYNAMIC ASSESSMENTS** Only in part B of this study, PD assessments using both the NeuroCart[25] and the Cambridge Neuropsychological Test Automated Battery (CANTAB)[26] were performed. The Neurocart and CANTAB are test batteries that include cognitive tests that can be used to examine effects of CNS-active drugs on a wide range of cognitive domains. NeuroCart and CANTAB tests have previously been shown to be sensitive to cholinergic modulation[27-29]. The NeuroCart also includes neurophysiological measurements. Blood pressure and pulse rate were considered both as safety and PD measurements.

The following Neurocart tests were performed: the adaptive tracking test measured attention and visuomotor coordination [25, 30, 31], the Milner maze test was used to evaluate spatial working memory, learning and executive function[32], the n-back task was used to asses (short-term) working memory[33-35], pupil size was measured to monitor any drug effects on the sympathetic nervous system[36,37], synaptic activity was assessed using electrophysiology and included resting electroencephalography (EEG, power in delta, theta, alpha, beta and gamma bands) and the event-related potentials (ERP) P300 and Mismatch negativity (MMN)[38,39]. P300 is related to an early attention process and is used as marker for attention[40] and memory[40,41]. MMN is related to central auditory processing and is used as marker for auditory memory[42]. Visual verbal learning test (VVLT) measured the whole scope of learning behaviour (i.e., acquisition, consolidation, storage and retrieval)[25], and a visual analogue scale was used to evaluate subjective nausea. The Leeds Sleep Evaluation Questionnaire (LSEQ) was used to assess changes in sleep quality[43]. The following CANTAB tests were performed: the paired associates learning test assessed visual memory, new learning and evaluated episodic memory[44], the rapid visual information processing test was used to measure sustained attention[45], and the spatial working memory test required retention and manipulation of visuospatial information[46]. Detailed task descriptions are provided as supplement.

PD tests were performed repeatedly and the timing was based on PK characteristics of HTL0009936 measured in a previous study in humans (maximum drug levels were measured in the CSF 1-2 hrs after plasma $T_{max}$). PD assessments were conducted at baseline (pre-dose) and between 1 hr and 8 hr post treatment. While the electrophysiological assessments ERPS MMN and P300, and EEG and Neurocart assessments were performed during steady-state levels of HTL0009936, due to heavy study burden, the three CANTAB assessments were performed at 5 hr post start of

treatment when infusion was stopped and plasma levels of HTL0009936 were declining below target exposure levels. All post-drug assessments for physostigmine were performed after infusion was stopped at 50 min post dose when plasma levels were declining and low.

**STATISTICS** No formal power calculations were performed to assess sample size in part A. The sample size of ten subjects was considered adequate and a compromise between minimizing exposure and the need to provide sufficient data in order to find a well-tolerated dosing regimen for part B and asses the bioavailability of oral HTL0009936. In part B, a sample size of 30 elderly subjects was defined to have 80% power to detect a difference of 1.53%-point on the adaptive tracking task, assuming a standard deviation of 2.9, using a paired t-test with a two-sided significance level of 0.05. Adaptive tracking was chosen to set the sample size in this exploratory study because it was the task shown previously to be most sensitive to cholinergic stimulation in studies of donepezil[29].

The PD analysis population per treatment session comprised all subjects who had at least one post-baseline assessment of any parameter being analysed. Repeatedly measured PD variables (Neurocart tests, CANTAB tests, blood pressure, and pulse rate) were analysed with a mixed model analysis of covariance with treatment, period, time, and treatment by time as fixed factors and subject, subject by treatment and subject by time as random factors and the average baseline measurement as covariate. The single measured PD variables were analysed with a mixed model analysis of variance with treatment and period as fixed factors and subject as random factor and the baseline measurement, if available, as covariate. The mean outcomes are presented as least square means (LSMs). Only PD data that was measured within 8 hours after starting the HTL0009936 administration and within 2 hours after start of the physostigmine administration was included in the analyses. PD tests performed within 2 hours after start of physostigmine were adaptive tracking test, VAS nausea, n-back test, pupillometry, EEG and ERP (P300 and MMN). The following contrasts were calculated: HTL0009936 versus placebo and physostigmine versus placebo. All calculations were performed using SAS (version 9.4).

## RESULTS

**SUBJECTS** Subject demographics and baseline characteristics are summarised in Table 1. A total of ten subjects participated in part A. No subjects dropped out of part A after drug administration.



In part B 33 subjects were enrolled. Eight subjects withdrew or were withdrawn before the end of part B for personal reasons (n=4) and safety reasons (n=4) and (as per protocol) three of them were replaced. Of the four subjects that were withdrawn due to safety reasons, one subject presented with a raised serum creatinine after completing the 13.5 mg dose before starting the 2nd dosing day; one subject completed three dosing days (placebo, physostigmine and 79.5 mg HTL0009936 respectively) before withdrawal due to a second degree atrioventricular block on the Holter registration; one subject was being withdrawn after completing the placebo and 13.5 mg HTL0009936 dosing day because of ST-segment depression seen on Holter registration; one subject completed the 40 mg, 79.5 mg, physostigmine and placebo dosing days before withdrawal due to ST segment depression on the Holter registration.

All treatment infusions were started by at least 28 subjects and completed by at least 26 subjects (Figure 1).

**SAFETY AND TOLERABILITY** In seven cases study drug administration had to be prematurely stopped due to a clinically significant rise in blood pressure. In part A there was one such case. Of the six cases of clinically significant rises in blood pressure in part B one was related to administration of physostigmine, the remaining five were attributed to administration of HTL0009936 (three of which were experienced in the same subject). No subject was withdrawn from the study as a result of increased blood pressure.

In both part A and part B only mild or moderate self-limiting treatment emergent adverse events (TEAEs) were reported and there were no serious adverse events. The most frequently reported TEAEs in part B following HTL0009936 administration were, headache (14 AEs), hyperhidrosis (6 AEs), and nausea (6 AEs).

One subject was withdrawn from the study because an ST-depression was recorded during the Holter monitoring between 2 and 3 hrs after starting the 13.5 mg HTL0009936 dose. There were no relevant changes in ECG, physical examination findings or laboratory values.

**PHARMACOKINETICS** The PK profile of HTL0009936 was well-characterized after IV infusion and oral dosing in elderly subjects (Figure 2 Table 2, Table 3, Table 4). In part B targeted $C_{mean}$ were reached. Systemic exposure after IV dosing was dose-proportional over a wide dose range and showed an inter-subject variability of ~30 %CV, irrespective of CYP2D6 intermediate or extensive metabolizer predicted phenotype. Plasma clearance was 68–81 L/hr with a volume of distribution of 222–262 L consistent with a short half-life (2.2–2.6 hrs). Renal clearance was a significant route of elimination of unchanged HTL0009936 (CLr 8.0 L/hr, range 3.4 to 14.2 L/

hr) with about 10% of the dose excreted unchanged after IV dosing. Absolute oral bioavailability was established to be about 15% ranging from 8.7 to 27%. Variability after oral administration (~50 %CV) was higher compared to IV infusion and CYP2D6 predicted phenotype was found to be related to systemic exposure and clearance of HTL0009936, with higher clearance and lower exposure in EM subjects compared with IM subjects (supplementary table S4).

Physostigmine plasma concentrations increased immediately after dosing with the mean $T_{max}$ at 50 minutes. It was rapidly eliminated from plasma with a mean t½ of 0.37 hr (CV 31%) with observed concentrations ≤ 1 ng/mL and typically < 0.5 ng/mL by 1.5 hrs after the start of infusion (see supplementary figure S5).

**PHARMACODYNAMICS** Dose-related increases in both systolic and diastolic blood pressure were observed following administration of 40 mg and 79.5 mg HTL0009936 compared to placebo (Figure 3). There were no increases in systolic or diastolic blood pressure at the 13.5 mg dose. The mean systolic blood pressure increased 3.87 mm Hg following 40 mg HTL0009936 (95% CI [0.70–7.05]) and 7.12 mm Hg after 79.5 mg HTL0009936 (95% CI [3.99-10.24]) compared with placebo. Mean diastolic blood pressure increased 3.83 mm Hg following 40 mg HTL0009936 (95% CI [1.64–6.01 ]) and 5.32 mm Hg after 79.5 mg HTL0009936 (95% CI[3.18–7.47]) compared with placebo. Similarly, there was a dose-related increase in heart rate. There were no significant increases in pulse rate at the 13.5 mg and 40 mg doses. Administration of 79.5 mg HTL0009936 resulted in increased pulse rate of 4.75 bpm when compared with placebo (95% CI [3.14–6.36]).

Overall, single doses of HTL0009936 showed no consistent acute effects on measures of cognitive or neurophsyiological function as measured by NeuroCart, CANTAB, EEG and ERPs compared with placebo (supplementary Table S6). However,13.5 mg HTL0009936 resulted in a mean increase in P300 maximum amplitude of 0.56 uV over the Cz lead compared to placebo administration (95% CI [0.139–0.971]), although similar increases were not observed at the Fz and Pz leads (Figure 4). No clinically relevant effects were observed on the VAS nausea scale and the LSEQ compared with placebo.

Physostigmine administration led to an improvement of 1.5%-point (95% CI 0.216–2.734,) on the adaptive tracking test performance within 2 hours post dose (Figure 4). No improvements in adaptive tracking were observed with HTL0009936.



## DISCUSSION

The objective of the study was to assess safety, tolerability and PK in elderly subjects and the effect of HTL0009936 on cognitive performance in elderly subjects with below average cognitive function. In part A, focussing on safety, tolerability and PK in normal healthy elderly, HTL0009936 was administered IV over a dose range of 0.1 mg (over 30 min) up to 83 mg (over 5 hr) and 24 mg orally. In part B, focussing on safety, tolerability, PK and PD in elderly with below average cognitive function, HTL0009936 was administered IV over a dose range of 13.5 to 79.5 mg and compared to placebo and physostigmine infusions in a double dummy manner. The infusion in part B consisted of a 1 hr loading dose in order to reach the target steady-state plasma concentration followed by a 4 hr maintenance dose designed to maintain the target steady-state concentration to ensure sustained exposure within the CNS over the period of cognitive testing.

All doses of HTL0009936 were associated with mild to moderate self-limiting TEAEs. Fewer subjects reported TEAEs after HTL0009936 (50-56.7% of the subjects) than after physostigmine (85.7% of the subjects) (supplementary information S3). The observed small increases in systolic (3.87 mm Hg) and diastolic (5.32 mm Hg) blood pressure and pulse rate (4.75 bpm) were dose-dependent and consistent with expected effects of $M_1$ MAChR stimulation on the peripheral cardiovascular system[47]. Importantly the effects of blood pressure and heart rate were acute, returning to normal soon after HTL0009936 infusion was stopped suggesting there were no persistent effects. Overall, HTL0009936 was considered safe and well-tolerated in elderly subjects at exposures predicted to have central physiological effects.

The PK of HTL0009936 were well-characterised up to single doses of 83 mg. IV infusion in part B resulted in stable and sustained exposure of HTL0009936. The PK variability after IV administration was lower than after oral administration (i.e. 30% vs 50% respectively).

Overall, no definitive positive or negative PD effects were observed on behavioural and electrophysiological biomarkers of cognitive function. Potential reasons for a lack of a clear PD effect are discussed below, which impacts the conclusions that can be made on the PD effects of HTL0009936. However, HTL0009936 showed a selective pro-cognitive effect as shown by an increase in P300 amplitude at the 13.5 mg doses, suggesting an improvement in early attentional processing. However, these data need to be interpreted with caution as the effects were only noted at the Cz lead, and not at the Fz lead (leads with the greatest signal change with P300 generated using a passive odd ball task).

In order to reduce the ceiling effects that cognitive tests have in healthy optimal cognitive functioning subjects, we aimed to investigate HTL0009936 in a study population in which the ceiling effects could be expected to be more limited, based on lower cognitive test scores. The percentage of subjects with impairments were 39% for memory, 36% for executive function and 42% for attention. One limitation of using this approach is that not all subjects were impaired on all tests and the percentage of subjects impaired in any one test or on all tests was low. This may have led to a variable cognitive baseline for the study population. Hence detecting drug effects may have been difficult for some domains of cognition. Alternatively, as subjects had no evidence of cholinergic deficiency, it is possible that they were not an appropriate population for study for this mechanism of action.

In addition to the potential limitation discussed above, the study was powered to detect a significant change in the adaptive tracking and therefore not to detect statistically significant changes in EEG/ERP or other cognitive tests in which either smaller treatment effects or larger variability could have been present. In addition, multiple PD assessments were not performed at the optimal time of target concentration of HTL0009936 (for the CANTAB tests performed at 5 hrs post dose) and physostigmine (for EEG and all cognitive tests performed after 1 hr post dose). This was due to stopping the infusion of HTL0009936 at 5 hrs and physostigmine at 50 min and the rapid drop in exposures of both drugs post cessation of infusion during the time of these assessments. The main reason for the latter was concerns with side effects associated with prolonged exposure to physostigmine. Additionally, subject discontinuation in the study due to significant burden due to the number of assessments required a change to the protocol in order to reduce the frequency of CANTAB tests. These limitations in the execution of the study are likely to have contributed to the lack of clear PD effects on the neurophysiological and neurocognitive tests after administration of HTL0009936 or physostigmine. However, physostigmine was associated with a significant but small improvement in adaptive tracking (reflecting psychomotor function and sustained attention). The improvement in adaptive tracking and the lack of effect on other tests may be due to the adapting tracking being performed close to the time when the physostigmine infusion was stopped (i.e. 10 min after infusion was stopped). As this study was powered on the adaptive tracking test, it is likely that this is a cholinergic relevant pharmacological effect of physostigmine and supports previous studies that have similarly shown positive effects of a cholinesterase inhibitor galantamine (35). The absence of an effect on adaptive tracking performance during HTL0009936 exposure based on visual inspection of the graphs, might be due to specificity of the cognitive processes modulated by $M_1$ receptor modulation. It is possible psychomotor/attentional processes



are less affected whereas memory is more affected by $M_1$ receptor modulation. In support, a study with the $M_1$ agonist GSK1034702 showed improvement in episodic memory but not psychomotor speed or attention[48]. Furthermore, preclinical studies with HTL0009936 showed reversal of scopolamine induced impairment in the novel object recognition and passive avoidance tests of memory and improvement in working memory in aged Beagle dogs[49]. On the other hand, the $M_1/M_4$ muscarinic antagonist biperiden led to a decrease in performance in the adaptive tracking task at dose levels that didn't lead to clinically overt (subjective or objective) sedation (results in preparation to be published). Given the limitations discussed which may have impacted the ability of HTL0009936 to exert effects of cognitive and neurophysiological function, no clear conclusions can be made with regard to the PD effects of HTL0009936 in this study. This would require further investigation in an appropriately designed and adequately powered study.

In summary, this safety, tolerability, PK and exploratory PD study of HTL0009936 showed that the drug had well-characterized PK and was generally well-tolerated in the dose range studied in elderly subjects. The incidence of adverse events were mild and dose-related. No clear PD effects of HTL0009936 could be observed, except a potential increase (i.e. improvement) in P300 amplitude, a measure of cognitive function, and a lack of effect of attention and psychomotor speed as measured by the adaptive tracking test. However overall, no conclusions can be made with regard to positive or negative effects of HTL0009936 on neurophysiological and neurocognitive function, given the limitations in the execution of this study including multiple cognitive tests performed at suboptimal exposures which may have impacted the ability to detect a drug effect. While the PD effects of HTL0009936 require further investigation, the good safety profile of HTL0009936 supports further safety and PD investigation in patients with AD and other dementias.

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TABLE 1 **Summary demographics and baseline characteristics, mean (SD).**

| | Part A (n=10) | Part B (n=33) |
|---|---|---|
| Age, years | 70.2 (3.6) | 70 (5.0) |
| Weight, kg | 74.8 (12.3) | 74.2 (8.7) |
| BMI, $kg/m^2$ | 25.5 (3.7) | 25.5 (2.5) |
| Gender, n (%) | | |
| Female | 5 (50) | 17 (52) |
| Male | 5 (50) | 16 (48) |
| CYP2D6 predicted phenotype, n (%) | | |
| Extensive metabolizer | 10 (100) | 27 (82) |
| Intermediate metabolizer | 0 | 6 (18) |
| Cognitive score at screening < 1 SD, n (%) | | |
| Word fluency | N/A | 12 (36) |
| AVLT | N/A | 13 (39) |
| Adaptive tracking test | N/A | 14 (42) |

TABLE 2 **Summary of HTL0009936 exposures after IV infusion in part A, mean (%CV) or [range].**

| Dose (mg) | Observed $C_{mean}$ (ng/mL) | $T_{max}$ (hr) | $C_{max}$ (ng/mL) | $AUC_{0-24}$ (hr.ng/mL) | $AUC_{0-\infty}$ (hr.ng/mL) | $t_{½}$ (hr) | CLp (L/hr) | CLr (L/hr) |
|---|---|---|---|---|---|---|---|---|
| 10a | n/a | 0.50 [0.33 - 0.58] | 59.5 (35) | 120 (24) | 124 (24) | 2.2 (12) | 81 (24) | 8.7 (27) |
| 49.2b | 97 (22) | 0.50 [0.17 - 5.5] | 125 (33) | 684 (24) | 691 (24) | 2.3 (35) | 71 (24) | 7.2 (41) |
| 83c | 172 (17) | 2.0 [2.0 - 3.0] | 197 (20) | 1130 (17) | 1140 (16) | 2.4 (25) | 73 (17) | 7.8 (25) |

*Geometric mean and (geometric %CV) except $T_{max}$ median [minimum - maximum] for n=6 per dose except n=5 at 83 mg. $AUC_{0-\infty}$ = area under the plasma concentration-time curve from zero extrapolated to infinity; $AUC_{0-24}$ = area under the plasma concentration-time curve from zero to 24 hours post dose; $C_{max}$ = maximum plasma concentration; $C_{mean}$ = mean plasma concentration during maintenance infusion; CLp = total plasma clearance; CLr = renal clearance; $T_{max}$ = time to $C_{max}$; $t_{½}$ = apparent terminal half-life; a 10 mg over 0.5 hr at 33.2 mL/h; b 14.1 mg over 0.5 hr at 47 mL/hr + 35.1mg over 4.5 hr at 13 mL/hr; c 43 mg over 2 hr at 64.8 mL/hr + 40mg over 3hr at 40.2 mL/hr.*



TABLE 3 **Oral PK of HTL0009936 at 24 mg, mean (%CV) or [range] for n=6.**

| Dose (mg) | $T_{max}$ (hr) | $C_{max}$ (ng/mL) | $AUC_{0-24}$ (h.ng/mL) | $AUC_{0-\infty}$ (hr.ng/ml) | $t_{½}$ po (hr) | Fpo (%)[a] |
|---|---|---|---|---|---|---|
| 24 | 1.0 [0.50 - 1.5] | 14.1 (49) | 44.1 (48) | 47.2 (41) | 2.4 (28) | 14.8 (44) [8.7 - 27] |

*Geometric mean and (geometric % CV) except $T_{max}$ median [minimum - maximum] for n=6. $AUC_{0-\infty}$ = area under the plasma concentration-time curve from zero extrapolated to infinity; $AUC_{0-24}$ = area under the plasma concentration-time curve from zero to 24 hours post dose; $C_{max}$ = maximum plasma concentration; Fpo = oral bioavailability and [minimum - maximum]; $T_{max}$ = time to $C_{max}$; $t_{½}$ po = apparent terminal half-life after oral administration; a = oral bioavailability estimated in comparison with 10 mg IV single infusion.*

TABLE 4 **Summary table of HTL0009936 exposures in part B (CYP2D6 EM and IM subjects combined), mean (%CV) and [range].**

| Dose (mg)[a] | $C_{mean}$ (ng/mL)[b] | $T_{max}$ (hr) | $C_{max}$ (ng/mL) | $AUC_{0-24}$ (hr.ng/mL) | $AUC_{0-\infty}$ (hr.ng/mL) | $t_{½}$ IV (hr) | CLp (L/hr) | CLr (L/hr) |
|---|---|---|---|---|---|---|---|---|
| 13.5 (4.5+9) | 27.1 (20) | 1.0 [0.52 - 5.1] | 33.8 (21) | 192 (27) | 197 (26) | 2.2 (28) | 69 (26) | 8.6 (23) |
| 40 (13.3 + 26.7) | 78.2 (18) | 1.0 [0.58 - 5.3] | 97.6 (21) | 550 (24) | 564 (24) | 2.3 (33) | 71 (24) | 8.2 (27) |
| 79.5 (26.5+53) | 166 (20) | 1.1 [0.83 - 5.6] | 203 (20) | 1200 (31) c | 1170 (25) | 2.6 (27) | 68 (25) | 7.3 (30) |

*Geometric mean and (geometric % CV) except $T_{max}$ median [minimum - maximum] for n=25 - 28 observations excluding subjects where infusion was stopped early or interrupted. $AUC_{0-\infty}$ = area under the plasma concentration-time curve from zero extrapolated to infinity; $AUC_{0-24}$ = area under the plasma concentration-time curve from zero to 24 hours post dose; $C_{max}$ = maximum plasma concentration; $C_{mean}$ = mean plasma concentration during 4 hour maintenance infusion; CLp = total plasma clearance; CLr = renal clearance; $T_{max}$ = time to $C_{max}$; $t_{½}$ IV = post-infusion intravenous apparent half-life; a loading dose (1 hr at 83.3 mL/hr) + maintenance dose (4 hr at 41.7 mL/hr); b steady-state concentration maintained between 1 and 5hr after the start of dosing; ; c includes a subject with a large value of $AUC_{0-t}$ due to limited available PK sampling times but for whom a value of $AUC_{0-inf}$ could not be estimated, therefore the group mean value of $AUC_{0-t}$ was greater than $AUC_{0-inf}$.*

FIGURE 1 Study design of part A (four-treatment open label sequential design) and B (five-treatment randomized, placebo and positive comparator-controlled crossover design) and the number of subjects that started and completed the treatment.

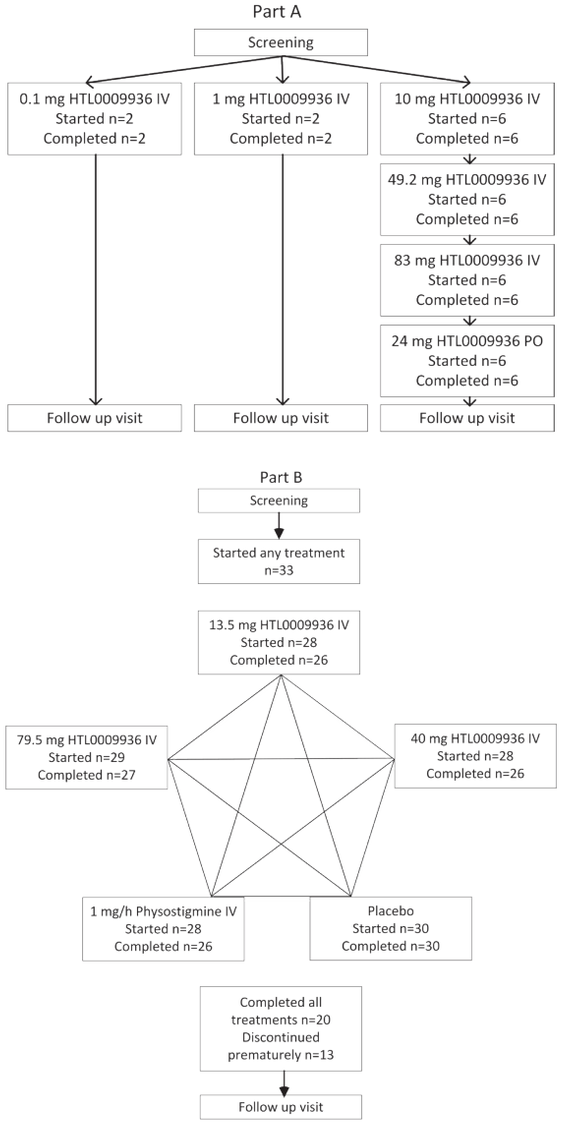
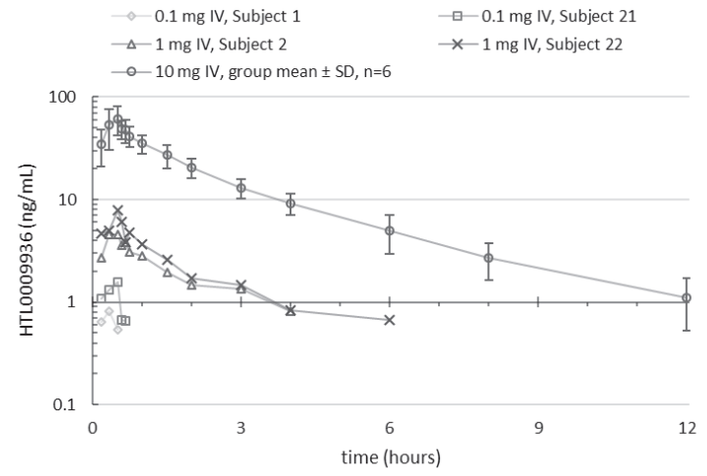





FIGURE 2 A. Concentration–time profiles of HTL0009936 single IV infusion at 0.1 mg (n=2), 1 mg (n=2) and 10 mg in part A (mean ± SD for n=6). B. Concentration–time profiles at 13.5, 40 and 79.5 mg HTL0009936 by dual IV infusion in part B (arithmetic mean ± SD; n=28–29). Profile truncated at 8 hours to show plateau during maintenance dose.



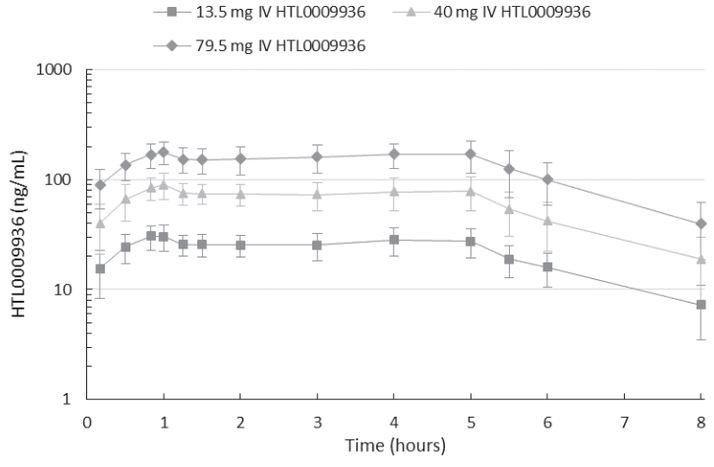
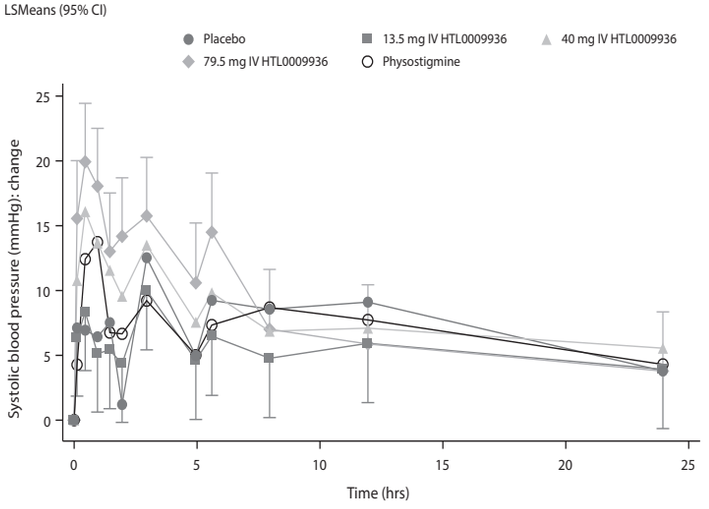



FIGURE 3 A. Systolic blood pressure (mm Hg) shown as change from baseline and B. Diastolic blood pressure (mm Hg) shown as change from baseline (mean, 95% CI error bars).



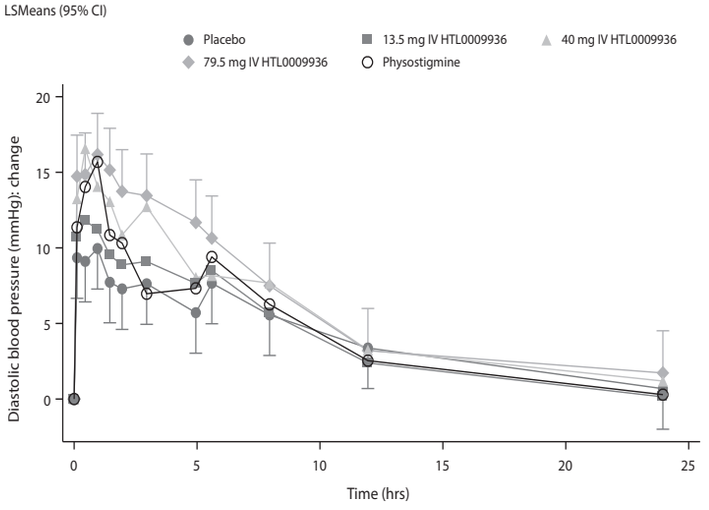
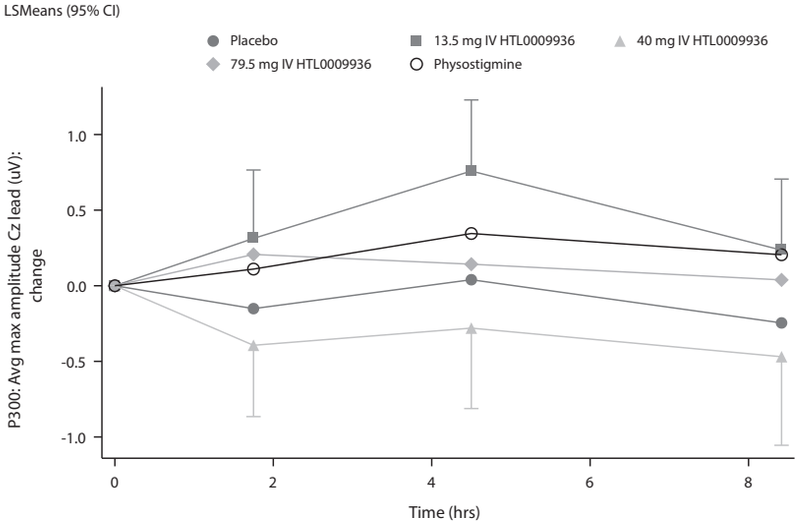
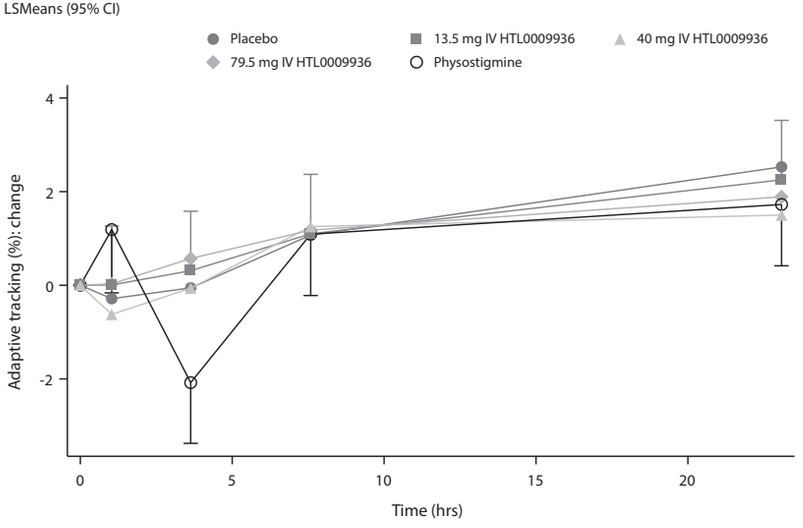



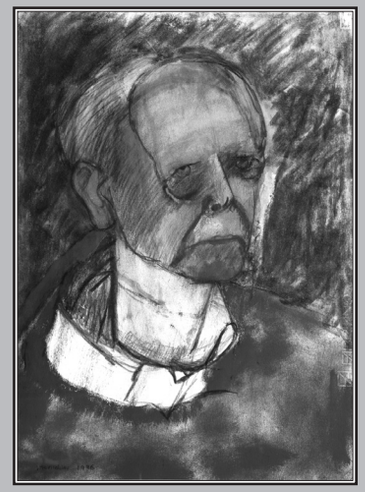



FIGURE 4 A. P300 results shown as change from baseline and B. Adaptive tracking test results shown as change from baseline (mean, 95% CI error bars).



CHAPTER III



# FIRST-IN-MAN STUDY TO INVESTIGATE SAFETY, PHARMACOKINETICS AND EXPLORATORY PHARMACODYNAMICS OF HTL0018318, A NOVEL $M_1$-RECEPTOR PARTIAL AGONIST FOR THE TREATMENT OF DEMENTIAS

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## ABSTRACT

**AIMS** HTL0018318 is a selective $M_1$ receptor partial agonist currently under development for the symptomatic treatment of cognitive and behavioural symptoms in Alzheimer's disease (AD) and other dementias. We investigated safety, tolerability, pharmacokinetics (PK) and exploratory pharmacodynamics (PD) of HTL0018318 following single ascending doses.

**METHODS** This randomized, double-blind, placebo-controlled study in 40 healthy younger adult and 57 healthy elderly subjects, investigated oral doses of 1-35 mg HTL0018318. PD assessments were performed using a battery of neurocognitive tasks and electrophysiological measurements. CSF concentrations of HTL0018318 and food effects on PK of HTL0018318 were investigated in an open label and partial cross-over design in 14 healthy subjects.

**RESULTS** Pharmacokinetics of HTL0018318 were well-characterized showing dose proportional increases in exposure from 1-35 mg. Single doses of HTL0018318 were associated with mild dose-related adverse events of low incidence in both younger adult and elderly subjects. The most frequently reported cholinergic AEs included hyperhidrosis and increases in blood pressure up to 10.3 mm Hg in younger adults (95% CI [4.2–16.3], 35 mg dose) and up to 11.9 mm Hg in elderly subjects (95% CI [4.9–18.9], 15 mg dose). There were no statistically significant effects on cognitive function but the study was not powered to detect small to moderate effect sizes of clinical relevance.

**CONCLUSIONS** HTL0018318 showed well-characterized pharmacokinetics and following single doses were generally well tolerated in the dose range studied. These provide encouraging data in support of the development for HTL0018318 for AD and other dementias.




## INTRODUCTION

Alzheimer's disease (AD) and Dementia with Lewy Bodies (DLB) are common neurodegenerative disorders associated with cognitive decline and the onset of behavioural and psychiatric symptoms in the elderly. One of the pathological characteristics is dysfunction of the cholinergic system[1] due to damage of the synapses and a progressive and irreversible loss of cholinergic neurons of the nucleus basalis of Meynert and medial septum (i.e. basal forebrain) that provide major source of cholinergic innervation to the neocortex and hippocampus[2-6]. These pathological changes lead to disturbed cholinergic signalling, which plays a critical role in the clinical characteristics of AD, including a decline of cognitive processes such as attention, learning and memory[7-9] as well as some of the behavioural and psychiatric symptoms including hallucinations[10].

The currently available treatment for AD and DLB is solely symptomatic, leading to temporary improvement of cognitive functioning without affecting the underlying pathophysiological processes and therefore without affecting disease progression. In patients with mild to moderate AD, treatment consists of the NMDA receptor antagonist memantine or of acetylcholinesterase inhibitors (AChEI) that inhibit the breakdown of the neurotransmitter acetylcholine, such as rivastigmine, donepezil and galantamine. AChEIs increase concentrations of acetylcholine at the synapse which subsequently activate cholinergic muscarinic and nicotinic receptors in the neocortex and hippocampus. The efficacy of these treatments are modest and dosing is limited by side effects consisting mainly of gastrointestinal adverse events (nausea, vomiting, diarrhoea) that are a consequence of the increased acetylcholine level hyperstimulating peripheral $M_2$ and $M_3$ receptors[11]. The modest efficacy of AChEIs is in part related to their primary action of inhibiting ACh breakdown in degenerating pre-synaptic cholinergic neurons with reduced ACh synthesis capacity with disease progression.

An alternative and potentially more effective strategy is to target post-synaptic $M_1$ receptors (nomenclature[12]). The $M_1$ receptor is the predominant muscarinic receptor in the central nervous system and is highly expressed in the neocortex and hippocampus[13]. It has been demonstrated that this receptor is involved in memory and learning processes[14,15] and therefore drugs that stimulate the $M_1$ receptor have a cognitive enhancing potential[16-19]. Additionally, in contrast to other acetylcholine receptors, the $M_1$ receptor is relatively preserved in AD including severe AD[20], which could allow treatment in more advanced stages of AD. Muscarinic receptor agonists including the $M_1/M_4$ agonist Xanomeline and the $M_1$ bitopic agonist GSK1034702 have shown promising early clinical effects[17,21]. The Phase 2 study of xanomeline in AD patients showed statistically significant effects on cognitive function (measured

using the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-COG), general clinical status (measured using the Clinician's Interview-Based Impression of Change (CIBIC+)),and behavioural symptoms such as delusions, hallucinations, agitation (measured using the Alzheimer's Disease Symptomatology Scale (ADSS))[21]. However, treatment with xanomeline was associated with the emergence of clinically significant, dose-dependent side effects (e.g. gastrointestinal effects and syncope) that were believed to be largely mediated through non-selective stimulation of $M_2$ and $M_3$ muscarinic receptors by the drug[21,22]. Similarly, the $M_1$ bitopic agonist GSK1034702 was shown to improve episodic memory (measured using the Cogstate International shopping list task) in a nicotine abstinence model of cognitive dysfunction, but this compound failed to progress to Phase 2 studies due to cardiovascular adverse events[17].

HTL0018318, in this study administered as HCl salt (ethyl (3-endo)-3-(3-oxo-2,8-diazaspiro[4.5]dec-8-yl)-8-azabicyclo [3.2.1]octane- 8-carboxylate hydrochloride), is a selective $M_1$ receptor partial agonist that is being developed to treat the symptomatic decline of cognitive function in dementias associated with cholinergic degeneration including AD and DLB. Pre-clinical studies demonstrated that HTL0018318 has approximately a two-fold selectivity for the $M_1$ over $M_4$ receptors with no detectable functional agonist activity at human $M_2$ and $M_3$ receptors[23]. Additionally, reversal of scopolamine-induced deficits have been shown in passive avoidance learning in rats consistent with pro-cognitive effects reported with other $M_1$ agonists on tests of learning and memory[23]. In this first in human study we aimed to investigate the safety, tolerability and pharmacokinetics (PK) of single ascending doses of HTL0018318 in healthy subjects. Exploratory pharmacodynamic (PD) measures were also included to assess effects of HTL0018318 on synaptic and cognitive markers relevant for central target engagement.

## METHODS

This study was approved by the medical ethics review board of the foundation Beoordeling Ethiek Biomedisch Onderzoek (BEBO, Assen, The Netherlands) and conducted according to the principles of the Declaration of Helsinki and the ICH GCP guidelines[24].

**DESIGN** This study consisted of three Parts. Part A used a double-blind, placebo controlled, randomized, single ascending dose design and consisted of five cohorts of eight healthy younger adult male subjects (6 active and 2 placebo per cohort). Part B used an open label and partial cross-over design where 14 healthy younger adult male subjects were administered HTL0018318 in the fasted state, and 6 subjects dosed as a cross-over from the previous occasion in the fed state, separated by a washout period of two to four weeks. A single CSF sample was collected from 12 of the fasted subjects in Part B.

Part C used a double-blind, placebo controlled, randomized, single ascending dose design and consisted of five cohorts of 12 healthy elderly subjects, both male and female (9 active and 3 placebo per cohort).

**PARTICIPANTS** Younger adult subjects aged 18-55 years, inclusive, and elderly subjects aged ≥65 years and over took part in the study. All subjects had to be healthy with no current or past history of any physical, neurological or psychiatric illness interfering with the study objectives and had to have a maximum resting blood pressure of up to 140/90 mmHg and a heart rate between 45-100 bpm at screening. Younger adult subjects were free of any medication. In elderly subjects, medication was allowed at discretion of the investigator, but antihypertensive drugs were not allowed (supplementary overview S1). Consumption of alcohol and caffeine containing products, the use of nicotine-containing products and products that influence CYP3A4 and CYP2C9 were not allowed prior to and during the study.

**MATERIALS** HTL0018318 was administered as an oral aqueous solution in 100 ml. Dose levels in Part A were 1 mg, 3 mg, 9 mg, 20 mg and 35 mg, in Part B 20 mg, and in Part C 9 mg, 15 mg, 23 mg, 30 mg and 35 mg. The 1 mg dose level is the human equivalent to the no effect level (NOEL) in the most sensitive preclinical study (dog cardiovascular study) with a 10-fold safety margin. There was no further dose escalation after the 35 mg dose level as it was decided to not exceed a $C_{max}$ of 267 ng/ml in humans due to observed increases in blood pressure and change in heart rate in the pre-clinical study. Water was used as placebo. To mask the difference in taste, if any, between HTL0018318 and placebo, a peppermint strip (Listerine) was administered at one minute before and after the administration of the oral solution.

**SAFETY AND TOLERABILITY** The primary safety and tolerability end points investigated were treatment emergent adverse events (TEAEs), safety laboratory, vital signs, electrocardiogram (ECG), 24-hour Holter and pulmonary function test (PFT). TEAE and serious adverse event (SAE) data were collected and recorded on the first dosing visit, continuing until the follow-up visit. Systolic and diastolic blood pressure (SBP and DBP), pulse rate, and single 12-lead ECGS were recorded at regular intervals. Twenty-four-hour Holter continuous ambulatory ECG monitoring was performed for approximately 24 hours at screening and at each dosing visit (starting just prior to dosing).

**PHARMACOKINETIC ASSESSMENTS** In all Parts, blood samples for determination of plasma HTL0018318 levels were collected at pre-dose and 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 9 h, 12 h, 24 h, 30 h, 48 h, 72 h and at follow-up (5-7 days post-dose). Urine was collected at pre-dose, up to 72 hours post-dose and at follow-up. Plasma and urine samples were analyzed for HTL0018318 using a validated bioanalytical method based on protein precipitation, high performance liquid chromatography with tandem mass spectrometric detection. Each bioanalytical run used to support PK endpoints met pre-defined acceptance criteria for quality control (± 15% of the nominal concentration) and calibration standards (± 15% except ± 20% at the lower limit of quantification (LLOQ)). The quantification range was 0.5-1000 $ng.ml^{-1}$. The following PK parameters were estimated from the plasma and urine concentration for HTL0018318 by non-compartmental analysis: the area under the plasma concentration-time curve (AUC) calculated from 0 to the last measurement point ($AUC_{0}$-last), from 0 to 24 h ($AUC_{0-24}$) and AUC to infinity ($AUC_{0}$-inf), maximum plasma concentration ($c_{max}$), time of the maximum plasma concentration ($T_{max}$), apparent half-life values ($t_1/_2$), apparent plasma clearance (CLp/F), amount of unchanged drug excreted into the urine (Ae) and renal clearance (CLr). The effect of food on exposure was assessed in terms of $T_{max}$, $c_{max}$, $AUC_{0-t}$, and $t_1/_2$.

CSF samples were collected only in Part B at 2, 4, 6 and 8 hours post-dose. One CSF sample was taken from each of 12 fasted subjects to create a composite concentration-time profile with triplicate measures at each time point. CSF samples were analysed for HTL0018318 using a suitably qualified bioanalytical method similar to that used for plasma and urine. CSF concentrations were used to calculate the HTL0018318 unbound CSF to unbound plasma ratio at each time point and the apparent $c_{max}$ and $T_{max}$ for CSF exposure.

**EXPLORATORY PHARMACODYNAMIC ASSESSMENTS** Exploratory PD measures were included to assess effects of HTL0018318 on synaptic and cognitive markers relevant for central target engagement as well as to assess any potential detrimental effects on brain function. The NeuroCart is a battery of tests for a wide range of CNS domains that was developed to examine different classes of CNS-active drugs[25]. In the present study the set of tests was customized to detect PD effects that can be expected with a drug modulating the cholinergic system. The adaptive tracking measured attention and visuomotor coordination. Subjects were asked to use a joystick to keep a randomly moving target on the screen inside a circle during three minutes. The percentage accuracy was recorded[25-28]. The Milner maze test (MMT) was used to evaluate spatial working memory, learning and executive function. Subjects were required to complete a maze by using trial and error learning to locate a 28-step pathway that was hidden beneath a 10×10 grid of tiles. There were three types of trials in the MMT: Immediate for imprinting (five times the same path version), Delayed (the same path once) and Reversed (the same path once in reversed direction)[29]. The n-back test was used to evaluate (short-term) working memory and executive function. Subjects had to remember and correlate a sequence of letters presented in a random order[30-32]. Synaptic activity was assessed using electrophysiology and included resting EEG (power in delta, theta, alpha, beta and gamma bands) and Event Related Potential (ERP) P300 and Mismatch Negativity (MMN). Other PD measurements included the Leeds Sleep Evaluation Questionnaire (LSEQ) to assess sleep quality[33], the visual analogue scale (VAS) according to Bond and Lader to assess subjective mood states[34-36] (including a VAS Nausea scale to assess subjective nausea) and pupil size (measured using a digital camera (Canon EOS1100D)) to monitor any drug effects on the sympathetic nervous system. The pupil size was calculated as the ratio of the pupil diameter over the cornea diameter of each eye[28,37]. In addition, pulmonary function (assessed by the spirometry system Spirostik) and saliva production (measured by the increase in weight of three Salivettes dental rolls that were put into the oral cavity for three minutes) were also examined.

In Parts A and C, all tests were performed twice at baseline and repeated at 1 h, 3 h, 5 h, 6 h and 9 h after administration of HTL0018318 or placebo. The only exceptions were EEG/ERP measurements, which was also performed 2.5 hours post dose, and the MMT, which was not performed 6 hours post dose. The extra EEG/ERP measurement was performed since effects were expected based on a previous study with an $M_1$ receptor agonist (data unpublished). The MMT was not performed in order to reduce the subject burden. Pulmonary function test and saliva production measurements were performed at regular intervals.

**STATISTICS** No formal hypothesis testing was conducted. Sample size was chosen as a compromise between minimizing the exposure of human subjects to a new chemical entity and the need to provide sufficient data. Hence the study was not powered to detect any significant treatment related effects of small to moderate effect sizes. To establish whether significant treatment effects could be detected, repeatedly measured variables were analyzed with a mixed model analysis of covariance with treatment, time and treatment by time as fixed factors, and subject as random factor and the (average) baseline measurement as covariate. Single measured variables were analyzed with a one-way analysis of covariance with fixed factor treatment and the baseline measurements as covariate. In these analysis models, all means are estimated. These are called the least square means. All calculations were performed using SAS for windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

ERP data (P300 and MMN) were excluded from statistical analysis due to data quality and technical issues with stimuli timing and recording. Hence only resting state EEG power data is reported.

## RESULTS

**SUBJECTS** In Part A 40 subjects received a single dose of HTL0018318 (n=30) or placebo (n=10). The mean (range) age was 29.1 years (18–53), bodyweight was 79.1 kg (54.8–105.6) and mean body mass index (BMI) was 23.5 $kg.m^{-2}$ (18.7–31.1).

In Part B 14 subjects completed the study. The mean age (range) was 29.0 years (18–51), weight was 77.3 kg (55.4–99.8) and the BMI was 24.3 $kg.m^{-2}$ (18.6–32.5). These 14 subjects include two additional subjects who were enrolled because CSF-sampling could not be performed in two initially included subjects.

In Part C 57 subjects received a single dose of HTL0018318 (n=43) or placebo (n=14). The mean age was 71.0 years (range 65–82), the bodyweight was 74.2 kg (range 54.8–105.6), the BMI was 24.7 $kg.m^{-2}$ (range 19.4–31.6) and 33.3% were female. In the 30 mg cohort only nine subjects were included (7 active : 2 placebo) due to recruitment difficulties.

**SAFETY AND TOLERABILITY** All TEAEs were mild or moderate in intensity in both younger adult and elderly subjects who received HTL0018318. In Part A, the most common TEAEs reported in younger adult subjects were gastrointestinal symptoms (i.e. diarrhoea, nausea or vomiting), headache and hypertension (see Table 1). One subject reported salivary hypersecretion after the 35 mg dose. The incidence of TEAEs in Part A appeared to be dose-related both in terms of number of TEAEs and number of subjects reporting TEAEs.

In Part B of the study relatively more subjects (71.4%) reported back pain, which was likely related to CSF sampling. In Part C the most common TEAEs reported in elderly subjects were headache, hyperhidrosis, gastrointestinal symptoms (i.e. diarrhoea, nausea or vomiting) and hypertension (see Table 2). There was no dose related increase in frequency of TEAEs, however, in the 35 mg cohort more hyperhidrosis and hypertension were reported. As such, these specific symptoms may be related to (increasing) dose of HTL0018318.

In younger adult subjects in Part A, no consistent effects on systolic blood pressure (SBP), diastolic blood pressure (DBP) or pulse rate measured in supine position were observed in the 1 mg–30 mg dose range. However, following the 35 mg dose, there was a 10.3 mm Hg (95% CI [4.2–16.3], p=0.0015) increase in mean SBP, a 9.2 mm Hg (95% CI [3.2–15.1], p=0.0038) increase in mean DBP, and a 9.8 bpm increase in mean pulse rate (95% CI [4.4–15.2], p=0.0008) relative to placebo (Figure 1). Hypertension was considered an TEAE in one subject following a 9 mg dose and three subjects who received the 35 mg dose. In these four subjects, the SBP increased between 14 and 40 mm Hg from baseline, and the DBP increased between 0 and 27 mm Hg from baseline between 25 minutes and 2 hours post dose. The highest SBP considered to be an TEAE was 145 mm Hg post dose which was 105 mm Hg at baseline. The highest DBP was 90 mm Hg post dose, which was 63 mm Hg at baseline.

In elderly subjects in Part C, the mean SBP was significantly higher than placebo following 15 mg HTL0018318 (difference of 11.9 mm Hg, 95% CI [4.9–18.9], p=0.0012), 23 mg (difference of 9.3 mm Hg, 95% CI [2.2–16.5], p=0.0114) and 30 mg (difference of 7.8 mm Hg, 95% CI [0.3–15.4], p=0.0430). The mean DBP was significantly higher following 15 mg (difference of 6.1 mm Hg, 95% CI [1.4–10.8], p=0.0118) and 23 mg (difference of 5.0 mm Hg, 95% CI [0.2–9.7], p=0.04). Hypertension was considered an TEAE in one subject following 9 mg HTL0018318, one subject following 15 mg, and three subjects following 35 mg administration. In these five subjects, the SBP increased between 14 and 51 mm Hg from baseline and the DBP increased between 10 and 31 mm Hg between 25 minutes and 3 hours post-dose. The highest blood pressure considered to be an TEAE was 181/98 mm Hg, this was 156/82 mm Hg at baseline.

No consistent clinically relevant abnormalities in chemistry and haematology blood results, urinalysis, electrocardiograms and 24-hour Holter monitoring were observed in both younger adult and elderly subjects.

**PK ASSESSMENTS** The plasma and CSF PK variables of HTL0018318 are shown in Table 3 and Figure 2. Plasma concentration increased immediately after dosing with median $T_{max}$ at 1.5 hours post-dose (range 0.5–6.0 hours). The PK profile appeared biphasic after $C_{max}$. Renal elimination was a significant route of clearance. The renal clearance was slightly higher in younger adults (8–9 $L.h^{-1}$) compared with elderly subjects (5–8$L.h^{-1}$). The mean $t_1/_2$ was 12 hours in younger adults and 16 hours in elderly subjects, which resulted in a slight increase in dose-normalized AUC in elderly subjects. Based on the recovery of unchanged HTL0018318 in urine over 72 hours, absolute oral bioavailability was at least 18–64% in younger adults and 28–88% in elderly subjects. Exposure in terms of $C_{max}$ and $AUC_{0\text{-}inf}$ appeared to be dose-linear over the range 1–35 mg. The highest individual plasma concentration measured was 231 $ng.ml^{-1}$ in younger adults and 260 $ng.ml^{-1}$ in elderly, both following 35 mg administration.

The CSF to unbound plasma concentration ratio was 0.16 at 2 hours rising to 0.82 at 9 hours (Figure 3), using a HTL0018318 fraction unbound of 0.94 in human

plasma. The CSF concentration increased from 2 to 3 hours post-dose and remained at approximately the same (mean 22.6 to 30.3 ng.ml$^{-1}$) to the last sampling point at 9 hours post-dose, with the rise in apparent unbound partition coefficient (kpuu) being primarily a function of decreasing plasma HTL0018318 concentration.

Dosing an oral solution of HTL0018318 with an FDA-style high calorie breakfast caused a trend towards delay in median $T_{max}$ from 0.75 to 2.25 hours and a 20% decrease in mean $C_{max}$ (ratio: 79.35%, 90% CI [70.09–89.83]) with an unchanged $AUC_{0-inf}$ (ratio: 103.11%, 90% CI [95.74–111.06]) and $t_1/_2$ (ratio: 98.91 %, 90 % CI [75.38–129.78 ]).

**PD ASSESSMENTS** Overall, single doses of HTL0018318 showed no acute effects on measures of synaptic and cognitive function. While the study was not powered to detect small to moderate pro- cognitive effects of HTL0018318, selective statistically significant effects were noted for some endpoints (table S2 and S3 in supplement). However, these effects appeared to be independent of the cognitive domains assessed, EEG frequency band, dose of HTL0018318, electrode position and cohort type. Interestingly some trend level significant improvements (i.e. effect sizes above 0.4 and p values under 0.2) in certain cognitive processes including memory/executive function (Milner maze) was observed, particularly in the elderly.

In both younger adults and elderly, isolated significant differences were observed in the VAS Bond and Lader, VAS Nausea and LSEQ outcomes between HTL0018318 and placebo treatment (table S2 and S3 in supplement). These differences were inconsistent and the magnitude of the change were less than 5 mm change on a 100 mm VAS scale and therefore considered clinically insignificant.

In the healthy elderly, HTL0018318 caused a small but consistent increase in pupil/iris ratio in left eye and right eye. In the 15 mg, 23 mg, 30 mg, and 35 mg cohorts, a significant increase in pupil/iris ratio (left eye) was observed compared to placebo, and in the 15 mg, 23 mg, and 30 mg cohorts, a significant increase in pupil/iris ratio (right eye) was observed compared to placebo, indicating an increase in pupil size. In younger adult and elderly subjects, administration of all dose levels of HTL0018318 did not lead to significant increases in saliva production and did not significantly affect pulmonary function compared to placebo.

## DISCUSSION

This first-in-man study investigated the safety and tolerability, PK and exploratory PD effects of the $M_1$ receptor partial agonist HTL0018318, administered as an oral solution in healthy younger adult and elderly subjects.



Single doses (1-35 mg) of HTL0018318 were associated with mild dose-related TEAEs (with low incidence) in both younger adult and elderly subjects. The most frequently reported TEAEs likely to be cholinergic-mediated included hyperhidrosis and increases in blood pressure, particularly following the 35 mg dose (younger adults) and 23 mg and 35 mg doses (elderly). In younger adult subjects, doses up to 20 mg were not associated with changes in systolic and diastolic blood pressure and heart rate. However, the 35 mg dose was associated with an increase in mean systolic and diastolic blood pressure (up to 10 mm Hg) and mean heart rate (up to 9.8 bpm). In elderly subjects, significant increases in mean systolic and diastolic blood pressure (up to 11.9 mm Hg) and mean heart rate (up to 6.3 bpm) were observed in the 15–35 mg dose range, with no clear evidence of dose-dependency. The increase in blood pressure and heart rate is consistent with expected effects of $M_1$ receptor stimulation on the cardiovascular system[38]. Development of $M_1$ orthosteric and allosteric agonists is often limited by cholinergic side effect, as was the case in the development of Xanomeline, PF-06767832, AZD6088 and GSK1034702[21,39-41]. More recently, the $M_1$ positive allosteric modulator MK7622 was also associated with more adverse events (including 2-3 times more cholinergic related adverse events) in AD patients and more study discontinuations than placebo. This is intriguing given the widely suggested hypothesis that allosteric modulation of the muscarinic $M_1$ receptor would provide improved therapeutic margins. While the profile of adverse events observed in this single dose study in healthy younger adults and elderly subjects is generally consistent with that reported clinically with other muscarinic receptor orthosteric and allosteric agonists[17,21,42], we report low incidence of cholinergic adverse events with HTL0018318 with doses below 35 mg. The higher incidence of adverse events and increase in blood pressure and heart rate at the 35 mg dose suggests that, at least in healthy younger adult and elderly subjects, single doses above 35 mg may be less well-tolerated. In the current study, while doses up to 35 mg were well-tolerated, it remains to be determined if doses up to and including 35 mg are better tolerated following repeat dosing in healthy subjects as well as patients with Alzheimer's Disease who reportedly have lower autonomic function[43]. It is likely that the safety profile of $M_1$ agonists including HTL0018318 may vary depending on the patient population.

The pharmacokinetics of HTL0018318 were well-characterized in younger adult and elderly subjects up to a 35 mg single dose. Exposure was dose-proportional over the range 1-35 mg. Absorption was rapid with $T_{max}$ typically around 1–2 hours post-dose and a typical oral PK profile which was biphasic after $C_{max}$. In general, elderly subjects appeared to have marginally higher AUC values and lower oral clearance than younger adults (CLp/F 15–21 L.h$^{-1}$ in younger adult and 12–17 L.h$^{-1}$ in elderly subjects). HTL0018318 was found to distribute into CSF with a CSF:plasma ratio of

about 30% based on $C_{max}$ and AUC (16-82 % in CSF as fraction of unbound plasma HTL0018318 concentration, from 2–9 hrs respectively). The CSF to unbound plasma ratio for HTL0018318 is comparable or higher than the equivalent ratio for drugs approved for symptomatic treatment described in literature[44-47]. The concentration of donepezil in CSF achieved 11.25% 12 hours post administration and 25.97% 24 hours post administration, compared with plasma concentrations[44] while approximately 30–40% of rivastigmine plasma concentrations were detected in the CSF[45]. These data are encouraging in relation to achieving sufficient brain exposure to exert pro-cognitive effects and indicate the potential for HTL0018318 to persist in the CSF as plasma HTL0018318 concentration decline after dosing.

The mean apparent oral half-life of HTL0018318 in healthy subjects was 12 h in younger adult subjects and 16 h in elderly subjects predicting minimal (< 2-fold) accumulation at steady-state and appeared independent of dose. The longer half-life resulted in a slight increase in dose-normalized exposure in elderly subjects. This half-life would support once daily dosing, which would favour compliance in elderly patients with dementia. Variability in exposure ($C_{max}$, AUC, t½) was modest, with inter-individual variability typically 20–40 %CV. A substantial portion of the dose was eliminated unchanged in urine with renal clearance being slightly higher in younger adults (8–9 $L.h^{-1}$) compared with elderly subjects (5–8 $L.h^{-1}$). Based on the recovery of HTL0018318 in urine, minimum absolute oral bioavailability was at least 18–64% in younger adults and 28–88% in elderly subjects. Dosing an oral solution of HTL0018318 with an FDA-style high calorie breakfast caused a trend towards delay in $T_{max}$ (group median 0.75 h to 2.25 h) and a 20% decrease in mean $C_{max}$ with an unchanged AUC and half-life.

While the current study was not powered to examine pharmacodynamic effects of clinical relevance, exploratory biomarkers of synaptic and cognitive function were assessed in order to provide early evidence of CNS target engagement as well as any potential adverse effects (i.e. cognitive safety). Single doses of HTL0018318 up to 35 mg had a no deleterious effects on biomarkers of synaptic or cognitive function suggesting a favourable cognitive safety profile. Such effects are important to examine in single dose studies given the potential inverted U dose response effects on cognition often reported for drugs targeting receptors on cortical pyramidal cells including $M_1$ receptors[48]. HTL0018318 across different doses had selective statistically significant effects on some biomarkers of synaptic and cognitive function as shown in the supplement table, however these effects were fairly isolated and inconsistent with regard to the dose of HTL0018318, cognitive domains modulated, the EEG frequency band affected including the electrode position and the cohort type. Hence no meaningful conclusions could be drawn from the observations



regarding consistent improvement in cognitive function. Interestingly some trend level improvements (i.e. effect sizes above 0.4 and p values under 0.2) were noted on certain cognitive processes including memory/executive function (Milner maze) particularly in the elderly. While overall these data are interesting and encouraging, given the very small sample size of the study and lack of multiplicity corrections, we simply note these observations with a view to further exploring these biomarkers of synaptic and cognitive function in future studies in healthy subjects and patients with Alzheimer's disease.

There were some notable effects (and lack of effects) of HTL0018318 in this study that warrant further discussion. In the healthy elderly, HTL0018318 caused a small but consistent increase in pupil/iris ratio in left eye and right eye. In the 15 mg, 23 mg, 30 mg, and 35 mg cohorts, a significant increase in pupil/iris ratio (left eye) was observed compared to placebo, and in the 15 mg, 23 mg, and 30 mg cohorts, a significant increase in pupil/iris ratio (right eye) was observed compared to placebo, indicating an increase in pupil size. The human eye has varying expressions of muscarinic receptors including $M_1$ receptors in the in the ciliary processes and iris[49,50]. It is possible that the small increase in pupil/iris ratio reflecting mydriasis is associated with sympathetic activation of the dilator muscle in the iris. Increased saliva production was to be expected in the current study, based on the fact that saliva production is modulated by a number of muscarinic receptors including $M_1$ and $M_3$ receptors[51], and because salivary hypersecretion has been described in other studies investigating $M_1$ receptor agonists[17,42,52]. Interestingly, no significant increase in saliva production was observed in the current study. The measurement technique of saliva production and materials (Saliva Collection Aid (Salimetrics, UK)) are widely used and hence the sensitivity of the assay is unlikely to be the reason for not observing a change in saliva secretion. It is more likely that the influence of HTL0018318 on saliva production was too small to observe and therefore clinically irrelevant. It also confirms the selectivity of HTL0018318 as salivary secretion is predominantly mediated by $M_3$ receptors[51]. Finally no clinically relevant abnormalities in chemistry, liver enzymes, haematology blood markers, urinalysis, electrocardiograms and 24-hour Holter registrations were observed in both young and elderly subjects.

In summary, HTL0018318 showed well-characterized pharmacokinetics and was generally well-tolerated in the dose range studied in healthy younger adults and elderly subjects. The incidence of adverse events including cholinergic adverse events were mild and dose-related with low incidence. These findings provide encouraging safety and pharmacokinetic data in support of the development of HTL0018318 as a symptomatic treatment for cognitive impairment in dementia including AD and DLB.

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TABLE 1 **Most reported treatment-emergent adverse events (TEAEs) by younger adult subjects; number of subjects (%) per treatment group.**

| | Placebo n = 10 | 1 mg n = 6 | 3 mg n = 6 | 9 mg n = 6 | 20 mg n = 6 | 35 mg n = 6 | All HTL0018318 n = 30 |
|---|---|---|---|---|---|---|---|
| All TEAEs | 6 (60.0) | 0 | 2 (33.3) | 3(50) | 3 (50.0) | 6 (100.0) | 14 (46.6) |
| Diarrhoea/nausea/vomiting | 1 (10.0) | 0 | 0 | 0 | 1 (16.7) | 2 (33.3) | 3 (10.0) |
| Hypertension | 0 | 0 | 0 | 1 (16.7) | 0 | 3 (50.0) | 4 (13.3) |
| Headache | 3 (30.0) | 0 | 0 | 0 | 1 (16.7) | 2 (33.3) | 3 (10.0) |

TABLE 2 **Most reported treatment-emergent adverse events (TEAEs) by elderly subjects; number of subjects (%) per treatment group.**

| | Placebo n = 14 | 9 mg n = 9 | 15 mg n = 9 | 23 mg n = 9 | 30 mg n = 7 | 35 mg n = 9 | All HTL0018318 n = 43 |
|---|---|---|---|---|---|---|---|
| All TEAEs | 3 (21.4) | 6 (66.7) | 4 (44.4) | 6 (66.7) | 6 (85.7) | 7 (77.8) | 29 (67.4) |
| Diarrhoea/nausea/vomiting | 0 | 1 (11.1) | 1 (11.1) | 2 (22.2) | 1 (14.3) | 1 (11.1) | 6 (14.0) |
| Hypertension | 0 | 1 (11.1) | 1 (11.1) | 0 | 0 | 3 (33.3) | 5 (11.6) |
| Hyperhidrosis | 0 | 0 | 0 | 3 (33.3) | 2 (28.6) | 5 (55.6) | 10 (23.3) |
| Headache | 0 | 2 (22.2) | 2 (22.2) | 2 (22.2) | 2 (28.6) | 2 (28.6) | 10 (23.3) |

TABLE 3 **Pharmacokinetic parameters of HTL0018318 in CSF and plasma in younger adults after 20 mg HTL0018318. Group mean.**

| matrix | $C_{max}$ | $T_{max}$ | Clast | Tlast | AUC0-last | CSF/plasma(u) ratio (%) | |
|---|---|---|---|---|---|---|---|
| | (ng.ml-1) | (h) | (ng.ml-1) | (h) | (ng.h.ml-1) | $C_{max}$ | AUC |
| CSF | 30.3 | 6 | 27.4 | 9 | 184 | | |
| Plasma | 103 | 1 | 40.6 | 9 | 615 | | |
| Plasma(u) | 97 | | 38.1 | | 578 | 31 | 32 |

*(u) = unbound concentration based on human plasma fu = 0.94*



FIGURE 1 **Vital signs in adult subjects (A,B,C) and elderly subjects (D,E,F) presented as change from baseline (mean, 95% CI error bars).** – *see inside front cover for these images in full color.*

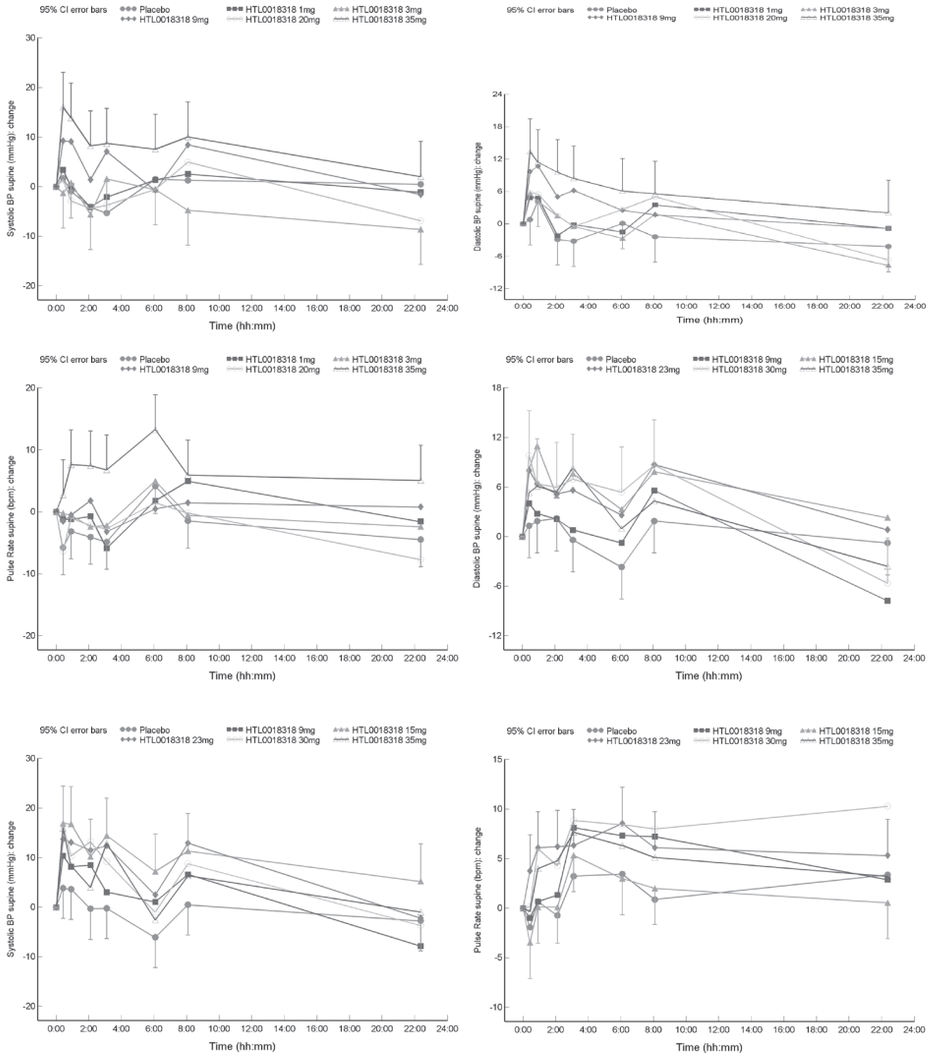


FIGURE 2 HTL0018318 arithmetic mean (± standard deviation) plasma concentration against time after dose following single oral doses of HTL0018318 in healthy younger adults (A) and elderly (B) subjects.

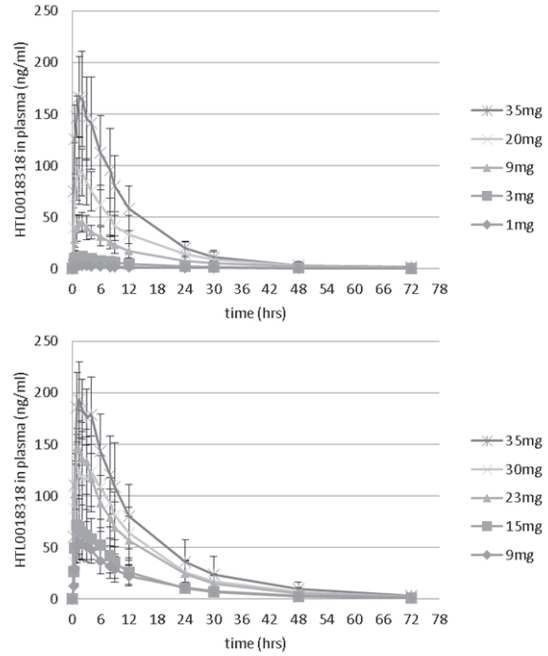
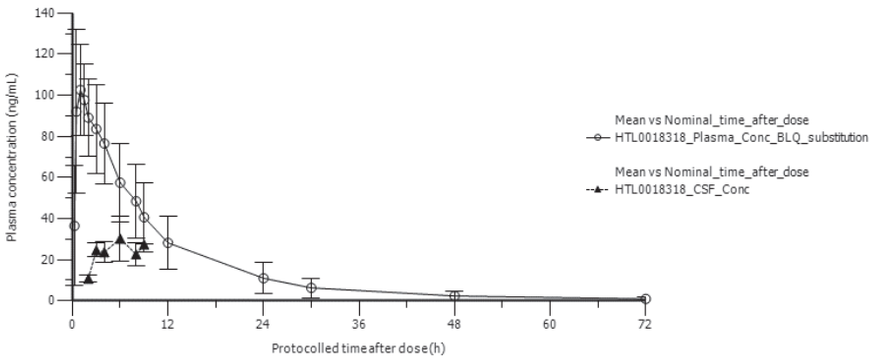
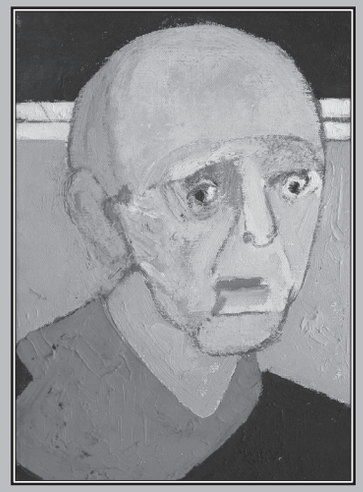



FIGURE 3 HTL0018318 plasma and cerebrospinal fluid (CSF) concentration–time profile after 20 mg HTL0018318 in fasted state. Group mean ± standard deviation.






CHAPTER IV



# SAFETY, PHARMACOKINETICS AND EXPLORATORY PRO-COGNITIVE EFFECTS OF HTL0018318, A SELECTIVE $M_1$ RECEPTOR AGONIST, IN HEALTHY YOUNGER ADULT AND ELDERLY SUBJECTS: A MULTIPLE ASCENDING DOSE STUDY

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## ABSTRACT

**BACKGROUND** The cholinergic system and $M_1$ receptor remain an important target for symptomatic treatment of cognitive dysfunction. The selective $M_1$ receptor partial agonist HTL0018318 is under development for the symptomatic treatment of Dementia's including Alzheimer's disease (AD) and dementia with Lewy bodies (DLB). We investigated the safety, tolerability, pharmacokinetics and exploratory pharmacodynamics of multiple doses of HTL0018318 in healthy younger adults and elderly subjects.

**METHODS** This randomized, double blind, placebo-controlled study was performed, investigating oral doses of 15-35 mg/day HTL0018318 or placebo in 7 cohorts of healthy younger adult (n=36; 3 cohorts) and elderly (n=50; 4 cohorts) subjects. Safety, tolerability and pharmacokinetic measurements were performed. Pharmacodynamics were assessed using a battery of neurocognitive tasks and electrophysiological biomarkers of synaptic and cognitive functions.

**RESULTS** HTL0018318 was generally well-tolerated in multiple doses up to 35 mg/day and were associated with mild or moderate cholinergic adverse events. There were modest increases in blood pressure and pulse rate when compared to placebo treated subjects, with tendency for the blood pressure increase to attenuate with repeated dosing. There were no clinically significant observations or changes in blood and urine laboratory measures of safety or abnormalities in the ECGs and 24-hour Holter assessments. HTL0018318 plasma exposure was dose-proportional over the range 15-35 mg. Maximum plasma concentrations were achieved after 1-2 h. The apparent terminal half-life of HTL0018318 was 16.1 h (± 4.61) in younger adult subjects and 14.3 h (± 2.78) in elderly subjects at steady state. HTL0018318 over the 10 days of treatment had significant effects on tests of short-term (working) memory (n-back) and learning (Milner maze) with moderate to large effect sizes.

**CONCLUSION** Multiple doses of HTL0018318 showed well characterised pharmacokinetics and were safe and generally well-tolerated in the dose range studied. Pro-cognitive effects on short term memory and learning were demonstrated across the dose range. These data provide encouraging data in support of the development of HTL0018318 for cognitive dysfunction in AD and DLB.




## BACKGROUND

Alzheimer's disease (AD) and Dementia with Lewy Bodies (DLB) are progressive neurodegenerative disorders caused by complex pathophysiological processes[1], leading to degeneration of the cholinergic neurons in the basal forebrain and their projections to the cortex and the hippocampus[2]. These cholinergic deficits play a key role in the underlying cognitive impairments as well as some of the behavioural and psychiatric symptoms including visual hallucinations[3-7].

The complex pathology of AD and DLB has hampered progress towards a curative treatment. Currently, the only available treatment is symptomatic and consists mostly of acetylcholinesterase inhibitors (ChEIs). ChEIs inhibit the breakdown of the neurotransmitter acetylcholine (ACh) and subsequently prolongs the availability of ACh at the synapse. This leads to activation of cholinergic muscarinic and nicotinic receptors in the neocortex and hippocampus, which are involved in cognitive function. Despite their ability to improve cognition, ChEIs demonstrate only modest clinical efficacy, likely due to ongoing neurodegeneration of cholinergic neurons in dementia including AD and associated decrease of ACh synthesis, and by a limited dosing range of ChEIs because of side effects including gastrointestinal side effects linked to indirect stimulation of peripheral muscarinic $M_2$ and $M_3$ receptors[8-11]. Therefore, despite efforts to develop disease modifying treatments for AD, there is a need for improved symptomatic treatments for AD and other dementia's targeting not only cognitive symptoms but behavioural and psychiatric symptoms. Optimization of the current treatment options can be achieved by targeting post synaptic muscarinic receptors, in particular $M_1$ receptors involved in cognitive function relative to other muscarinic receptors (i.e. $M_2$ and $M_3$ receptors) associated with peripheral side effects. Such selectivity might allow for higher dosing, which could contribute to improved efficacy for certain cognitive and/or behavioural symptoms. Hence selective $M_1$ receptor agonists may be promising drugs for the treatment of cognitive, behavioural and psychological symptoms in psychiatric and neurological disorders (for a review see Erskine et al., 2019[12]).

The $M_1$ muscarinic acetylcholine receptor (MAChR) is the predominant MAChR in the central nervous system and is highly expressed in the neocortex and hippocampus[13,14]. Pre-clinical studies suggest $M_1$ agonists can improve cognitive function including learning and memory[15-19]. Consistent with this evidence, muscarinic receptor agonists including the $M_1/M_4$ agonist Xanomeline and the $M_1$ agonist GSK1034702 have shown promising early clinical effects[20,16]. Xanomeline showed improvement in global cognitive function (i.e ADAS-COG), general clinical status (i.e. CIBIC+), and behavioural symptoms such as delusions, hallucinations,

agitation[20]. Similarly, the $M_1$ agonist GSK1034702 was shown to improve episodic memory (international shopping list task of the Cogstate battery) in a nicotine abstinence model of cognitive dysfunction[16].

HTL0018318 ((ethyl (3-endo)-3-(3-oxo-2,8-diazaspiro[4.5]dec-8-yl)-8-azabicyclo[3.2.1]octane-8-carboxylate hydrochloride), is a partial $M_1$ MAChR agonist that has been developed for the symptomatic treatment of cognitive, behavioural and psychiatric symptoms in dementias including AD and DLB. In pre-clinical studies, HTL0018318 was found to be highly selective for the $M_1$ receptor with an $EC_{50}$ of approximately 100 nM and with two-fold selectivity for the $M_1$ over $M_4$ receptors with no detectable functional agonist activity at human $M_2$ and $M_3$ receptors. Preclinical studies have shown HTL0018318 to reverse scopolamine-induced deficits in passive avoidance learning in rats consistent with pro-cognitive effects reported with other $M_1$ agonists on tests of learning and memory (Congreve M, Brown AJH, J C.: Identification of novel muscarinic $M_1$ agonist HTL0018318 using structure based drug design, in preparation). The single ascending dose (SAD) study with HTL0018318 has shown that single doses of HTL0018318 up to 35 mg were relatively well-tolerated in healthy younger adult and elderly subjects[21]. HTL0018318 was absorbed rapidly, peak plasma concentration was typically reached 1-2 hours post-dose and the average elimination half-life was 12-16 hours. Approximately 30 percent of the plasma unbound concentration entered the cerebral spinal fluid. No consistent significant effects on exploratory pharmacodynamic (PD) tests were observed. The SAD study was followed by the current multiple-dose escalation study, in which we aimed to investigate the safety, tolerability, pharmacokinetics (PK) and exploratory PD of HTL0018318 in healthy subjects.

## METHODS

**DESIGN** This was a double-blind, randomized, placebo-controlled, parallel group study that consisted of 7 cohorts in total: 3 cohorts of 12 younger adult healthy subjects, and 4 cohorts of 12 healthy elderly subjects. The study design is shown in figure 1. The study was approved by the medical ethics review board of the foundation Beoordeling Ethiek Biomedisch Onderzoek (BEBO, Assen, The Netherlands) and conducted according to the principles of the Declaration of Helsinki and the ICH GCP guidelines[22].

**PARTICIPANTS** Healthy younger adult subjects aged 18-55 years and healthy elderly subjects aged 65 years and over, both male and female, were enrolled to participate in the study. Subjects were eligible if they were non-smokers, in good health, with a resting systolic blood pressure between 90-140 mm Hg, diastolic blood pressure between 50-90 mm Hg and a heart rate between 45-100 bpm at screening. Main exclusion criteria were current or past history of any physical, neurological or psychiatric illness and currently on any medication including antihypertensive drugs.

**INVESTIGATIONAL PRODUCT** HTL0018318, in this study administered as the HCl salt, was administered as a 100 ml oral solution. Water was used as placebo. To mask the difference in taste between HTL0018318 and placebo, a peppermint strip (Listerine) was administered one minute before and after the administration of HTL0018318. Subjects were not asked if they could guess whether they received HTL0018318 or placebo as the study was a parallel group design with taste related unmasking having minimal impact on unblinding.

Dose levels of 15 mg, 20 mg, 25 mg or placebo were administered in a ratio 9:3 (active:placebo) once a day for 10 consecutive days in both younger adult and elderly subjects. The dose level of 35 mg was only studied in a cohort with elderly subjects (8 subjects on HTL0018318 and 4 subjects on placebo) and administered to 3 subjects (including one replacement), however lack of tolerability led to a titration regimen of 20 mg HTL0018318 once a day for 5 consecutive days followed by 35 mg HTL0018318 once a day for 10 consecutive days (7 subjects on HTL0018318 and 3 subjects on placebo). Before implementing this new dosing regimen, the protocol was amended and approved.

HTL0018318 dosages were based on the tolerated dose range of 1-35 mg in the SAD study.

**SAFETY AND TOLERABILITY ASSESSMENTS** The investigated safety end points were adverse events (AEs) collected and recorded on the first dosing day, continuing until the follow-up visit, safety laboratory sampled at regular intervals, vital signs and electrocardiogram (ECG) conducted daily pre-dose and one hour post dose, and with a higher frequency on the 1st, 5th and 10th dosing day, 24-hour Holter registration performed at the 1st and 10th dosing day and pulmonary function tests (PFT). Cholinergically-mediated AEs (i.e. AEs with a (possible) relationship to increased cholinergic stimulation) were identified. AEs in this category included; hyperhidrosis, salivary hypersecretion, hypertension, nausea, diarrhoea, vomiting, constipation, insomnia, somnolence, dizziness, muscle spasms, hot flush, cold sweat and piloerection.

**PHARMACOKINETICS ASSESSMENTS** Venous blood samples for PK analysis were obtained pre-dose, 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6h, 8 h, 9 h, 12 h

post dose on dosing day 1 and 10. On dosing day 5 blood PK samples were obtained pre-dose and at 1 h, 3 h, 6 h and 9 h post dose. On the remaining days only trough samples were taken. Urine for PK analyses was collected up to 24 hours after the first drug administration and up to 72 hours after the last drug administration. In subjects who were administered 35 mg HTL0018318 according to the up-titration schedule, extra PK samples were collected pre-dose on each day and 1, 3, 6 and 9 hours after the 5th administration of 20 mg. Plasma and urine concentrations of HTL0018318 were determined using validated bioanalytical methods involving protein precipitation and liquid chromatography coupled with tandem mass spectrometry. The analytical range of the assay was 0.5–1000 ng/ml.

PK parameters included in the analysis were the maximum observed plasma concentration ($C_{max}$), time to $C_{max}$ ($T_{max}$), area under the plasma-concentration-time curve (AUC) zero to the last measurement (AUClast), from zero to the end of the dose interval ($AUC_{0\text{-}tau}$), from zero to infinity ($AUC_{0\text{-}inf}$); time of the minimum concentration (tlast), the minimum concentration within the dosing interval ($C_{min}$), apparent elimination half-life ($t_1/_2$), apparent oral clearance (CL/F), apparent volume of distribution (Vz/F), renal clearance (CLr) and percentage of dose excreted renally as unchanged drug (Ae%), and coefficient of variation (%CV). Non-compartmental analyses were performed on the PK data using Phoenix 64 build 6.4.0.768 using WinNonlin 6.4. Statistical analysis was performed in R version 3.3.1 (2016-06-21).

**EXPLORATORY PHARMACODYNAMIC ASSESSMENTS** To assess the acute effects of HTL0018318 on central nervous system (CNS) functioning, exploratory PD tests were performed with use of the NeuroCart, a test battery assessing a wide range of CNS domains, developed to examine the acute PD effects of CNS-active drugs and previously shown to be sensitive to cholinergic modulation[23-27]. A customized set of tasks to detect PD effects to be expected from a cholinergic drug was performed pre-dose, 1 h, 3 h, 5 h and 9 h post dose on dosing day 1, 5 and 10. On dosing day 1 the pre-dose measurements were performed twice.

The following NeuroCart tests were performed: the adaptive tracking test measured attention and visuomotor coordination, the Milner maze test (MMT) was used to evaluate spatial working memory, learning and executive function, the n-back task was used to assess (short-term) working memory, pupil size was measured to monitor any drug effects on the sympathetic nervous system, synaptic/network activity was assessed using electrophysiology and included resting electroencephalogram (EEG) (power in delta, theta, alpha, beta and gamma bands) and event-related potentials (ERP) (P300 and Mismatch negativity (MMN)), and a visual analogue scale (VAS) according to Bond and Lader was used to subjectively assess alertness, mood and calmness and a VAS nausea was used to evaluate subjective nausea. The Leeds Sleep Evaluation Questionnaire (LSEQ) was used to assess changes in sleep quality. Detailed task descriptions are provided in the supplement methods section.

Saliva production was assessed by measuring the change in weight of three Salivette® dental rolls put into the oral cavity for 3 minutes. Pulmonary function was measured using the Spirostik (distributed by Accuramed), a PC-based open spirometry system. Vital signs were also analysed.

**STATISTICS** No formal hypothesis testing was conducted. The sample size was chosen based on a compromise between minimizing the exposure of human subjects to a new chemical entity and the need to provide sufficient data. Hence the study was not powered to detect any significant treatment effects of small to moderate effect sizes.

The repeatedly measured PD endpoints on dosing days 1, 5 and 10 were analysed with a mixed model analysis of covariance (ANCOVA) with treatment, time, treatment by time, group, treatment by group, group by time and treatment by group by time as fixed factors and subject as random factor and the average baseline measurement as covariate. Least-square means (LSMS) and 95% confidence intervals (CIS) were estimated from the ANCOVA models. The repeatedly measured PD parameters of the subjects dosed 35 mg according to an up-titration schedule were analysed together with the placebo subjects of the other elderly cohorts to increase the power. The data after the up-titration was analysed with a mixed model ANCOVA with treatment, time and treatment by time as fixed factors, subject as random factor and the average baseline measurement before the up-titration as covariate. All subjects who received at least one dose of study treatment were included in the safety and PD analysis set. PD data of the two subjects who were administered 35 mg HTL0018318 not according to an up-titration schedule were not analysed. The following contrasts were calculated for dosing day 1, 5 and 10 separately for every dose level: HTL0018318 (younger adults + elderly subjects) vs placebo (all placebo subjects pooled together); HTL0018318 (younger adult subjects) vs placebo (younger adult placebo subjects pooled together); HTL0018318 (elderly subjects) vs placebo (elderly placebo subjects pooled together)

For all outcome parameters the mean, standard deviation, 95% CI and effect sizes were calculated. All calculations were performed using SAS for windows V9.4 (SAS Institute, Inc., Cary, NC, USA). MMN data were excluded from statistical analysis due to limited data quality and technical issues with stimuli timing and recording. Hence only resting state EEG power data and P300 data were reported here.

## RESULTS

**SUBJECTS** A total of 36 healthy younger adult subjects with a mean age of 30 years (range 18-53) and with a mean body mass index (BMI) of 23.7 kg/m$^2$ (range 18-31) were enrolled. A total of 50 healthy elderly subjects, with a mean age of 69.9 years (range 65-83) and with a mean BMI of 25.9 kg/m$^2$ (range 20.5-32.5) were included. See figure 2 for subject disposition flow chart. The AEs leading to withdrawal of six subjects are described below. In one elderly subject the third dose of 25 mg HTL0018318 was not administered due to infection-like symptoms and elevated c-reactive protein. The subject subsequently recovered spontaneously and dosing was resumed.

**SAFETY AND TOLERABILITY** Multiple doses of 15, 20 and 25 mg HTL0018318 were generally well-tolerated by healthy younger adult and elderly subjects. The dose level of 35 mg without up-titration period was not tolerated by elderly subjects. The 2 subjects dosed with 35 mg without up-titration period were withdrawn from the study due to AEs (hypertension and cold sweat) after the 1$^{st}$ or 2$^{nd}$ administration of HTL0018318. Consequently, it was decided to stop dosing 35 mg without an up-titration period in the remaining subjects of this cohort, and to add an up-titration period preceding the 35 mg doses. This was relatively well-tolerated with only mild AEs and no withdrawn subjects. Overall, more subjects dosed with HTL0018318 reported AEs compared to subjects dosed with placebo (table 1 and 2). In younger adults, the number of subjects reporting AEs in general and the number of cholinergically-mediated AEs appeared to be treatment-related. However, no clear dose-response relationship was observed. In elderly, the number of subjects reporting AEs in general and the number of cholinergically-mediated AEs appeared to be treatment- and dose-related.

The most frequently occurring cholinergically-mediated AEs were nausea, hyperhidrosis, chills, cold sweat, somnolence and feeling cold (see table 1 and 2).

In total 6 subjects were withdrawn from the study because of AEs. One elderly subject experienced severe cold sweats and chills after 1 administration of 35 mg HTL0018318 without up-titration period. One elderly subject was withdrawn from the study after 2 administrations of 35 mg HTL0018318 without up-titration period due to hypertension (supine systolic blood pressure of 168/84 mm Hg, increase of >40% from baseline). One elderly subject was withdrawn after 1 administration of 20 mg during the period preceding the 35 mg dose due to a 40% increase of supine blood pressure to 196/99 mg Hg compared to baseline (140/62 mm Hg). One subject was withdrawn from further participation after 6 administrations of 25 mg HTL0018318 due to elevated liver enzymes AST (76 U/L) and ALT (127 U/L). The AEs of these 4 subjects were considered to be related to the study drug. One younger adult subject was withdrawn after 4 administrations of 25 mg HTL0018318 because of episodes of bradycardia down to 38 bpm in combination with nausea and fatigue. These episodes of bradycardia were also observed on the 24-hour Holter monitoring which was part of the screening and were therefore not considered to be drug-related. One elderly subject was withdrawn because of orthostatic hypotension 24 hours after the first 25 mg administration. The supine blood pressure decreased from 106/48 mm Hg to 66/37 mm Hg. As there was no clear relation to peak plasma HTL0018318 concentration, this AE was not considered to be related to the study drug.

No consistent clinically relevant abnormalities in haematology blood results, urinalysis, ECGs and 24-hour Holter monitoring were observed in both younger adult and elderly subjects. No serious AEs or deaths occurred. There were no chemistry blood results that showed an apparent trend toward increased incidence with ascending dose levels of HTL0018318 during the study.

**PHARMACOKINETIC PARAMETERS** The PK parameters and mean concentration-time profiles of HTL0018318 are shown in Figure 3 and 4. These figures shows HTL0018318 arithmetic mean plasma concentration against time of 10 daily oral doses of 15, 20 or 25 mg for younger adult subjects (Figure 3) and additionally mean (± SD) plasma concentrations of 35 mg in elderly subjects (Figure 4). The absorption was rapid with a median $T_{max}$ of 1 hour post-dose (range 0.5 to 4 hours). The oral PK profile was biphasic after $C_{max}$. The mean (SD) apparent terminal half-life in healthy subjects was 16.1 h (± 4.61) in younger adult subjects and 14.3 h (± 2.78 h) in elderly subjects determined up to 72 hours post-dose. A mean accumulation index of 1.32 was seen in younger adult and 1.29 in elderly subjects following the 10$^{th}$ dose, in terms of $AUC_{0\text{-}24}$. Steady state was reached in approximately two or three doses. The ratio between the mean multiple dose $AUC_{0\text{-}tau}$ to the $AUC_{0\text{-}inf}$ after the 1$^{st}$ dose was 1.06 following the 5$^{th}$ and 1.14 following the 10$^{th}$ dose in younger adults and 1.03 following the 5$^{th}$ and 1.07 following the 10$^{th}$ dose in elderly subjects. On average, elderly subjects appeared to have lower oral clearance than the younger adults (mean (SD) CLss/F 18.3 (± 6.68) L/h in younger adult and 16.0 (± 6.79) L/h in elderly subjects). The inter-individual variability in $C_{max}$, AUC and t½ was moderate, with a %CV typically < 30 and not larger than 66 for any variable.

Exposure to HTL0018318 was dose-proportional over the range 15 to 35 mg. Exposure in elderly subjects given 20 mg was higher than expected, however dose-exposure proportionality did not deviate from linearity assessed using the power model[28]. The reason for higher exposure at 20 mg in elderly subjects could not be determined.

Renal elimination was a major route of clearance. The mean percentage recovery of HTL0018318 over the 24 h dose interval following the 10th dose was 58.1 % in younger adults (range 22.3 to 95.3) and 50.4 % in elderly subjects (range 25.8 to 95.7 %), which represents the minimum absolute oral bioavailability. The mean renal clearance was 8.62–8.84 L/h in younger adults and 6.03–6.23 L/h in elderly subjects.

**CENTRAL PHARMACODYNAMIC BIOMARKERS** HTL0018318 daily dosing for 10 days showed no consistent effects on EEG/ERP, saliva production, LSEQ, pupil size or VAS scores compared to placebo (table in result supplement). Although the study was not powered to detect small to moderate pro-cognitive effects of HTL0018318, some significant differences compared to placebo were observed on a number of cognitive tests including adaptive tracking (a measure of psychomotor function and attention), the n-back test (a measure of working memory) and the Milner Maze Test (a measure of learning and memory).

- **Adaptive tracking test**

Overall, HTL0018318 had no significant effects on the adaptive tracking test in young and elderly subjects across doses and testing days. However, after administration of 20 mg HTL0018318 the time correctly tracked was improved by 3.605 %-point (95% CI [0.672–6.539], p=0.0167) compared with placebo, on dosing day 1 in the elderly subjects (see Figure 5, data shown as estimate of the change from baseline performance.).

- **N-back test**

Overall, an improvement in performance in both younger adult and elderly subjects on the n-back test (0, 1 and 2-back conditions) was observed following administration of all dose levels of HTL0018318 compared with placebo. The effect on the performance on the most relevant 2-back working memory "accuracy" measure is reported here. In all observations, a higher (number correct-number incorrect)/total was observed indicating better performance. See supplement results section for data on the 0-back and 1-back (accuracy and reaction time) and 2-back (reaction time) conditions.

After administration of 15 mg HTL0018318 the 2-back accuracy score was 0.076 higher on dosing day 1 (95% CI [0.028–0.125], p=0.0022) and 0.069 higher on dosing day 10 (95% CI [0.020–0.118], p=0.0058) compared with placebo. Following administration of 20 mg HTL0018318, the 2-back accuracy score was 0.108 higher on dosing day 1 (95% CI [0.059–0.157], p=<.0001), 0.068 higher on dosing day 5 (95% CI [0.019–0.117], p=0.0073) and 0.066 higher on dosing day 10 (95% CI [0.016–0.115], p=0.0095), compared with placebo. After administration of 25 mg HTL0018318 the 2-back accuracy score was 0.068 higher on dosing day 1 (95% CI [0.020–0.117], p=0.0063) compared with placebo. These data were analysed separately for younger adult and elderly subjects. This is presented in table 1 and figure 6.

- **Milner Maze Test**

Overall, administration of HTL0018318 in both younger adult and elderly subjects was associated with a reduction in total exploratory errors and total moves (on immediate, reversed and delayed conditions) on the Milner maze test (MMT). There was an overall significant treatment effect of HTL0018318 (including dose level 15 mg, 20 mg, 25 mg and 35 mg) on the performance of the MMT immediate and reversed condition (MMT immediate exploratory error; MMT immediate total moves; MMT reversed exploratory error; MMT reversed total moves). The data from exploratory errors are reported here. See supplement results section for data on the total moves outcome measure which was generally consistent with the data on exploratory errors for immediate and delated conditions. Overall, HTL0018318 had no significant or consistent effects on exploratory errors in the Milner Maze delayed condition across doses and days in young and elderly subjects. However selective effects were observed (see supplementary results).

- **MMT IMMEDIATE: EXPLORATORY ERRORS**

HTL0018318 had some significant and consistent effects on exploratory errors in the Milner maze test at the 15 mg 20 mg, 25 mg and 35 mg doses across days, particularly in the elderly subjects. In all observations, a lower number of errors were observed indicating better performance

Administration of 15 mg HTL0018318 was associated with 3.6 fewer exploratory errors on dosing day 1 (95% CI [-7.1–-0.2], p=0.0380), 6.7 fewer exploratory errors on dosing day 5 (95% CI [-10.1–-3.2], p=0.0002) and 4.8 fewer exploratory errors on dosing day 10 (95% CI [-8.2–-1.3], p=0.0073), compared with placebo. Following 20 mg HTL0018318, 3.6 fewer exploratory errors were observed on dosing day 5 (95% CI [-7.1–-0.1], p=0.0460), compared with placebo. No significant effect was observed after administration of 25 mg HTL0018318 on the number of exploratory errors compared with placebo. Data were analysed separately for younger adult and elderly subjects. These are presented in table 2 (HTL0018318 compared to placebo, results expressed in exploratory errors), and figure 7.

- **MMT REVERSED: EXPLORATORY ERRORS**

Overall, HTL0018318 had some significant and consistent effects on exploratory errors in the Milner maze test at the 15 mg 20 mg, 25 mg and 35 mg doses across days,

in younger adult and elderly subjects. In all observations, a lower number of errors were observed indicating better performance. Administration of 15 mg HTL0018318 was associated with 1.7 fewer exploratory errors on dosing day 1 (95% CI [-2.9–-0.4], p=0.0086), compared with placebo. After 20 mg HTL0018318 1.4 fewer exploratory errors were observed on dosing day 1 (95% CI [-2.7–-0.2], p=0.0275), compared with placebo. Administration of 25 mg HTL0018318, was associated with 2.1 fewer exploratory errors on dosing day 1 (95% CI [-3.3–-0.9], p=0.0011) and 1.7 fewer exploratory errors on dosing day 10 (95% CI [-2.9–-0.4], p=0.0119), compared with placebo. The MMT reversed condition data was analysed separately for younger adult and elderly subjects. This is presented in table 3 (HTL0018318 compared to placebo, results expressed in exploratory errors), and figure 8.

- **EEG/ERPS**

In general, HTL0018318 had no consistent effects on EEG power. For several EEG bands some statistically significant effects in subjects treated with HTL0018318 compared with placebo were observed, however, these were no consistent across treatment, electrode position or days of treatment. Similarly, there was not consistent effect of HTL0018318 on P300 amplitude or latency, although a significant improvement in P300 amplitude was noted with the 20 mg dose in the elderly on dosing day 1 (mean difference of 3.670 μ V, 95% CI [0.554–6.786], p=0.0222).

**OTHER PHARMACODYNAMIC BIOMARKERS: BLOOD PRESSURE AND PULSE RATE** Systolic and diastolic blood pressure were not consistently higher or lower in the subjects treated with HTL0018318 than in the placebo subjects. However, some doses of HTL0018318 did demonstrate statistically significant differences versus placebo at some time points. The magnitude and direction of change in blood pressure following HTL0018318 treatment was similar for younger adult and elderly subjects. The statistically significant differences in blood pressure on dosing day 1 were relative increases and on dosing day 5 and 10 were relative decreases compared to placebo. This pattern was consistent for supine and standing systolic blood pressure and diastolic blood pressure, but with more significant effects being noted on diastolic blood pressure than on systolic blood pressure.

Mean systolic blood pressure increased up to 8.7 mm Hg (95% CI[1.6, 15.8], p=0.0116, dosing day 1, 25 mg in younger adults) and in mean diastolic blood pressure up to 7.0 mm Hg (95% CI [2.4, 11.7], p=0.0036, dosing day 1, 15 mg in elderly subjects) (Figure 8a and 8b, results shown as estimate of the change from baseline.). No evidence of a dose response was observed.



There was a statistically significantly higher pulse rate for the majority of treatment groups versus placebo across duration of dosing, particularly in supine pulse rate and in elderly subjects. The maximum increase in mean supine pulse rate was 10 bpm (95% CI [4.7–15.3], p=0.0007, dosing day 5 of 35 mg regimen by up-titration). Similar results were observed in standing pulse rate. See supplement results section for data on systolic and diastolic blood pressure and pulse rate.

## DISCUSSION

We previously reported the safety and tolerability of HTL0018318 following ascending doses in healthy subjects[21]. In this study we report the safety and tolerability of HTL0018318 following multiple ascending dosing over ten days in healthy younger adult and elderly subjects. We also report exploratory PD effects on biomarkers of cognitive function. Overall, HTL0018318 was generally well tolerated at the doses tested and there was some evidence for pro-cognitive effects, particularly on tests of short term (working) memory and learning.

Systemic exposure of HTL0018318 showed dose-proportional increases and reproducible PK in the 15-35 mg dose range. The plasma concentrations of HTL0018318 reached a maximum typically 1-2 hours post-dose and the apparent half-life was approximately 16 hours in younger adult subjects and 14 hours in elderly subjects. Elimination of unchanged drug in urine was a major pathway with renal clearance being similar to the age-adjusted glomerular filtration rate. PK characteristics were expected based on the results of the SAD study[21]. Overall, these data suggest that HTL0018318 has a PK profile consistent with a once daily regimen with no clear PK differences between healthy younger adult and elderly subjects.

Multiple doses of HTL0018318 up to 25 mg were well-tolerated and associated with mild and moderate treatment-related cholinergically-mediated AEs (reported subjectively) in healthy younger adult and elderly subjects. The highest dose level of 35 mg tested in 2 participants without an up-titration period was not tolerated by elderly subjects, however, this dose was generally well tolerated with the dose titration regimen. In the SAD study, the severity of the AEs was lower, although in the SAD study 3 of the 9 elderly subjects dosed with 35 mg had an increase in blood pressure and more cholinergically-mediated AEs compared with other dose levels, suggesting a lower tolerability at this high dose.

Clinically-relevant hypertension (an increase of >40% compared to the baseline measurement or a blood pressure >180/115 mm Hg) occurred in 3 elderly subject following 20 mg, 25 mg or 35 mg without up-titration, which is comparable to the 5 (out

of 57) elderly subjects presenting with increased blood pressure in the SAD study[21]. The observed increases in systolic blood pressure of up to 4.6 mm Hg in the elderly and up to 8.7 mm Hg in the younger adult subjects in the current study and increases up to 11.9 mm Hg in the SAD study were both modest increases and showed no dose-dependency. The mean supine pulse rate was significantly higher in the majority of treatment groups in the current study (up to 9.6 bpm in the elderly and up to 7.5 bpm in the younger adult subjects) and some evidence for dose dependency. However, it should be noted that there was a reduction in pulse rate post-dose in the placebo treated participants and therefore the higher pulse rate in the HTL0018318 groups demonstrated less of a reduction (relative to placebo) rather than an increase in pulse rate from baseline with HTL0018318 treatment. While the exact mechanisms associated with the blood pressure and pulse rate changes are not known it is possible that this may be related to $M_1$ mediated modulation of postganglionic sympathetic neurons that innervate the heart[29]. Additionally, there appeared to be a decrease of the cholinergic side effects following repeated dosing of HTL0018318. For example, the increases in blood pressure seen on dosing day 1 attenuated over time (See Figure 8), suggesting that there may tolerance to the blood pressure increases with repeat dosing. This phenomenon probably contributes to the better tolerance of 35 mg HTL0018318 preceded by the up-titration period compared to 35 mg HTL0018318 without up-titration period.

Central PD effects were assessed with a range of cognitive tasks probing psychomotor function/attention, working memory and learning as well as electrophysiological biomarkers including P300, a marker of attention and working memory updating. In general, there were no consistent effects on the electrophysiological biomarkers, although this is likely to have been due to poor quality of data and contamination of the P300 data due a voltage from the trigger pulses leading to high variability. Furthermore, many data sets had to be partially or fully removed due to the artefact (or missing data). This reduced the usable sample considerably reducing the statistical power of the analysis. Hence, these data should be interpreted with caution when interpreting central PD effects measured with EEG/ERP biomarkers including P300.

HTL0018318 over 10 days of treatment was associated improvements in number of cognitive tests including adaptive tracking in elderly subjects (a measure of psychomotor function and sustained attention), the n-back test in both younger adults and elderly subjects (a measure of working memory) and the MMT in elderly subjects (a measure of learning and memory). Overall, HTL0018318 had more consistent effects across cognitive domains in the elderly compared to younger adults, although the study was not adequately powered to investigate differential effects between the



two groups. The magnitude of effects on adaptive tracking (i.e. 3.6%-point improvement) was comparable to that previously reported with donepezil (10 mg) in healthy subjects[23] but were only observed at the 20 mg dose and only on dosing day 1 in elderly subjects suggesting that effects on psychomotor speed and sustained attention were not robust and consistently modulated by $M_1$ receptor modulation with HTL0018318. This is consistent with the lack of effects we previously reported with the $M_1$ agonist HTL0009936 on adaptive tracking performance[30]. It is possible that cholinergic and $M_1$ receptor modulation of attentional processing may depend on "attentional effort" or activation of attentional systems by motivation, particularly in the face of challenges such as distractors where a high level of attentional control is needed[31]. In this context, the adaptive tracking task may have not been challenging enough to require sufficient attentional effort for $M_1$ activation to modulate performance. The effects on tests of memory (n-back and MMT) were however more consistent in younger adult and elderly subjects across doses and over the 10 days of treatment with clinically relevant effects of moderate to large effect sizes. These effects in healthy normal subjects (presumably with minimal cholinergic dysfunction) is encouraging and may suggest $M_1$ receptor modulation may have significant effects on learning and memory in disorders of cholinergic dysfunction such as AD and other dementias. The n-back test is a working memory test associated with prefrontal function[32,33], while the MMT is a learning and memory test associated with hippocampal function[34]. Both the prefrontal cortex and hippocampus are areas rich in muscarinic $M_1$ receptors[35,13]. The sensitivity of these tests to muscarinic (and $M_1$) receptor modulation is supported by previous studies with the non-selective muscarinic antagonist scopolamine and the $M_1$ antagonist biperiden which have been shown to impair performance on tests comparable to the n-back test (45, 46) and MMT (47). The findings of the current study demonstrating positive effects of HTL0018318 on tests of short-term memory and learning are also consistent with the pre-clinical[36,37] and clinical studies[16,38] that have similarly shown improvements tests of learning and memory with selective $M_1$ receptor agonists. These findings, while pre-liminary, provide encouraging data in support of the development of HTL0018318 for cognitive dysfunction in AD and other dementias.

The effects of HTL0018318 was also examined on other PD markers including saliva production, LSEQ, pupil size or VAS scores, but overall no significant changes were observed (table in result supplement). While our data showed no effects on saliva production, hypersalivation was observed in other studies investigating other less selective $M_1$ MAChR agonists[16,39-41] and could be explained by their relatively small effects on the $M_3$ receptors[41]. The observation in the current study confirms the selectivity of HTL0018318 for muscarinic $M_1$ receptors.

**LIMITATIONS** There are some limitations of the study that warrant discussion. This study was primarily a safety and tolerability study and the PD measurements were exploratory. As such, these data need to be interpreted with caution, given the small sample size and the lack of power in the study to detect pro-cognitive effects of small to moderate magnitude. While effect sizes were calculated to nuance the PD results calculated by the statistic model (table in result supplement), it is possible that the small sample size could over- or underestimate the pro-cognitive effects of HTL0018318. As discussed above, the EEG/ERP were of poor data quality driven by a voltage noise from the trigger pulses leading to high variability and significant loss of data. Hence, no definite conclusions can be made with regard to the absence of effects of HTL0018318 on the EEG and ERP biomarkers of cognitive function.

## CONCLUSIONS

In conclusion, HTL0018318 was generally well-tolerated in multiple doses up to 25 mg/day and dosed up to 10 days (in adult and elderly subjects) or up to 15 days according to a titration regimen of 20 mg/day for 5 days followed by 35 mg/day for 10 days in elderly subjects. The multiple dose PK of HTL0018318 were well-characterized. Treatment related AEs including cholinergically-mediated AEs were mild and transient. Modest changes in blood pressure were observed after the first dose administration, which returned to normal after multiple doses. Consistent and pro-cognitive effects of moderate to large magnitude on short-term memory and learning were demonstrated across the dose range over the 10 days of treatment providing encouraging data in support of the development of HTL0018318 for cognitive dysfunction in dementias.



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**TABLE 1** **Overview of (potentially cholinergically mediated) AEs reported by younger adult subjects.**

| MedDRA preferred term | Placebo n=9 | 15 mg n=9 | 20 mg n=9 | 25 mg n=9 | All HTL0018318 n=27 |
|---|---|---|---|---|---|
| All AEs | 5 ( 55.6) | 8 ( 88.9) | 6 ( 66.7) | 6 ( 66.7) | 20 (74.1) |
| All cholinergic AEs | 1 (11.1) | 5 (55.6) | 5 (55.6) | 6 (66.7) | 16 (59.3) |
| Hypertension | 0 | 0 | 0 | 0 | 0 |
| Nausea | 0 | 3 ( 33.3) | 2 ( 22.2) | 4 ( 44.4) | 9 (33.3) |
| Diarrhoea | 0 | 1 ( 11.1) | 1 ( 11.1) | 0 | 2 (7.4) |
| Vomiting | 0 | 0 | 0 | 1 ( 11.1) | 1 (3.7) |
| Hypersalivation | 0 | 0 | 0 | 1 (11.1) | 1 (3.7) |
| Hyperhidrosis | 0 | 1 ( 11.1) | 4 ( 44.4) | 3 ( 33.3) | 8 (29.6) |
| Constipation | 0 | 0 | 0 | 0 | 0 |
| Chills | 0 | 1 ( 11.1) | 0 | 0 | 1 (3.7) |
| Cold sweat | 0 | 0 | 0 | 0 | 0 |
| Feeling cold | 0 | 0 | 1 ( 11.1) | 0 | 1 (3.7) |
| Feeling hot | 0 | 0 | 1 (11.1) | 0 | 1 (3.7) |
| Feeling of body temperature change | 0 | 0 | 0 | 0 | 0 |
| Hot flush | 0 | 0 | 1 (11.1) | 2 (22.2) | 3 (11.1) |
| Piloerection | 0 | 0 | 2 (22.2) | 0 | 2 (7.4) |
| Peripheral coldness | 0 | 0 | 0 | 0 | 0 |
| Insomnia | 1 (11.1) | 0 | 0 | 0 | 0 |
| Dizziness | 0 | 0 | 1 (11.1) | 1 (11.1) | 2 (7.4) |
| Muscle spasm | 0 | 1 (11.1) | 0 | 0 | 1 (3.7) |

*Data are shown as number (percentage) of subjects reporting AEs.*

TABLE 2 Overview of (potentially cholinergically mediated) AEs reported by elderly subjects.

| MedDRA preferred term | Placebo n=14 | 15 mg n=9 | 20 mg n=9 | 25 mg n=9 | 35 mg n=2 | 35 mg + up-titration n=7 | All HTL0018318 n=36 |
|---|---|---|---|---|---|---|---|
| All AEs | 8 (57.1) | 7 (77.8) | 7 (77.8) | 9 (100.0) | 2 (100.0) | 7 (100.0) | 32 (88.9) |
| All cholinergic AEs | 2 (14.3) | 1 (11.1) | 5 (55.6) | 7 (77.8) | 2 (100) | 7 (100) | 22 (61.1) |
| Hypertension | 0 | 0 | 0 | 1 | 1 | 1 | 3 (8.3) |
| Nausea | 0 | 1 (11.1) | 1 (11.1) | 2 (22.2) | 0 | 0 | 4 (11.1) |
| Diarrhoea | 0 | 0 | 0 | 1 (11.1) | 0 | 0 | 1 (2.8) |
| Vomiting | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Constipation | 1 (7.1) | 0 | 0 | 0 | 0 | 0 | 0 |
| Hypersalivation | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hyperhidrosis | 0 | 0 | 3 (33.3) | 5 (55.6) | 0 | 2 (28.6) | 10 (27.8) |
| Chills | 0 | 0 | 0 | 1 (11.1) | 0 | 4 (57.1) | 5 (13.9) |
| Cold sweat | 0 | 0 | 0 | 0 | 1 (50.0) | 2 (28.6) | 3 (8.3) |
| Feeling cold | 1 (7.1) | 0 | 1 (11.1) | 2 (22.2) | 1 (50.0) | 2 (28.6) | 6 (16.7) |
| Feeling hot | 0 | 0 | 0 | 2 (22.2) | 0 | 0 | 2 (5.6) |
| Feeling of body temperature change | 0 | 0 | 0 | 1 (11.1) | 0 | 0 | 1 (2.8) |
| Hot flush | 0 | 0 | 2 (22.2) | 1 (11.1) | 0 | 2 (28.6) | 5 (13.9) |
| Peripheral coldness | 0 | 0 | 0 | 0 | 1 (50.0) | 0 | 1 (2.8) |
| Piloerection | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Insomnia | 0 | 0 | 1 | 0 | 0 | 0 | 1 (2.8) |
| Dizziness | 1 | 0 | 0 | 3 | 0 | 0 | 3 (8.3) |
| Muscle spasm | 0 | 0 | 1 | 0 | 0 | 0 | 1 (2.8) |

*Data are shown as number of subjects reporting adverse events (percentage of subjects).*



TABLE 3 Effects on the accuracy of the 2-back performance compared with placebo.

| | Younger adults | | | Elderly | | |
|---|---|---|---|---|---|---|
| Parameter | Day 1 | Day 5 | Day 10 | Day 1 | Day 5 | Day 10 |
| **15 mg HTL0018318** | | | | | | |
| N-back corr-incorr/total 2 | 0.079 (0.009, 0.148) **p=0.0265** ES=1.06 | 0.074 (0.005, 0.143) **p=0.0361** ES=1.00 | 0.102 (0.033, 0.172) **p=0.0041** ES=1.38 | 0.074 (0.007, 0.142) **p=0.0318** ES=1.00 | 0.008 (-0.060, 0.076) p=0.8070 ES=0.11 | 0.036 (-0.033, 0.105) p=0.3016 ES=0.49 |
| **20 mg HTL0018318** | | | | | | |
| N-back corr-incorr/total 2 | 0.070 (0.001, 0.140) **p=0.0476** ES=0.95 | 0.059 (-0.010, 0.129) p=0.0947 ES=0.80 | 0.081 (0.011, 0.151) **p=0.0237** ES=1.09 | 0.145 (0.077, 0.213) **p=<.0001** ES=1.96 | 0.076 (0.008, 0.145) **p=0.0299** ES=1.03 | 0.051 (-0.019, 0.120) p=0.1503 ES=0.68 |
| **25 mg HTL0018318** | | | | | | |
| N-back corr-incorr/total 2 | 0.027 (-0.042, 0.097) p=0.4337 ES=0.37 | 0.033 (-0.038, 0.103) p=0.3640 ES=0.44 | 0.073 (0.002, 0.144) **p=0.0436** ES=0.99 | 0.109 (0.041, 0.177) **p=0.0020** ES=1.47 | 0.029 (-0.042, 0.099) p=0.4243 ES=0.38 | 0.027 (-0.047, 0.101) p=0.4690 ES=0.37 |
| **(20+35mg) HTL0018318** | | | | | | |
| N-back corr-incorr/total 2 | | | | 0.061 (0.030, 0.152) p=0.1846 ES=0.67 | 0.030 (-0.062, 0.122) p=0.5144 ES=0.33 | 0.009 (-0.083, 0.101) p=0.8429 ES=0.10 |

*Mean estimated difference (95% CI), p-value, effect size*

TABLE 4 **Effects of HTL0018318 on the performance of the Milner maze test immediate condition.**

| | Younger adults | | | Elderly | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Day 1** | **Day 5** | **Day 10** | **Day 1** | **Day 5** | **Day 10** |
| **15 mg HTL0018318** | | | | | | |
| MMTImm: Expl Error | -1.2 (-6.1, 3.7) p=0.6303 ES=0.23 | -1.8 (-6.7, 3.1) p=0.4678 ES=0.34 | -2.6 (-7.5, 2.4) p=0.3050 ES=0.49 | -6.1 (-10.8, -1.3) **p=0.0133** ES=1.15 | -11.5 (-16.3, -6.7) **p=<.0001** ES=2.19 | -7.0 (-11.8, -2.1) **p=0.0052** ES=1.32 |
| **20 mg HTL0018318** | | | | | | |
| MMTImm: Expl Error | -0.9 (-5.9, 4.1) p=0.7204 ES=0.17 | -0.4 (-5.4, 4.6) p=0.8637 ES=0.08 | 3.3 (-1.7, 8.3) p=0.1930 ES=0.63 | -3.1 (-7.9, 1.7) p=0.2061 ES=0.59 | -6.7 (-11.5, -1.9) **p=0.0066** ES=1.28 | -4.3 (-9.2, 0.5) p=0.0802 ES=0.83 |
| **25 mg HTL0018318** | | | | | | |
| MMTImm: Expl Error | 2.6 (-2.4, 7.5) p=0.3047 ES=0.49 | 3.6 (-1.4, 8.6) p=0.1565 ES=0.69 | 1.4 (-3.6, 6.4) p=0.5720 ES=0.27 | -4.4 (-9.3, 0.4) p=0.0718 ES=0.84 | -7.7 (-12.6, -2.7) **p=0.0025** ES=1.46 | -4.8 (-9.9, 0.3) p=0.0632 ES=0.92 |
| **(20+35mg) HTL0018318** | | | | | | |
| MMTImm: Expl Error | | | | -8.9 (-15.7, -2.1) **p=0.0118** ES=1.32 | -8.2 (-15.0, -1.4) **p=0.0196** ES=1.22 | -1.6 (-8.4, 5.2) p=0.6317 ES=0.24 |

*Mean estimated difference (95% CI), p-value, effect size*

TABLE 5 **Effects of HTL0018318 on the performance of the Milner maze test reversed condition.**

| | Younger adults | | | Elderly | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Day 1** | **Day 5** | **Day 10** | **Day 1** | **Day 5** | **Day 10** |
| **15 mg HTL0018318** | | | | | | |
| MMTRev: Expl Error | -1.9 (-3.7, -0.1) **p=0.0359** ES=1.00 | -0.5 (-2.2, 1.3) p=0.6108 ES=0.24 | -1.0 (-2.8, 0.8) p=0.2737 ES=0.52 | -1.4 (-3.2, 0.3) p=0.1011 ES=0.76 | -1.9 (-3.6, -0.2) **p=0.0322** ES=1.00 | -0.7 (-2.5, 1.0) p=0.4115 ES=0.38 |
| **20 mg HTL0018318** | | | | | | |
| MMTRev: Expl Error | -1.1 (-2.9, 0.7) p=0.2347 ES=0.57 | -0.0 (-1.8, 1.7) p=0.9592 ES=0.02 | 0.3 (-1.5, 2.1) p=0.7500 ES=0.15 | -1.8 (-3.5, -0.0) **p=0.0487** ES=0.92 | -2.0 (-3.7, -0.2) **p=0.0284** ES=1.03 | -1.3 (-3.0, 0.5) p=0.1593 ES=0.66 |
| **25 mg HTL0018318** | | | | | | |
| MMTRev: Expl Error | -2.2 (-3.9, -0.4) **p=0.0174** ES=1.14 | -0.1 (-1.9, 1.7) p=0.9334 ES=0.04 | -1.4 (-3.2, 0.4) p=0.1219 ES=0.75 | -2.0 (-3.8, -0.3) **p=0.0217** ES=1.08 | -1.7 (-3.5, 0.1) p=0.0577 ES=0.90 | -1.9 (-3.7, -0.1) **p=0.0431** ES=0.99 |
| **(20+35mg) HTL0018318** | | | | | | |
| MMTRev: Expl Error | | | | -2.9 (-5.7, -0.1) **p=0.0457** ES=1.05 | -3.3 (-6.1, -0.5) **p=0.0230** ES=1.20 | -2.6 (-5.4, 0.2) p=0.0718 ES=0.93 |

*Mean estimated difference (95% CI), p-value, effect size*

FIGURE 1 Study design

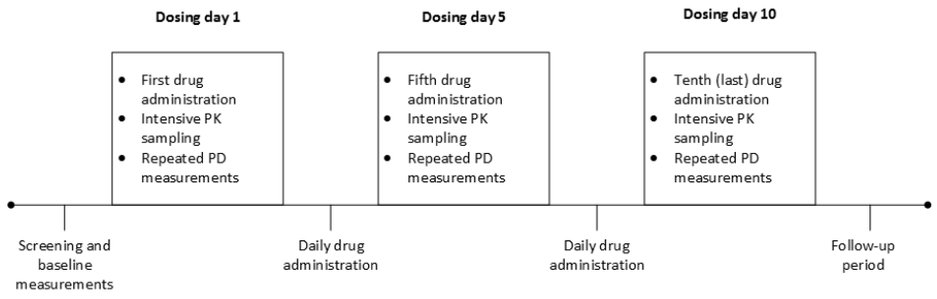





FIGURE 2 Subject disposition flow chart n=number of subjects

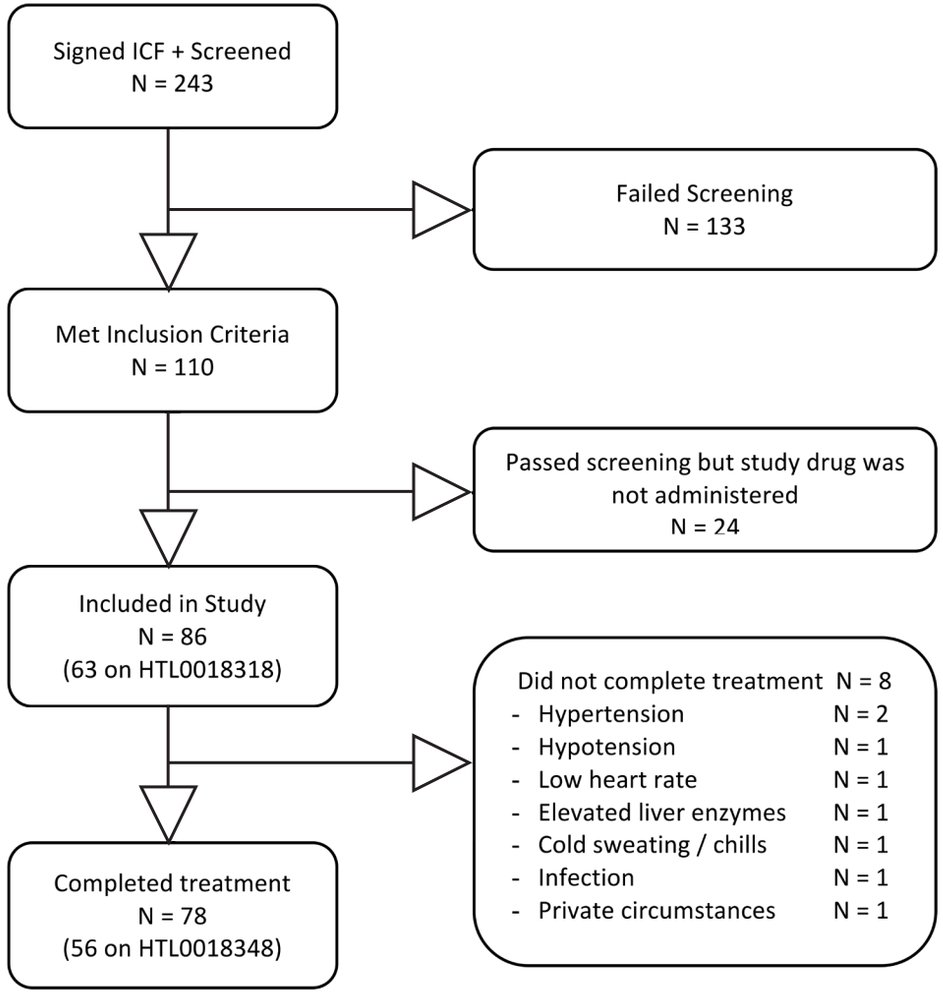


FIGURE 3 HTL0018318 mean ± SD plasma concentration after 15, 20 or 25 mg for younger adult subjects

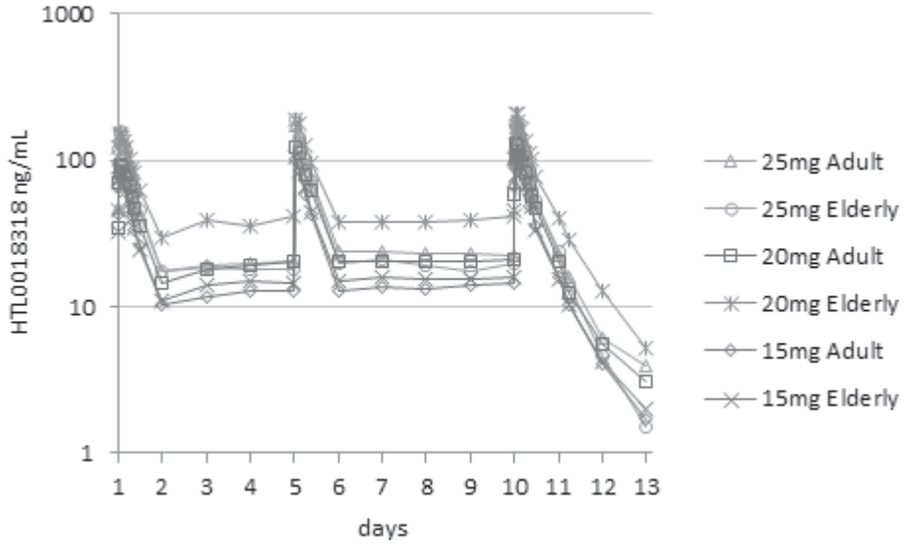



FIGURE 4 HTL0018318 mean ± SD plasma concentration after 15, 20, 25 or 35 mg for elderly subjects

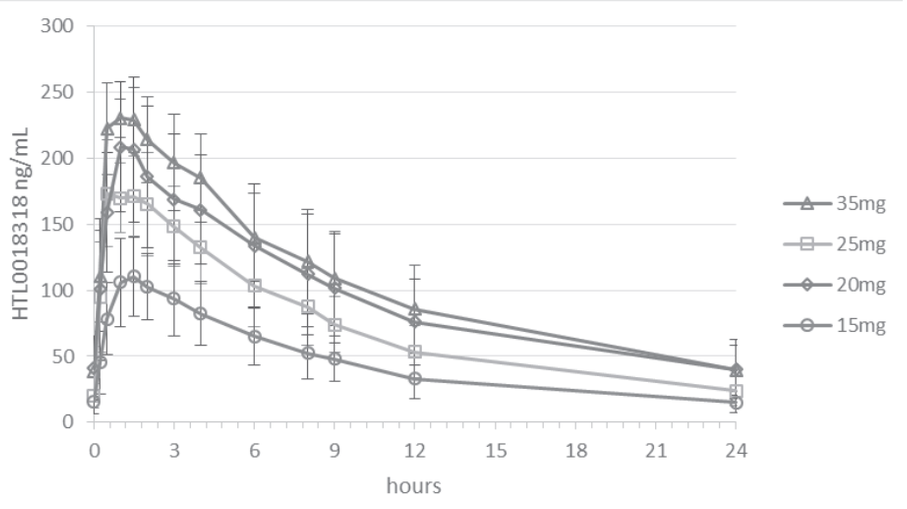





FIGURE 5 Effects on adaptive tracking (% correctly tracked) in younger adults (A) and elderly subjects (B) shown as change from base line (mean, 95% CI error bars)

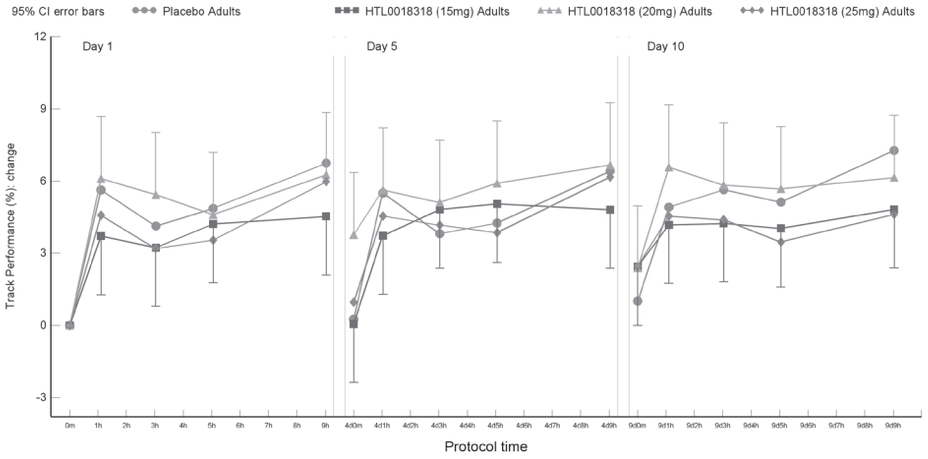



FIGURE 6 Effects on the 2-back accuracy in younger adults (A) and elderly subjects (B) shown as change from base line (mean, 95% CI error bars)

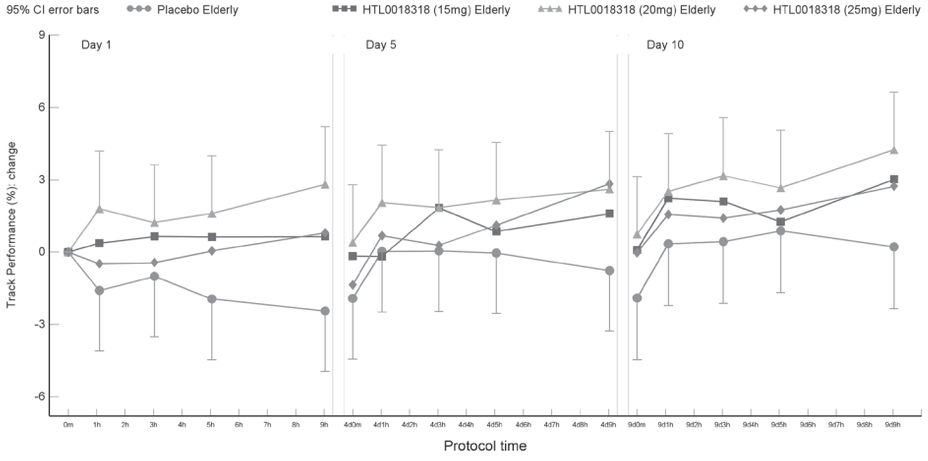


FIGURE 7 Effects on the MMT immediate condition performance in younger adult (A) and elderly subjects (B) shown as change from base line (mean, 95% CI error bars)

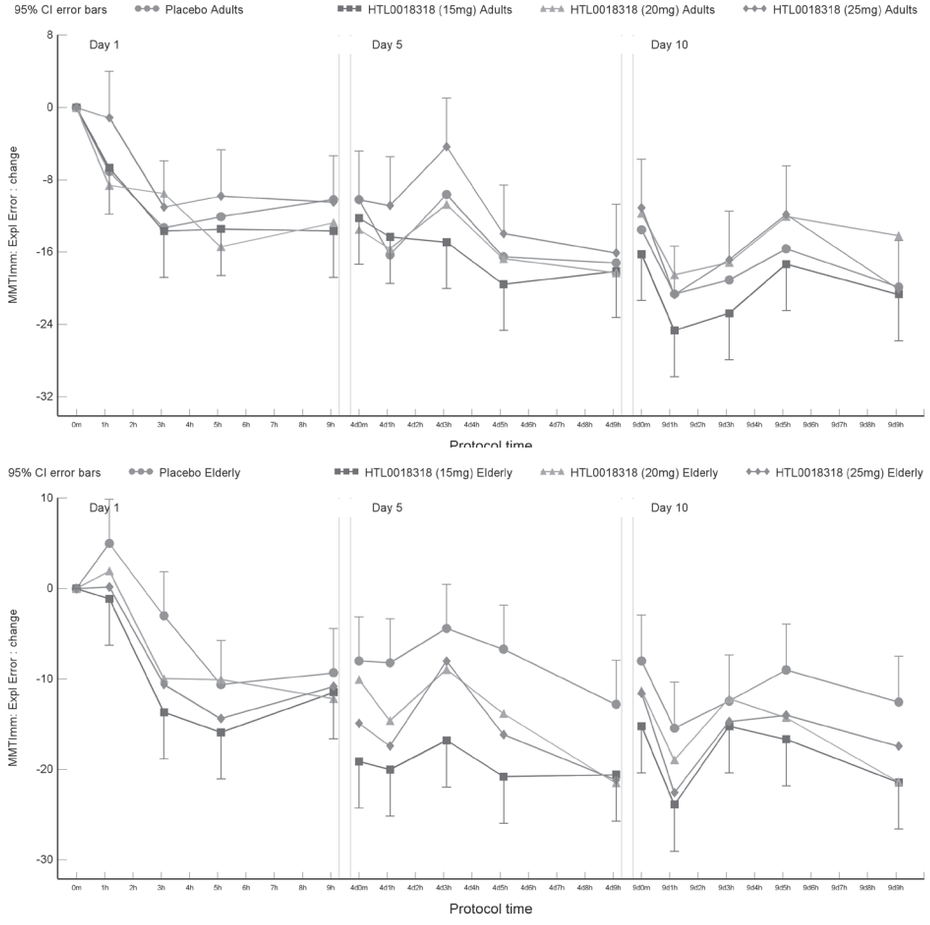





FIGURE 8 Effects on the MMT reversed condition performance in younger adults (A) and elderly subjects (B) shown as change from base line (mean, 95% CI error bars)

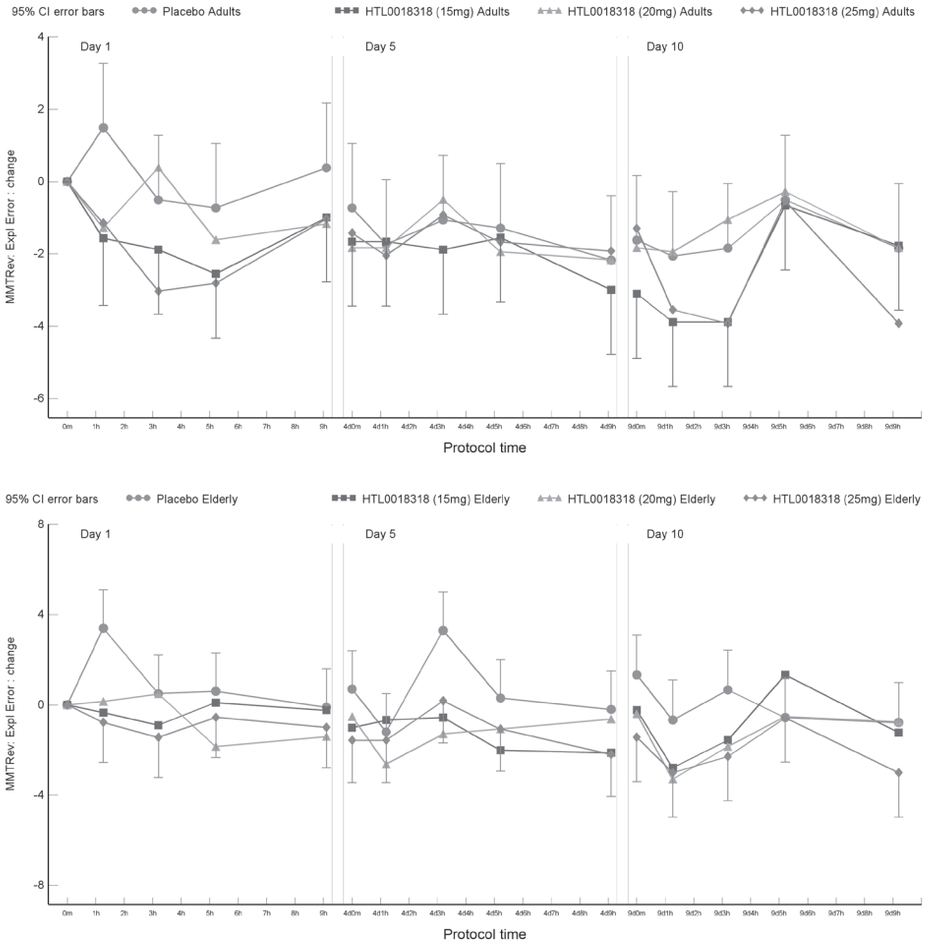
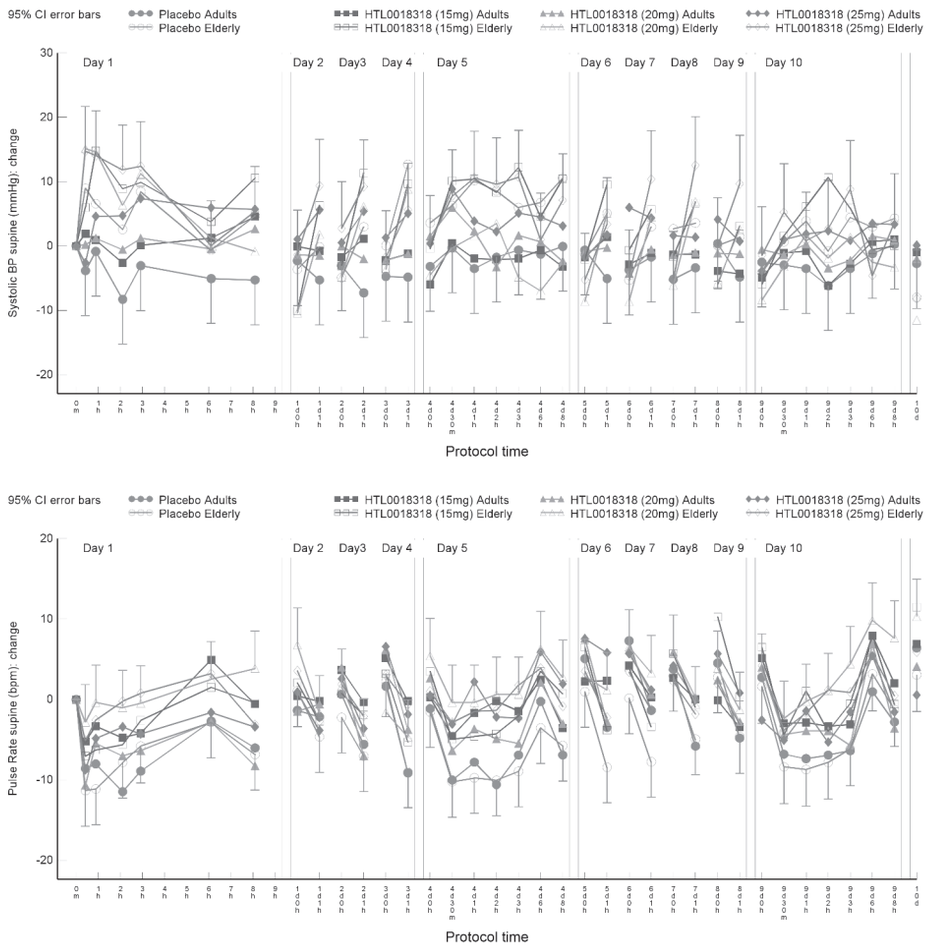
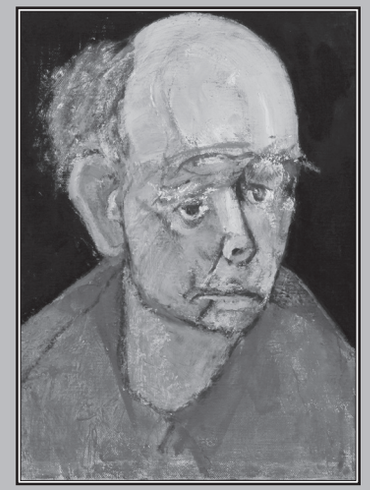


FIGURE 9 Effects on systolic blood pressure (A) and pulse rate (B) shown as change from base line (mean, 95% CI error bars)






CHAPTER V



# SAFETY AND PHARMACOKINETICS OF HTL0018318, A NOVEL $M_1$ RECEPTOR AGONIST, GIVEN IN COMBINATION WITH DONEPEZIL AT STEADY STATE: A RANDOMIZED TRIAL IN HEALTHY ELDERLY SUBJECTS

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## ABSTRACT

**INTRODUCTION** HTL0018318 is a selective muscarinic $M_1$ receptor partial agonist under development for the symptomatic treatment of dementias including Alzheimer's Disease. Clinically, HTL0018318 would likely be used alone or in conjunction with cholinesterase inhibitors (e.g. donepezil). We investigated the safety, tolerability and pharmacokinetics of HTL0018318 given alone and in combination with donepezil.

**METHODS** This was a randomized, double-blind, placebo-controlled trial in 42 (to deliver 36 with combination treatment) healthy elderly subjects investigating effects of 15 mg and 25 mg oral HTL0018318 given alone and combined with 10 mg donepezil at steady state on adverse events (AEs), vital signs, saliva production, sleep quality, pulmonary function, subjective feelings and pharmacokinetics.

**RESULTS** AEs were reported by lower percentages of subjects after HTL0018318 alone than after donepezil alone. There was no increase in percentage of subjects reporting AEs after co-administration than donepezil alone. Supine systolic blood pressure was 1.6 mm Hg (95% CI [-3.1; -0.1]) lower after HTL0018318 alone compared with combination treatment. This was comparable with placebo alone, which is 1.7 mm Hg (95% -3.2;0.2) lower versus combination treatment. Supine pulse rate was 3.3 bpm (95% CI [1.5; 5.1]) higher after HTL0018318 alone compared with co-administration. HTL0018318 and donepezil were found not to impact meaningfully each other's pharmacokinetics.

**CONCLUSION** HTL0018318 was well tolerated when given alone and when given together. HTL0018318 and donepezil do not demonstrate a pharmacokinetic or pharmacodynamic interaction, indicating that HTL0018318 can be safely co-administered in combination with donepezil.




## INTRODUCTION

Alzheimer's disease (AD) is characterised by a significant and progressive loss of acetylcholine producing neurons in the brain[1] which is correlated with the degree of cognitive decline[2,3]. The current standard of care consists of cholinesterase inhibitors, such as donepezil, that reduce the breakdown of synaptic acetylcholine and consequently enhance cholinergic transmission in the brain. The efficacy of cholinesterase inhibitors are modest and dosing is limited by side effects caused by non-selective enhancement of cholinergic transmission at other acetylcholine receptor subtypes located throughout the body[4,5]. Another approach to improve cholinergic function in AD might be agonism or modulation of the $M_1$ subtype of the muscarinic acetylcholine receptors ($M_1$ MAChR). The $M_1$ MAChR is the predominant subtype in the central nervous system and is expressed in areas of the brain associated with cognitive processes, such as the prefrontal cortex, neostriatum and hippocampus[6,7]. In AD patients, the $M_1$ MAChR is relatively well preserved[8]. Previously, muscarinic receptor agonists have been taken into development; the $M_1/M_4$ agonist xanomeline and the $M_1$ allosteric bitopic agonist GSK1034702 showed promising early clinical effects on cognitive function[9,10], however, further development of both compounds was terminated because of side effects caused by binding of the compounds to muscarinic receptors outside of the central nervous system.

HTL0018318 is a novel selective $M_1$ MAChR partial agonist. Pre-clinical data show that HTL0018318 has approximately a two-fold selectivity for the $M_1$ over $M_4$ receptors with no detectable functional agonist activity at human $M_2$ and $M_3$ receptors[11]. Multiple doses in healthy elderly humans resulted in an acceptable side effect profile with hyperhidrosis, nausea and hot flushes as most prevalent adverse events[12]. As a treatment for AD, HTL0018318 will very likely be given in combination with standard of care cholinesterase inhibitors such as donepezil. As both HTL0018318 and cholinesterase inhibitors increase cholinergic activity, the aim of this study is to investigate whether HTL0018318 can be safely co-administered in combination with donepezil. Although HTL0018318 was found not to interact with CYPs or drug transporters and donepezil is reported to be only a weak inhibitor of CYP2D6 or 3A4 ($IC_{50}$ 50–130 μM;[13]), suggesting a low probability for drug-drug interactions, this study evaluated what the effects are on pharmacokinetic (PK) characteristics of both HTL0018318 and donepezil when at steady state.

## METHODS

**TRIAL DESIGN AND SUBJECTS** This was a randomized, fixed-sequence, double-blind, placebo-controlled trial investigating multiple doses of 15 mg (12 subjects; 8 active and 4 placebo) and 25 mg (24 subjects; 16 active and 8 placebo) HTL0018318 given with and without donepezil at steady state in healthy elderly subjects. Open label donepezil (taken in the evening) was up titrated to steady state plasma concentrations by administering 5 mg donepezil for 5 consecutive days once daily, followed by 10 mg donepezil (therapeutic dose level) for 15 consecutive days once daily. Subsequently the donepezil treatment was combined with HTL0018318 or placebo dosed daily for 5 consecutive days (taken in the morning). This was followed by a wash-out period of 20 days and subsequent administration of HTL0018318 or placebo alone, daily for 5 consecutive days, was given at the same dose as previously administered in combination (figure 1). As it was expected that some subjects would withdraw from study participation due to side effects of donepezil during the donepezil run-in period, this treatment sequence prevented unnecessary exposure to HTL0018318 in subjects who had not previously completed the donepezil run-in phase. During the 2 periods in which HTL0018318 or placebo were administered, safety and PK measurements were performed daily. The study was run in three cohorts to allow within study modification of the dose of HTL0018318 in the event that an unexpected interaction occurred. According to protocol, the first cohort was administered 15 mg HTL0018318 and the second cohort 25 mg HTL0018318. The dose level of the third cohort (25 mg) was based on blinded safety and PK data of the first and second cohort.

Elderly subjects aged 65-80 years (inclusive) participated in the study. Subjects were eligible if in good health, with a maximum resting blood pressure of up to 150/90 mm Hg and a heart rate between 45-100 bpm at screening. Main exclusion criteria were current or past history of any illness interfering with the study objectives, the use of antihypertensive drugs, products that influence CYP3A4 or CYP2D6 and clinically relevant abnormalities on a 24-hour Holter ECG.

**MATERIALS** HTL0018318 was administered orally as an aqueous solution in 100 ml. Water was used as placebo. To mask the difference in taste between HTL0018318 and placebo, a peppermint strip (Listerine) was administered one minute before and after the administration of the oral solution. In humans, HTL0018318 the time to the maximum observed plasma concentration (TMAX) was 1-2 hours and a half-life of approximately 16 hours, which permits once daily dosing[12,14]. Steady state was reached after 2 or 3 daily doses[12]. Donepezil (manufactured by Aliud Pharma GmbH, Laichingen, Germany) was administered as 5 mg tablets. Donepezil has a $T_{max}$ of 3-4 hours and a half-life of approximately 70 hours[13].

**SAFETY AND TOLERABILITY ASSESSMENTS** A detailed overview of the timing of all measurements is provided in supplementary table S1. AEs were summarised per treatment (i.e. 15 mg HTL0018318, 25 mg HTL0018318 or placebo) and per study phase (i.e. donepezil alone, HTL0018318/placebo in combination with donepezil and HTL0018318/placebo alone). The AEs that were reported when donepezil was administered alone were summarised per treatment given after this phase (e.g. AEs reported when donepezil was administered alone by subjects that were to receive 15 mg HTL0018318 later on during the study). A subset of AEs was created that have a possible relation to increased cholinergic stimulation, being: hyperhidrosis, salivary hypersecretion, hypertension, tachycardia, bradycardia, nausea, diarrhoea, vomiting, constipation, insomnia, dizziness, muscle spasms, hot flush and cold sweat.

Systolic and diastolic blood pressure and pulse rate, all measured in supine and standing position, safety laboratory, electrocardiogram (ECG), and 24-hour Holter ECG were performed.

Saliva production was assessed by measuring the change in weight of three Salivette® dental rolls put into the oral cavity for 3 minutes. Pulmonary function was measured using the Spirostik (Accuramed), a PC-based open spirometry system. Subjective feelings were assessed using the visual analogue scale (VAS) according to Bond & Lader[15] and a VAS for nausea (0-100 mm). The Leeds Sleep Evaluation Questionnaire (LSEQ) was used to monitor changes in ease of initiating sleep, quality of sleep, ease of waking, and behaviour following wakefulness[16].

**PHARMACOKINETIC ASSESSMENTS** Plasma concentrations of donepezil and plasma and urine concentrations of HTL0018318 were determined using validated bioanalytical methods involving protein precipitation and liquid chromatography coupled with tandem mass spectrometry. The analytical range of the assay was 0.1–100 ng/mL (donepezil) or 0.5–1000 ng/mL (HTL0018318). To determine plasma donepezil concentrations, blood samples were collected after the 5$^{th}$ administration of 5 mg donepezil and during the donepezil administrations at therapeutic dose level as shown in figure 2. The time point 15 hours post donepezil dose corresponds with the time point immediately prior to HTL0018318 dosing.

To determine plasma HTL0018318 concentrations, blood samples were frequently taken on days when the first and fifth dose of HTL0018318 in combination with and without donepezil was administered. On the days between, only pre-dose PK

samples were taken. The last PK blood sample was taken between 7-14 days after the last HTL0018318 dose (Supplementary table S1).

To estimate HTL0018318 urine concentrations, all urine was collected within 24 hours after the first dose, and within 72 hours after the last dose of HTL0018318 in combination with and without donepezil.

PK parameters included in the analysis were the maximum observed plasma concentration ($c_{max}$), $T_{max}$, plasma concentration 24 hr post-dose ($C_{min}$), area under the plasma-concentration-time curve (AUC) from zero to 24 hr post dose ($AUC_{0-24}$), from zero to the end of the dose interval ($AUC_{0-tau}$), from zero to infinity ($AUC_{0-inf}$), apparent elimination half-life ($t_1/_2$), apparent oral clearance (CL/F), renal clearance (CLr) and percentage of dose excreted renally as unchanged drug (Ae%), and coefficient of variation (%CV). All PK analyses were performed in Phoenix 64 build 6.4.0.768 using WinNonlin 6.4.

**STATISTICAL ANALYSIS** A sample size was chosen typical of drug-drug interaction studies[17-19], the study was not statistically powered. The safety and tolerability assessments of saliva measurement, pulmonary function test, VAS Bond&Lader, VAS nausea, LSEQ and vital signs measured during the periods that HTL0018318 or placebo were administered in combination with and without donepezil were subjected to exploratory analysis. To this end a mixed model analysis of variance was used with treatment, period, time, treatment by period, period by time, treatment by time and treatment by period by time as fixed factors. Subject, subject by period and subject by time were random factors and the pre-HTL0018318 baseline measurement per period was a covariate. In these analysis models, all means are estimated (least square means, LSM). Statistical analysis was conducted with SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). The following contrasts were calculated: HTL0018318 alone vs placebo alone, HTL0018318 + donepezil vs placebo + donepezil, HTL0018318 + donepezil vs HTL0018318 alone. Analyses were performed for 15 mg and 25 mg HTL0018318 dose levels separately.

The effect of HTL0018318 on the PK of donepezil was analysed by comparing the plasma donepezil concentrations sampled pre-dose, 4 h and 15 h after the 20$^{th}$ donepezil dose (i.e. prior to HTL0018318 or placebo) with the plasma donepezil concentrations at the same times of the 21$^{st}$ and 24$^{th}$ donepezil dose. The 21$^{st}$ and 24$^{th}$ donepezil dose were administered after the first and fourth HTL0018318 administration, respectively.

The effects of donepezil on the PK of HTL0018318 were assessed by comparing the HTL0018318 $c_{max}$, $T_{max}$ and $AUC_{0-24}$ after the first dose of HTL0018318 in combination with donepezil with the same parameters when HTL0018318 was administered without donepezil. Also, the HTL0018318 $c_{max}$, $AUC_{0-tau}$, $T_{max}$ and $C_{min}$ after the last dose of HTL0018318 in combination with donepezil was compared with the same parameters when HTL0018318 was administered without donepezil. For these calculations, data of 15 mg and 25 mg HTL0018318 were grouped together.

The ratio of each above-mentioned parameter with and without donepezil co-dosing was calculated and the 90% CI of the geometric mean was assessed.

The degree of accumulation of exposure to HTL0018318 over the study period was assessed by calculating the ratio of $AUC_{0-tau}$ following repeat dosing to the $AUC_{0-tau}$ following the first dose. To assess the effect of donepezil co-administration on accumulation, these ratios calculated during the treatment period with co-administration of donepezil and without co-administration of donepezil were compared.

Statistical analysis was performed in R version 3.3.1 (2016-06-21) Copyright (C) 2016 The R Foundation for Statistical Computing (Platform: x86_64-w64-mingw32/x64 64-bit).

## RESULTS

**SUBJECTS** In total 42 subjects enrolled in this study, of whom three subjects withdrew due to side effects of donepezil and three subjects were withdrawn upon re-evaluation of eligibility, all prior to co-administration of HTL0018318. The remaining 36 subjects were randomized to placebo (n=12), 15 mg HTL0018318 (n=8), or 25 mg HTL0018318 (n=16) (table 1).

After the first dose of the HTL0018318/placebo in combination with donepezil five subjects dropped out due to a presumed viral gastro-enteritis (n=2 on placebo, n=3 on 25 mg HTL0018318) and one subject missed the fifth placebo dose due to this presumed viral gastro-enteritis. Another two subjects were withdrawn because of non-study drug related abnormal laboratory results after the washout period prior to first administration of HTL0018318/placebo without donepezil. In total 28 subjects completed the study.

**SAFETY AND TOLERABILITY** No clinically significant changes, related to treatment, were seen in any of the laboratory tests, ECG assessment and 24-hour Holter ECG results.

There were no significant changes in standing systolic blood pressure, supine and standing diastolic blood pressure, standing-supine blood pressure or standing pulse rate after HTL0018318 in combination with donepezil compared with HTL0018318 alone. Only effects on supine systolic blood pressure, supine pulse rate and standing-supine pulse rate were observed.

Supine systolic blood pressure was significantly lower after administration of 25 mg HTL0018318 without donepezil (118 mm Hg), but not after administration of 15 mg, compared with 25 mg and 15 mg HTL0018318 respectively in combination with donepezil (120 mm Hg, mean difference of 1.6 mm Hg, 95% CI [-3.1;-0.1], p=0.0378). After placebo without donepezil (118 mm Hg) the supine systolic blood pressure was significantly lower compared with placebo in combination with donepezil (120 mm Hg, mean difference of 1.7 mm Hg, 95% CI [-3.2; -0.2], p=0.0242). Administration of HTL0018318 (at both 15 mg and 25 mg) showed no significant effects on supine systolic blood pressure when compared with placebo either in combination with or without donepezil.

Supine pulse rate was significantly lower after administration of 15 mg and 25 mg HTL0018318 in combination with donepezil compared with HTL0018318 alone (15 mg HTL0018318 in combination with donepezil (64 bpm) vs 15 mg HTL0018318 without donepezil (67 bpm): mean difference of 3.3 bpm, 95% CI [1.5; 5.1], p=0.0009; 25 mg HTL0018318 in combination with donepezil (64 bpm) vs 25 mg HTL0018318 without donepezil (66 bpm): mean difference of 1.5 bpm, 95% CI [0.2; 2.9], p=0.0302). Administration of HTL0018318 (both 15 mg and 25 mg) showed no significant effects on supine pulse rate when compared with placebo either in combination with or without donepezil.

The change in pulse rate when standing from the pulse rate when supine (delta pulse rate) was significantly lower after administration of 25 mg HTL0018318 without donepezil (change of 10 bpm) compared with HTL0018318 25 mg in combination with donepezil (change of 12 bpm, mean difference of–1.6 bpm, 95% CI [-3.0; -0.2], p=0.0252). There were no significant changes in delta pulse rate after administration of 15 mg HTL0018318 or placebo without donepezil compared with the treatment in combination with donepezil. The delta pulse rate after 25 mg HTL0018318 without donepezil (change of 12 bpm) and in combination with donepezil (change of 10 bpm) was significantly lower compared with placebo without donepezil (change of 14 bpm, mean difference of -3.7 bpm, 95% CI [-6.6; -0.8], p=0.0137) and placebo in combination with donepezil (change of 15 bpm, mean difference of -3.4 bpm, 95% CI [-6.2; -0.6], p=0.0184).

Statistically significant changes were observed on saliva production, pulmonary function FEV1/FVC, LSEQ domain Quality of Sleep and LSEQ Awake following sleep (Supplementary table S2). All these changes were small and not considered to be clinically relevant.

There were no statistically significant effects on VAS alertness, calmness, mood, and nausea after HTL0018318 in combination with donepezil compared with HTL0018318 without donepezil.



All AEs were mild or moderate in intensity and there were no serious adverse events. The number of AEs reported when donepezil was administered alone did not increase after co-administering HTL0018318 15 mg and 25 mg. The percentages of subjects that reported AEs are shown in table 2. Compared with 15 mg HTL0018318 alone, co-administration of donepezil did increase the percentage of subjects reporting AEs. When 25 mg HTL0018318 was administered, a similar percentage of subjects reported AEs in the presence and absence of donepezil. The same pattern was observed in relation to percentages of subjects that reported AEs with a (possible) relation to increased cholinergic stimulation (table 3). The most frequently reported AEs were hot flushes, hyperhidrosis, nausea, vomiting, headache and somnolence. During the study period in which 25 mg HTL0018318 or placebo was dosed together with donepezil in subjects of cohort 2, there was an outbreak of a presumed gastrointestinal viral infection at the clinical research unit. When the gastrointestinal AEs related to the viral gastroenteritis were excluded from this analysis, no vomiting was reported in any of the treatment groups, and nausea was only reported by one subject dosed with placebo in combination with donepezil and by one subject dosed with 15 mg HTL0018138 in combination with donepezil. Additionally, when excluding the viral gastroenteritis AEs, the number of AEs in the gastrointestinal disorders class was slightly higher when HTL0018318 treatment was combined with donepezil compared with HTL0018318 alone (placebo + donepezil 4 AEs vs placebo alone 1 AE; HTL0018318 15 mg + donepezil 3 AEs vs HTL0018318 15 mg alone 1 AE; and HTL0018318 25 mg + donepezil 4 AEs vs HTL0018318 25 mg alone 3 AEs).

## PHARMACOKINETICS

- **HTL0018318 PK characteristics**

PK characteristics are shown in Table 4 and 5. Plasma HTL0018318 concentrations increased immediately following dosing and after $T_{max}$ (1.74-2.5 h), plasma concentrations declined in a biphasic manner. Pharmacokinetic steady-state was reached for HTL0018318 on or before the fifth daily dose of HTL0018318.

- **HTL0018318 accumulation**

The mean ratio of the $AUC_{0\text{-}tau}$ of HTL0018318 after the fifth dose of HTL0018318 to $AUC_{0\text{-}tau}$ after the first dose of HTL0018318 was 1.27 for 15 mg HTL0018318 and 1.23 for 25 mg HTL0018318. These ratios were comparable with donepezil co-dosed: 1.23 for 15 mg HTL0018318 and 1.21 for 25 mg HTL0018318.

The mean ratio of $AUC_{0\text{-}tau}$ of HTL0018318 after fifth dose of HTL0018318 to the $AUC_{0\text{-}inf}$ after the first dose of HTL0018318 was 1.04 following dosing with 15 mg

HTL0018318 and 1.06 after 25 mg HTL0018318. These ratios were comparable with donepezil co-dosed: 1.04 for 15 mg HTL0018318 and 1.03 for 25 mg HTL0018318.

- **Comparison of HTL0018318 PK characteristics in combination with and without donepezil**

The ratio of the PK parameters following the first dose of HTL0018318 in combination with donepezil compared with HTL0018318 alone were 1.05 (90 % CI [0.986–1.11]) for $C_{max}$, 1.01 (90 % CI [ 0.793–1.28]) for $T_{max}$ and 1.02 (90 % CI [0.975–1.07]) for $AUC_{0\text{-}24}$. The ratios following the fifth dose of HTL0018318 were 1.04 for $C_{max}$ (90 % CI [0.995–1.09]), 0.974 (90 % CI [0.744–1.28]) for $T_{max}$, 1.00 (90 % CI [0.969–1.03]) for $AUC_{0\text{-}tau}$ and 0.911 (90 % CI [0.854–0.972]) for $C_{min}$.

- **Donepezil**

The mean plasma donepezil concentration immediately before the first administration of 15 mg HTL0018318 (15 hours post donepezil dose) was 40.5 ng/ml (CV 25.0%), before 25 mg HTL0018318 was 37.4 ng/ml (CV 28.8%) and before placebo was 36.1 ng/ml (CV 29.6%). Plasma donepezil concentrations after the 18$^{th}$ to 24$^{th}$ doses suggested that donepezil was at pharmacokinetic steady-state by the time of the 18$^{th}$ donepezil dose. The geometric mean ratios of the donepezil concentration at 4, 15 or 24 hours post-dosing with HTL0018318 at 15 or 25 mg on the first dose of HTL0018318 or at steady-state versus donepezil plasma concentrations immediately before co-dosing (18$^{th}$ donepezil dose) was between 0.961 and 1.06 with the 90% CI including unity for all comparisons. The corresponding donepezil concentrations associated with dosing HTL0018318 placebo fell in the range 0.915 to 1.06 with the 90% CI including unity except at 24 hours post dose on Day 1 of placebo administration where the ratio was 0.915 (90% CI 0.871-0.962).

## DISCUSSION

This randomized, double-blind, placebo-controlled trial in 42 (to deliver 36) healthy elderly subjects investigated safety and tolerability and PK of repeated doses of HTL0018318 (15 mg or 25 mg) given without and in combination with donepezil (10 mg) at steady state. An effect on tolerability could have been predicted since both donepezil and HTL0018318 enhance cholinergic activity. There was no a priori expectation of a pharmacokinetic drug-drug interaction.

AEs were reported by a high proportion of the subjects during the donepezil run-in phase. Multiple doses of HTL0018318 in combination with donepezil were generally well tolerated. When 15 mg HTL0018318 and placebo were combined with donepezil, a greater proportion of subjects reported AEs compared with HTL0018318 or placebo alone. This difference is likely caused by donepezil, as donepezil alone resulted in more AEs than HTL0018318 without donepezil. Since 25 mg HTL0018318 without donepezil led to a comparable percentage of subjects experiencing AEs as donepezil alone, there was no difference when the treatments were combined.

The side effect profile observed in this study is comparable to that observed in the single ascending dose (SAD) and multiple ascending dose (MAD) studies with HTL0018318[12,14]. Only nausea and vomiting were reported more frequently than in the SAD and MAD study. During the study period in which 25 mg HTL0018318 or placebo were dosed in combination with donepezil in subjects of cohort 2, there was an outbreak of a presumed gastrointestinal viral infection at the clinical research unit. The presumption of viral gastroenteritis was based on the clinical presentation of the symptoms and the fact that staff of the clinical research organisation and placebo subjects were affected as well. Additionally, the onset of the symptoms of each individual followed one after the other and was not related to the timing of dosing.

Fewer AEs were reported after 15 mg HTL0018318 in combination with donepezil than after donepezil alone. A similar trend was observed in the placebo group. This may be explained by the high number of side effects that are associated with the start of intake of donepezil, which then decreases over time. In addition the duration of the run-in period (20 days) was longer than the treatment period of HTL0018318 in combination with donepezil (5 days).

The statistically significant increases in supine systolic blood pressure after administration of 25 mg HTL0018318 in combination with donepezil (1.6 mm Hg) and after placebo in combination with donepezil (1.7 mm Hg) are considered to be of small magnitude and not of clinical concern. The pulse rate data suggest that the combination of HTL0018318 and donepezil may decrease supine pulse rate, but not standing pulse rate compared with HTL0018318 without donepezil. Accordingly, the physiological heart rate increment after standing up was greater in those who had received HTL0018318 in combination with donepezil versus HTL0018318 without donepezil. However, these changes were similarly of small magnitude (up to 1.6 bpm) and of no clinical concern.

Increased saliva production was expected based on the mechanism of action of HTL0018318[20], and because salivary hypersecretion has been described in other studies investigating $M_1$ MAChR agonists [10, 21, 22], whereas it is not a common side effect of donepezil[23]. In the current study, the small changes on production of saliva are not considered of clinical importance (Supplementary table S2).

Acetylcholine can elicit bronchoconstriction and mucous secretion by activating the $M_2$ and $M_3$ MAChRs on the airway smooth muscle and mucous glands. The

$M_1$ mAChRs might play a minor role as agonism of the $M_1$ mAChRs at the postganglionic nerves facilitates acetylcholine release in the synaptic junction. This stimulates the $M_3$ mAChRs which contributes to bronchoconstriction and mucous secretion[24,25]. The observed increase of FEV1/FVC in the current study suggesting less constriction is therefore not considered to be a pharmacological effect and not clinically important.

The $M_1$ and $M_3$ mAChRs play an essential role in the rapid eye movement phase during the sleep wake cycle[26]. In the current study, no clinically relevant changes were observed on the LSEQ after administration of HTL0018318 alone or HTL0018318 in combination with donepezil (Supplementary table S2).

The pharmacokinetics of HTL0018318 were well-characterized in plasma and urine. The characteristics were comparable to the PK data observed in previous studies[14,12]. Median $T_{max}$ (1.74-2.5 h) and mean half-life following the fifth dose (10.5-13.7 h) did not appear to change with respect to HTL0018318 dose level and co-dosing with donepezil. There was no apparent change in renal elimination of HTL0018318 due to changing HTL0018318 dose level or due to co-dosing with donepezil. Variability of the HTL0018318 plasma PK $C_{max}$, $AUC_{0\text{-}tau}$ and apparent elimination half-life was similar between the 15 mg and 25 mg dose groups and similar between the periods with and without donepezil co-dosing (between 12.0% and 39.2%). There appeared to be no trend in degree of accumulation related to HTL0018318 dose level or related to co-dosing with donepezil. Comparisons of the ratios for $C_{max}$, $T_{max}$, $AUC_{0\text{-}24}$, $AUC_{0\text{-}tau}$ and $C_{min}$ (between 0.911 and 1.05) of HTL0018318 measured during the HTL0018318 dosing period with and without co-administration of donepezil indicate that donepezil does not have a meaningful impact on the PK of HTL0018318.

The plasma donepezil concentrations before the first administration of HTL0018318/placebo were considered to be therapeutic[27-30]. Comparisons of the plasma donepezil concentrations measured with and without co-administration HTL0018318 indicate that HTL0018318 does not impact the PK of donepezil (mean ratios between 0.915 and 1.06).

A potential limitation of this study is the fixed treatment sequence: in all subjects HTL0018318 in combination with donepezil was administered first, then HTL0018318 alone was investigated. As explained in the methods section, this treatment sequence prevented unnecessary exposure to HTL0018318 in subjects who were not able to complete the donepezil run-in phase due to donepezil related side effects. The impact of the sequence on the outcomes is considered to be low, because subjects were blinded to treatment allocation, which is the most important factor to prevent bias in safety reporting. The unforeseen outbreak of a presumed gastrointestinal viral infection at the clinical research unit complicated the interpretation of the safety data. However, the clinical presentation allowed to distinguish the symptoms related to the presumed gastrointestinal infection from drug related symptoms. In addition, more data on 25 mg HTL0018318 were able to be collected because this dose level was also investigated in the third cohort.

## CONCLUSION

Overall, HTL0018318 given in combination with donepezil to elderly healthy subjects was generally well tolerated, did not lead to clinical, safety or PK concerns and would be a viable combination treatment, at these dose levels, for the treatment of patients with Alzheimer's disease.

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TABLE 1 **Demographics.**

| | Placebo (n=12) | HTL0018318 15 mg (n=8) | HTL0018318 25 mg (n=16) |
|---|---|---|---|
| Age (years)<br>Mean (range) | 68.5<br>(65-71) | 70.4<br>(67-73) | 68.3<br>(65-75) |
| Weight (kg)<br>Mean (range) | 70.7<br>(55, 89.1) | 73.9<br>(64.05, 81.8) | 74.6<br>(60.2, 86.5) |
| BMI ($kg/m^2$)<br>Mean (Min, Max) | 25.2<br>(20.7, 31.2) | 25.8<br>(22, 27.6) | 24.8<br>(19.9, 33.6) |
| Sex<br>Female<br>Male | <br>6 (50%)<br>6 (50%) | <br>4 (50%)<br>4 (50%) | <br>6 (38%)<br>10 (63%) |
| CYP2D6 predicted phenotype<br>IM<br>EM<br>PM | Genotyping not performed | <br>1 (12%)<br>7 (88%)<br>0 | <br>2 (13%)<br>13 (81%)<br>1 (6%) |

*EM = Extensive metabolizer; IM = Intermediate metabolizer; PM = Poor metabolizer*

TABLE 2 **Percentage of subjects reporting adverse events (number of subjects reporting AEs/group size).**

| | Donepezil run in to steady state | Treatment in combination with donepezil | Treatment without donepezil |
|---|---|---|---|
| **Placebo** | | | |
| All AEs | 92% (12/13) | 75% (9/12 subjects) | 40% (4/10 subjects) |
| Gastrointestinal disorders | 46% (6/13) | 42% (5/12 subjects) | 10% (1/10 subjects) |
| Neurological disorders | 85% (11/13) | 33% (4/12 subjects) | 20% (2/10 subjects) |
| **HTL0018318 15 mg** | | | |
| All AEs | 80% (8/10) | 50% (4/8 subjects) | 43% (3/7 subjects) |
| Gastrointestinal disorders | 50% (5/10) | 13% (1/8 subjects) | 14% (1/7 subjects) |
| Neurological disorders | 60% (6/10) | 13% (1/8 subjects) | 14% (1/7 subjects) |
| **HTL0018318 25 mg** | | | |
| All AEs | 95% (18/19) | 88% (14/16 subjects) | 92% (11/12 subjects) |
| Gastrointestinal disorders | 53% (10/19) | 25% (4/16 subjects) | 17% (2/12 subjects) |
| Neurological disorders | 58% (11/19) | 38% (6/16 subjects) | 50% (6/12 subjects) |

*The donepezil run in period of 20 days was followed by the combination treatment of donepezil at steady state and HTL0018318/placebo (5 days). After a 20-day washout period HTL0018318/placebo was administered alone (5 days). AE = adverse event*



TABLE 3 **Percentage of subjects reporting cholinergic adverse events (number of subjects reporting AEs/group size).**

| | Donepezil run in to steady state | Treatment in combination with donepezil | Treatment without donepezil |
|---|---|---|---|
| Placebo | 62% (8/13) | 58% (7/12 subjects) | 20% (2/10 subjects) |
| HTL0018318 15 mg | 60% (6/10) | 13% (1/8 subjects) | 0% (0/7 subjects) |
| HTL0018318 25 mg | 63% (12/19) | 69% (11/16 subjects) | 67% (8/12 subjects) |

*The donepezil run in period of 20 days was followed by the combination treatment of donepezil at steady state and HTL0018318/placebo (5 days). After a 20-day washout period HTL0018318/placebo was administered alone (5 days). AE = adverse event*

TABLE 4 **Group summary data of HTL0018318 plasma and urine pharmacokinetic parameters following the first and fifth dose of HTL0018318 15 mg with and without donepezil co-dosing.**

| | Dosage | | $T_{max}$* (h) | $C_{max}$ (ng/mL) | Half-life (h) | $AUC_{0-inf}$ (ng.h/mL) | $AUC_{0-tau}$ (ng.h/mL) | CL/F (L/h) | Ae (%) | CLr (L/h) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1st dose | 15 mg only | N | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| | | Mean | 2.00 | 82.9 | 9.09 | 1090 | 900 | 13.7 | 38.6 | 6.43 |
| | | CV% | (1.00-4.00) | 12.0 | 20.5 | 29.4 | 23.0 | 29.4 | 18.7 | 21.5 |
| 5th dose | 15 mg only | N | 7 | 7 | 7 | | 7 | 7 | 7 | 7 |
| | | Mean | 2.05 | 100 | 13.7 | | 1130 | 13.3 | 46.4 | 6.17 |
| | | CV% | (1.00-4.00) | 25.3 | 10.9 | | 35.2 | 35.2 | 27.5 | 16.2 |
| 1st dose | 15 mg + DPZ | N | 8 | 8 | 8 | 8 | | 8 | 8 | 8 |
| | | Mean | 2.50 | 88.1 | 8.73 | 1070 | 903 | 14.0 | 38.5 | 6.40 |
| | | CV% | (1.00-4.00) | 28.3 | 25.7 | 32.6 | 27.5 | 32.6 | 27.4 | 22.0 |
| 5th dose | 15 mg + DPZ | N | 8 | 8 | 8 | | 8 | 8 | 7 | 7 |
| | | Mean | 1.74 | 107 | 11.5 | | 1110 | 13.5 | 50.1 | 6.40 |
| | | CV% | (0.50-4.00) | 22.0 | 12.2 | | 29.4 | 29.4 | 24.8 | 23.3 |

*Geometric mean, %CV except * median (min-max). Tau = 24hrs.*
*% CV = coefficient of variation; AE % = percentage of dose excreted renally as unchanged drug; AUC = area under the plasma-concentration-time curve; AUC0-inf = AUC from zero to infinity; $AUC_{0-tau}$ = AUC from zero to the end of the dose interval; CL/F = apparent oral clearance; CLr = renal clearance; $C_{max}$ = maximum observed plasma concentration; DPZ = donepezil; $t_{max}$ = time to $C_{max}$*

TABLE 5 **Group summary data of HTL0018318 plasma and urine pharmacokinetic parameters following the first and fifth dose of 25 mg HTL0018318 with and without donepezil co dosing.**

| | Dosage | | $T_{max}$* (h) | $C_{max}$ (ng/mL) | Half-life (h) | $AUC_{0\text{-}inf}$ (ng.h/mL) | $AUC_{0\text{-}tau}$ (ng.h/mL) | CL/F (L/h) | Ae (%) | CLr (L/h) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1st dose | 25 mg only | N | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | | Mean | 1.75 | 133 | 8.25 | 1520 | 1300 | 16.4 | 30.9 | 5.94 |
| | | cv% | (0.650-3.00) | 20.4 | 25.2 | 41.8 | 32.7 | 41.8 | 36.9 | 32.2 |
| 5th dose | 25 mg only | N | 12 | 12 | 12 | | 12 | 12 | 11 | 11 |
| | | Mean | 1.76 | 157 | 13.3 | | 1600 | 15.6 | 41.8 | 6.41 |
| | | cv% | (1.50-3.00) | 26.5 | 13.5 | | 39.2 | 39.2 | 36.2 | 23.9 |
| 1st dose | 25 mg + DPZ | N | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| | | Mean | 1.76 | 136 | 8.44 | 1580 | 1340 | 15.9 | 38.8 | 7.22 |
| | | cv% | (0.267-3.02) | 20.1 | 25.8 | 41.9 | 32.4 | 41.9 | 39.3 | 46.6 |
| 5th dose | 25 mg + DPZ | N | 13 | 13 | 13 | | 13 | 13 | 8 | 8 |
| | | Mean | 2.00 | 165 | 10.5 | | 1640 | 15.2 | 47.1 | 6.51 |
| | | cv% | (1.50-4.00) | 27.9 | 18.8 | | 35.7 | 35.7 | 17.8 | 32.6 |

*Geometric mean, %CV except * median (min-max). Tau = 24hrs.*
*% CV = coefficient of variation; AE% = percentage of dose excreted renally as unchanged drug; AUC = area under the plasma-concentration-time curve; $AUC_{0-inf}$ = AUC from zero to infinity; $AUC_{0-tau}$ = AUC from zero to the end of the dose interval; CL/F = apparent oral clearance; CLr = renal clearance; $C_{max}$ = maximum observed plasma concentration; DPZ = donepezil; $t_{max}$ = time to $C_{max}$*



FIGURE 1 **Study design. CRU clinical research unit, Dpz donepezil, ss steady state**

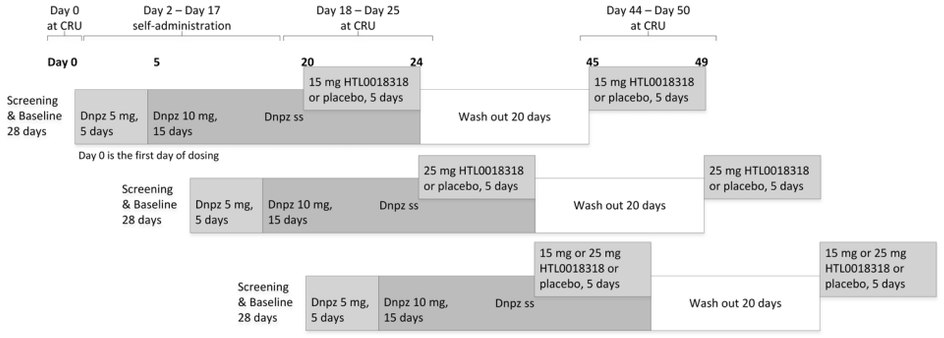
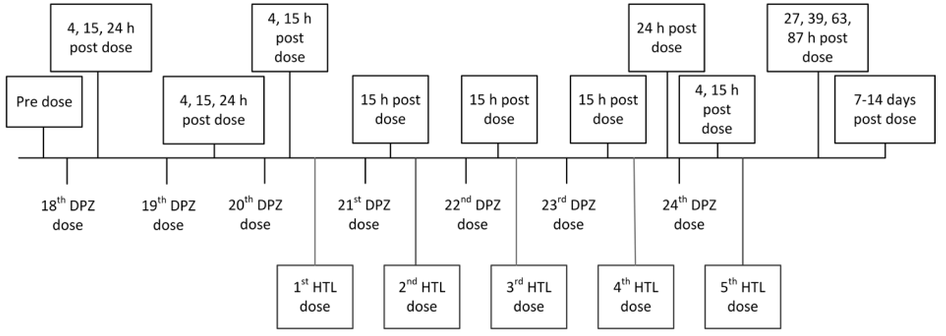
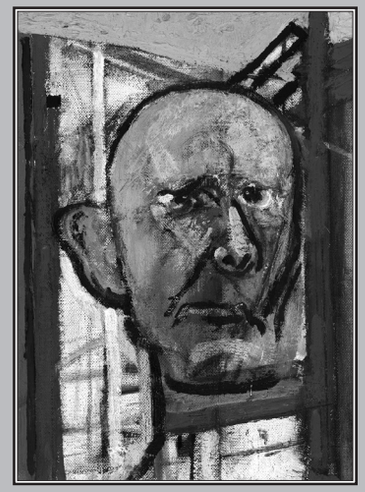



FIGURE 2 **Timing of donepezil pharmacokinetic samples. Dpz donepezil, h hour, HTL HTL0018318**



CHAPTER VI



# SAFETY, PHARMACOKINETICS AND PHARMACODYNAMICS OF GLN-1062, A PRODRUG OF GALANTAMINE

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## ABSTRACT

**INTRODUCTION** Gln-1062 (Memogain) is an intranasally administered lipophilic prodrug of galantamine. Based on high brain-to-blood concentrations observed in pre-clinical studies, Gln-1062 is expected to have superior cognitive efficacy than oral galantamine.

**METHODS** 48 healthy elderly subjects were randomised 12:4 to Gln-1062 (5.5, 11 or 22 mg b.i.d. for 7 days) or placebo. Safety, tolerability, pharmacokinetics and pharmacodynamics were assessed repeatedly. Pharmacokinetics were compared with 16 mg oral galantamine.

**RESULTS** Gln-1062 up to 22 mg b.i.d. was well tolerated. Gln-1062 plasma concentrations increased immediately following dosing (median $T_{max}$ of 0.5 hour (range 0.5-1.0)). $C_{max}$ and $AUC_0$-last increased in a dose linear manner over all three dose levels. Gln-1062 was rapidly cleaved into galantamine. Gln-1062 significantly improved adaptive tracking (sustained attention) with 1.95% (95% CI 0.630–3.279, p=0.0055) compared to placebo after correction for individual baseline performance.

**DISCUSSION** Gln-1062 was considered to be safe and caused fewer gastro-intestinal side effects than oral galantamine. Gln-1062 behaved pharmacokinetically as expected and improved performance on cognitive tests.




## INTRODUCTION

Alzheimer's disease (AD) is the most common manifestation of dementia. The first-line therapy for mild to moderate AD is symptomatic and consists of cholinesterase inhibitors (ChEIs). These ChEIs inhibit the cholinesterase enzyme from breaking down acetylcholine resulting in higher acetylcholine levels. Unfortunately, the efficacy of these ChEIs is moderate[1-3]. Raising the dose in order to increase efficacy leads to a marked increase in peripheral side effects such as nausea, vomiting and diarrhoea, which reduce the likelihood of the drugs' cognitive enhancing effects[4]. As there is no curative treatment for AD yet, it is important to optimise the symptomatic treatment.

Gln-1062 (Memogain) was developed as an augmented form of galantamine, a reversible, competitive ChEI. Galantamine is a quaternary ammonium and therefore does not pass the blood-brain-barrier easily. Gln-1062 is an inactive pro-drug of galantamine that is cleaved into active galantamine by a carboxy-esterase and butyrylcholinesterase. Due to its much higher lipophilicity it penetrates the blood brain barrier more easily than the parent drug galantamine[5]. Intranasal administration of 5.0 mg/kg and 20.0 mg/kg resulted in blood-to-brain ratios of 8.1 and 10.2 respectively[6], which is higher than the brain-to-blood ratio of galantamine 4 mg/kg in mice of 2.1[7]. Gln-1062 is administered intranasally to prevent cleavage to galantamine in the gastrointestinal tract.

Results from the first-in-human study with Gln-1062 dosed at 5.5, 11, 22, 33 and 44 mg showed that the drug led to dose-dependent improvements of sustained attention (adaptive tracking) and in verbal memory (visual verbal learning test, VVLT)[6]. Doses of 22 mg Gln-1062, which have the same molarity as 16 mg galantamine, were better tolerated with fewer peripheral side effects than 16 mg oral galantamine and other ChEIs[6]. In the current study, the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of Gln-1062 were investigated following multiple dose administrations and compared with placebo. Additionally, the PK of galantamine in plasma and in the cerebrospinal fluid (CSF) after 11 mg Gln-1062 (n=12) administration was compared to the galantamine PK after a 16 mg oral galantamine administration.

## METHODS

**TRIAL DESIGN AND SUBJECTS** This was a randomised, double-blind, placebo controlled study with multiple intranasal doses of Gln-1062 in healthy subjects (≥65 years, n=48). Subjects were randomised to Gln-1062 or placebo with a ratio of 12:4 per cohort. All 12 placebo subjects (cohorts 1 to 3) were pooled. Main exclusion

criteria were a mini-mental state examination of 25 or lower, impaired renal or liver function, use of interfering concomitant medication, and intranasal abnormalities.

Subjects were administered 5.5 mg, 11 mg or 22 mg Gln-1062 or placebo (NaCl 0.9%) b.i.d. for 7 subsequent days with a dosing interval of 6 hours between the morning- and afternoon dose. A follow-up visit took place 7 to 14 days after the last dose of Gln-1062 or oral galantamine.

During the clinical phase of the study, but after completion of all assessments of the Gln-1062/placebo occasion, the 11 mg cohort was unblinded to identify the 12 subjects who were administered active Gln-1062 11 mg. These subjects were administered a single oral dose galantamine hydrobromide 16 mg open label between 16 days to 28 days after the last Gln-1062 administration for determination of galantamine CSF concentration.

All subjects gave written informed consent for participation in the study. The study was approved by the ethics committee of the Foundation Beoordeling Ethiek Biomedisch Onderzoek (BEBO, Assen, The Netherlands), conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. The trial was registered in the Netherlands Trials Register (NL5557).

**SAFETY ASSESSMENTS** All subjects underwent medical screening, including (but not restricted to) medical history, physical examination, nasal examination, and vital signs measurement. During study periods, safety was assessed using monitoring of treatment-emergent adverse events (TEAEs), nasal examination, vital signs, ECG, and safety blood sampling (supplementary Table S1).

**PHARMACOKINETIC ASSESSMENT** To assess PK characteristics of Gln-1062, venous blood samples were obtained in all subjects, and in addition CSF samples were taken in the 11 mg Gln-1062 or placebo cohort (supplementary Table S1). Following oral galantamine administration one CSF sample was taken at 1, 3, 5 or 8 hours post dose, resulting in 3 CSF samples per time point. Blood samples were taken pre-dose, 30 minutes, 1 hour, 2 hours, 2.5 hours, 5 hours and 8 hours after oral galantamine administration to determine plasma concentrations of Gln-1062 and galantamine. Analysis was performed at the Analytical Biochemical Laboratory (Assen, the Netherlands) by a validated method using high performance liquid chromatography coupled to tandem-mass spectrometry. Non-compartmental analysis was performed using R, version 3.3.2.

As 11 mg Gln-1062 contains 5.379 mg galantamine and 16 mg galantamine hydrobromide contains 12.5 mg galantamine, the CSF and plasma data after oral galantamine were corrected for the difference in amount of administered galantamine in order to allow a direct comparison of the CSF and PK data after 16 mg oral galantamine and 11 mg Gln-1062.

**PHARMACODYNAMIC ASSESSMENTS** To assess the acute effects of Gln-1062 on central nervous system (CNS) functioning, PD effects were measured using the NeuroCart, a battery of neuropsychological and neurophysiological tests used to examine the effects of drugs that are active on a wide range of CNS domains[8]. A range of tasks was selected with high sensitivity to detect cognitive changes that could be expected from ChEIs.

The N-back test was used to evaluate verbal working memory[9-11]. It consists of 3 conditions with increasing working memory load. The adaptive tracking test measured attention and eye-hand coordination[12,13,6]. The subject was asked to keep a dot inside a moving circle by operating a joystick. The speed of the moving circle increased when the dot was contained in the circle, and reduced if the dot could not be maintained in the circle. Performance was measured as percentage of time correctly tracked over a 3.5-minute period including a run-in time of 0.5 minute. The visual analogue scale (VAS) according to Bond and Lader was used to assess subjective drug effects on mood, alertness and calmness on a 100-mm scale[13,14]. Nausea was assessed using a 100-mm VAS. Pharmaco-electroencephalography (EEG), eye movements, and pupil size were used to monitor drug effects that could be interpreted as evidence of blood-brain barrier penetration and cholinergic influence on pupil size[15-19]. In the VVLT, subjects were asked to recall immediately the 30 words that were presented repeatedly, which reflects the acquisition and consolidation of novel information. After 30 minutes, word retrieval from long term memory was assessed as well as recognition of learned words between a list of distractor words[20].

All PD tests except for the VVLT were performed repeatedly to assess the effects over time (supplementary Table S1). Extra adaptive tracking tests were performed in the cohort dosed 22 mg b.i.d. following a protocol amendment, which also removed the EEG measurements at 30 minutes and 3 hours post dose to ensure the NeuroCart repeated measurements were feasible to complete within time constraints. No PD measurements were performed after administration of oral galantamine.

**STATISTICS** A sample size of 12 healthy elderly subjects treated with Gln-1062 per cohort was defined to have 82% power to detect a difference of 1.8% on the adaptive tracking test performance, assuming a standard deviation of 1.47%-point, using a two-sample t-test with a two-sided significance level of 0.05, based on data of the first in human study[6].

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. VVLT data were compared with a mixed model analysis of variance with fixed factors treatment, day and treatment by day and random factor subject. The difference between the least square mean (LSM) of the treatment and placebo was calculated for all test endpoints. All calculations were performed using SAS for windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

**PK-PD ANALYSIS** The relationship between galantamine plasma and CSF levels and PD was investigated by analysing the PK-PD data using nonlinear mixed effect modelling (Phoenix 64 build 8.0.0.3176 using WinNonlin 8.0). PK and PD data of the first-in-human study were also included in this analysis[6]. PK models were tested with the assumptions that Gln-1062 could be metabolized to galantamine in plasma, to galantamine in CSF and eliminated unchanged. The Gln-1062 metabolism and elimination was assumed to be from the Gln-1062 plasma central compartment. PD models were tested with $I_{max}$ type models linking the galantamine plasma concentration to the size of the response; and direct effect, turnover and effect compartment models characterizing any temporal difference between the galantamine plasma concentration and the response. Inter-occasion variability and inter-individual variability were studied for significance. Covariates were stepwise introduced to the base model (PK and PD) and the covariates that were significant at $p < 0.01$ were added to the model. Once the full model was established, the significance of the potential covariates were evaluated using a backward reduction method. The least significant covariate not resulting in an increase of $p < 0.001$ was removed from the model.

## RESULTS

**SUBJECTS** A total of 48 healthy elderly subjects were enrolled in this study. The mean (± SD) age of the subjects was 70.0±3.4 years, the mean weight (± SD) was 72.0±10.6 kg, the mean BMI (± SD) was 25.0±2.6 kg/m$^2$. Of the 48 subjects 22 (46%) were female. Two subjects who were administered Gln-1062 were withdrawn from study participation at their own request; one subject due to intranasal pain after the second dose on the first dosing day, the other subject due to nausea, vomiting and diarrhoea after the first dose on the first dosing day. Data of these subjects were included in the analysis sets.



**SAFETY** Administration of multiple doses of Gln-1062 did not result in clinically significant changes in blood and urinary laboratory values, and ECG safety parameters. All TEAEs were mild or moderate of intensity and were self-limiting. There were no serious adverse events. For an overview of the most frequent occurring TEAEs (Table 1). There was an increase in the incidence of TEAEs related to nasal complaints and nasal exam abnormalities with increasing Gln-1062 dose. The nasal examinations showed abnormalities in the form of dry white plaques in the nose and red and irritated nasal mucosa. The incidence of gastrointestinal (GI) TEAEs was also dose dependent. However, all three Gln-1062 dose levels led to fewer GI TEAEs than oral galantamine (Table 2).

On the first dosing day a significant increase in the mean systolic blood pressure between 8.9 mm Hg (95% CI 2.8–15.0, p=0.005) and 10.9 mm Hg (95% CI 4.8–17.1, p=0.0007) was observed at all dose levels. The mean diastolic blood pressure increased only after 11 mg Gln-1062 on the first dosing day with 5.1 mm Hg (95% CI 1.4–8.7, p=0.0068). On the seventh day, only the systolic blood pressure was increased after 5.5 mg Gln-1062 with 7.6 mm Hg (95% CI 1.4–13.8, p=0.0170). No statistically significant effect of Gln-1062 on the pulse rate was observed.

**PHARMACOKINETICS** Gln-1062 plasma concentrations increased immediately following dosing with a median $T_{max}$ of 0.5 hour (range 0.5-1.0). Plasma concentrations of Gln-1062 declined in a monophasic manner following $C_{max}$. The mean (± SD) $t_1/_2$ of 5.5 mg Gln-1062 was 1.16 (± 0.37) hour, of 11 mg Gln-1062 was 1.57 (± 1.74) hour, and of 22 mg Gln-1062 was 2.09 (± 0.58) hour. The variability of plasma PK of Gln-1062 can be considered moderate in elderly subjects with a coefficient of variation (CV) of $C_{max}$ between 35 and 48%, a CV of $AUC_0$-last between 29% and 60% and a CV of the apparent elimination $t_1/_2$ between 27% and 49%. The $C_{max}$ and $AUC_0$-last of Gln-1062 increased in a dose linear manner over all dose levels. Gln-1062 was rapidly cleaved into galantamine (Figure 1).

Dose corrected plasma and CSF concentrations of galantamine cleaved from Gln-1062 were lower than from oral galantamine (Figure 2).

**PHARMACODYNAMICS** The PD effects of Gln-1062 compared with placebo are summarised in Table 3. An improvement in adaptive tracking performance was measured in the 22 mg cohort (Figure 3). EEG delta power was significantly decreased after 22 mg Gln-1062 at the frontal and parietal locations. Reaction time on the n-back task was decreased in the 1-back condition after 5.5 mg Gln-1062 and in the 2-back condition after 11 mg Gln-1062 .

No significant and consistent effects were observed on VVLT, eye movements and VAS mood, calmness, alertness and nausea, compared with placebo.

**PK-PD ANALYSIS** PK data was fit best by a 2 compartment model for Gln-1062 in plasma; 1 compartment for galantamine in plasma and 1 compartment for galantamine in CSF. The clearance of Gln-1062 from the plasma compartment is described well by metabolism to galantamine and by elimination of unchanged Gln-1062, both with linear clearance. Galantamine can distribute between the plasma and CSF compartments and is cleared linearly from the plasma compartment only. Absorption of intranasal Gln-1062 into the Gln-1062 plasma compartment occurs with a first order input without lag time. Absorption of oral galantamine into the galantamine plasma compartment is described with first order absorption and no absorption lag time. The concentration-effect relationship for adaptive tracking could be described by a direct effect of galantamine plasma concentration with a maximum increase ($E_{max}$) of 1.91 (95% CI 1.15 to 5.90) and a concentration resulting in half of the maximum ($EC_{50}$; mg/L) of 0.0231 (95% CI -0.0005 to 0.134) for Gln-1062 and 0.172 (95% CI -0.584 to 5.01) for oral galantamine.

## DISCUSSION

This was a randomised, double-blind, placebo controlled, multiple ascending dose study to assess the safety, tolerability, PK and PD of 7 days b.i.d. intranasal dosing of 5.5 mg, 11 mg or 22 mg Gln-1062 to 48 healthy elderly subjects. Gln-1062 is an inactive lipophilic pro-drug that is cleaved into galantamine. The PK in blood and CSF of 11 mg Gln-1062 were compared with 16 mg oral galantamine.

Overall, Gln-1062 was well tolerated. Often, side effects are a reason to stop CHEI treatment[1]. In general, most frequently reported adverse events after administration of CHEIs are GI related[21]. In the current study, the number of GI related TEAEs was dose dependent with the highest incidence in the group treated with 22 mg Gln-1062 b.i.d. Interestingly, after administration of a single dose of 16 mg oral galantamine resulted in more GI related TEAEs compared with 22 mg Gln-1062 b.i.d. for 7 consecutive days, which equals a total daily dose of 32 mg oral galantamine. The difference in gastrointestinal symptoms is probably due to the lower $c_{max}$ and more gradual increase of plasma galantamine concentrations as it is cleaved from Gln-1062. oral galantamine may have an increased burden on the GI tract compared to intranasal Gln-1062 administration. Nausea and vomiting were reported in the Gln-1062 cohorts, however there was no significant increase in the VAS nausea score for any dose level compared with placebo. It should be noted that the VAS nausea was taken on the first and seventh dosing day, whereas nausea AEs were reported in between these measurement days.

Nasal complaints were the most frequently reported TEAEs and were dose dependent. Although these symptoms were mild in our study population, they could lead to low compliance or discontinuation of the therapy in AD patients. The nasal complaints could be related to the active pharmaceutical ingredient of Gln-1062 or to the acid in the formulation. Which of these two caused the nasal complaints cannot be concluded from this study, as the placebo formulation was NaCl 0.9%, and did not contain this acid. Further investigations on an improved formulation that may increase tolerability of intranasal administration are ongoing.

Galantamine can induce syncope and bradycardia due to stimulation of the vagus nerve[22,23]. No such symptoms were reported in the present study and no statistically significant change in heart rate was observed. These results should be interpreted with caution as the chance of observing bradycardia in this small healthy sample is likely lower than in the clinical population[22].

Gln-1062 was rapidly absorbed and cleaved into galantamine. Non-compartmental analysis of the galantamine cleaved from Gln-1062 concentrations could not be performed due to sparse sampling of the decline in galantamine plasma concentration after $c_{max}$, which prevented the calculation of the AUC and $T_1/_2$. The concentration of galantamine cleaved from 11 mg Gln-1062 in plasma and CSF appeared to be lower than the (corrected) galantamine concentration in plasma and CSF following oral galantamine administration. CSF sampling was used in this study as a surrogate measurement of drug concentrations in the brain. Animal studies have shown that CSF concentration can give an indication, but not a reliable prediction of the brain interstitial fluid concentrations[24] due to differences between blood-CSF-barrier and blood-brain-barrier, brain blood flow, capillary surface area, brain tissue binding and extra-intracellular exchange[25,26]. In general, CSF drug concentrations were slightly higher than the brain concentrations, although also higher brain drug concentrations than CSF concentrations were observed[24,27-29]. Considering this and the higher lipophilicity of Gln-1062 it could be that the brain galantamine concentrations are higher than the observed concentrations in the CSF and thus higher than the concentrations after oral galantamine. Additionally, the intranasal administration route might contribute to higher brain concentrations compared with the oral route[30]. Another explanation for the lower CSF concentrations might be a lower absorption by the nasal mucosa. We were not able to compare the plasma to CSF ratio of galantamine cleaved from Gln-1062 and oral galantamine because the plasma curve of oral galantamine showed a clear peak after ingestion of galantamine as opposed to the plasma curve of galantamine cleaved from Gln-1062.

Demonstrating a difference in distribution of Gln-1062 compared with oral galantamine based on PK data was therefore not possible. In the PK modelling the same distribution of galantamine from plasma to the CSF was identified following Gln-1062 and oral galantamine administration. All Gln-1062 concentrations in CSF were below the limit of quantification, and therefore the PK model did not contain a CSF compartment for Gln-1062. This should not be taken as evidence that Gln-1062 is not present and converted to galantamine in the brain, but only that this route of metabolism was not supported by the current data. On the other hand, a lower $EC_{50}$ was identified for Gln-1062 dosing as compared to oral galantamine dosing, when relating the plasma concentration to effect. This implies that more galantamine is delivered to the site of action following Gln-1062 dosing as compared to oral galantamine dosing.

The lack of effect of Gln-1062 on VVLT, eye movements and VAS was consistent with the first in human study[6]. Also the improvement in performance of the adaptive tracking test after 22 mg Gln-1062 is consistent with findings from the previous study[6]. The PK-PD analysis supported an effect of galantamine on adaptive tracking, although the $EC_{50}$ could not be determined with high precision. The use of a direct effect model further suggests that there is no noteworthy delay between changes in galantamine plasma concentration and changes in adaptive tracking response. The improvement of sustained attention was expected based on the pharmacological mechanism and previous studies demonstrating that the adaptive tracking test is sensitive to anti-cholinergic[9] and pro-cholinergic compounds[6]. Furthermore the study was powered on the adaptive tracking test, the effects are supported by the PK-PD analysis and consistent with the first in human study. For these reasons, the performance improvement is likely to be a pharmacological effect and not a type 2 error. Sustained attention is one of the cognitive functions that is impaired in patients with AD, starting in the early phase of the disease[31]. It is challenging to improve sustained attention in healthy subjects with optimal cognitive performance due to ceiling effects of the cognitive tests. Since we observed improvement of sustained attention despite these ceiling effects, we expect that Gln-1062 can contribute to improvement of sustained attention in AD patients as well. Further investigation is warranted to determine the benefits in patients and to compare these to the currently approved ChEIs. We did not assess the effects of oral galantamine on the adaptive tracking test performance in this study due to the open label design of the galantamine study visit.

In general, delta oscillations appear to increase in states of motivational urges and are involved in attentional processes as reviewed in[32]. In AD patients also in resting state a higher power of widespread delta rhythms has been reported[33-36]. In the current study a decrease of EEG delta power at frontal and parietal locations was observed. This is consistent with the decrease in delta power after ChEI administration in healthy subjects[37] and in patients with AD[38,39]. To date, no clear relationship between ChEI-induced decrease of delta power and cognitive function has been demonstrated.

To conclude, bi-daily dosing of dose levels up to 22 mg Gln-1062 for 7 days is considered to be safe and well tolerated in healthy elderly subjects. Nasal complaints were the most frequently reported TEAEs. A coincidental finding was that the severity of GI related side effects reported after administration of Gln-1062 were milder compared to a single oral dose of 16 mg galantamine. Effects on sustained attention were most evident, were reproduced from the SAD study, and were supported by the PK-PD analysis[6]. The improved sustained attention is expected to contribute to better cognitive function when treated with Gln-1062 for a longer period of time.

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TABLE 1 **Most prevalent TEAEs following Gln-1062 administration for 7 consecutive days.**

| | Gln-1062 5.5 mg b.i.d. | Gln-1062 11 mg b.i.d. | Gln-1062 22 mg b.i.d. | placebo |
|---|---|---|---|---|
| Any events | 11 (91.7) | 12 (100.0) | 12 (100.0) | 11 (91.7) |
| Diarrhoea | - | 1 (8.3) | 2 (16.7) | 1 (8.3) |
| Nausea | 1 (8.3) | 2 (16.7) | 5 (41.7) | 1 (8.3) |
| Vomiting | 1 (8.3) | - | 3 (25.0) | - |
| Administration site irritation | 3 (25.0) | - | - | - |
| Administration site pain | 1 (8.3) | 3 (25.0) | 2 (16.7) | - |
| Headache | - | 5 (41.7) | 4 (33.3) | 4 (33.3) |
| Epistaxis | 4 (33.3) | 3 (25.0) | 9 (75.0) | - |
| Mucosal haemorrhage | 1 (8.3) | 7 (58.3) | 9 ( 75.0) | - |
| Intranasal paraesthesia | - | 2 (16.7) | - | - |
| Nasal congestion | 2 (16.7) | 2 (16.7) | 3 (25.0) | 1 (8.3) |
| Nasal discomfort | 3 (25.0) | 5 (41.7) | 2 (16.7) | - |
| Nasal mucosal disorder | 7 (58.3) | 9 (75.0) | 11 (91.7) | 3 (25.0) |
| Rhinalgia | - | 1 (8.3) | - | - |
| Rhinitis | 1 (8.3) | 1 (8.3) | - | - |
| Rhinorrhoea | 8 (66.7) | 8 (66.7) | 8 (66.7) | 4 (33.3) |
| Sinusitis noninfective | | 1 (8.3) | | - |
| Sneezing | 3 (25.0) | 2 (16.7) | 2 (6.7) | 1 (8.3) |

*Number of subjects, percentage in parentheses.*

TABLE 2 **Gastrointestinal related TEAEs within 24 hours after the first Gln-1062 administration and consequently 18 hours after the second Gln-1062 administration on dosing day 1 and within 24 hours after 16 mg galantamine administration (oral).**

| | Gln-1062 5.5 mg b.i.d. | Gln-1062 11 mg b.i.d. | Gln-1062 22 mg b.i.d. | Galantamine 16 mg | placebo |
|---|---|---|---|---|---|
| Any events | 7 ( 58.3) | 12 (100.0) | 12 (100.0) | 10 ( 83.3) | 6 ( 50.0) |
| All gastrointestinal disorders | 1 ( 8.3) | - | 6 ( 50.0) | 8 ( 66.7) | 1 ( 8.3) |
| Diarrhoea | - | - | 2 ( 16.7) | 1 ( 8.3) | 1 ( 8.3) |
| Nausea | 1 ( 8.3) | - | 5 ( 41.7) | 5 ( 41.7) | - |
| Vomiting | 1 ( 8.3) | - | 3 ( 25.0) | 5 ( 41.7) | - |

*Number of subjects, percentage in parentheses.*



TABLE 3 **Pharmacodynamic effects of Gln-1062 5.5 mg, 11 mg, and 22 mg, b.i.d., for 7 days compared with placebo.**

| Parameter | Gln-1062 5.5 mg<br>Placebo | Gln-1062 11 mg<br>Placebo | Gln-1062 22 mg<br>Placebo |
|---|---|---|---|
| Smooth Pursuit (%) | -1.28 (-5.10, 2.53)<br>p=0.5011 | -0.10 (-4.04, 3.84)<br>p=0.9597 | 2.00 (-1.88, 5.88)<br>p=0.3039 |
| Saccadic Inaccuracy (%) | 0.59 (-0.46, 1.64)<br>p=0.2627 | 0.20 (-0.89, 1.30)<br>p=0.7099 | 0.51 (-0.56, 1.58)<br>p=0.3393 |
| Saccadic Peak Velocity (deg/s) | -0.06 (-21.97, 21.85)<br>p=0.9958 | 5.03 ( -17.82, 27.87)<br>p=0.6588 | 8.63 (-13.65, 30.91)<br>p=0.4379 |
| Saccadic Reaction Time (sec) | **-0.0112 (-0.0224, -0.0001)**<br>**p=0.0481** | -0.0018 (-0.0134, 0.0098)<br>p=0.7546 | -0.0013 (-0.0126, 0.0101)<br>p=0.8228 |
| Adaptive tracking (%-point) | 0.520 (-0.779, 1.818)<br>p=0.4240 | 0.318 (-0.992, 1.627)<br>p=0.6266 | **1.954 ( 0.630, 3.279)**<br>**p=0.0055** |
| VAS Alertness (mm) | -1.09 (-3.74, 1.57)<br>p=0.4134 | 0.63 (-2.15, 3.41)<br>p=0.6514 | -0.21 (-2.86, 2.44)<br>p=0.8735 |
| VAS Calmness (mm) | 0.44 (-2.59, 3.48)<br>p=0.7694 | 0.27 (-2.86, 3.40)<br>p=0.8615 | 0.44 (-2.61, 3.49)<br>p=0.7734 |
| VAS Mood (mm) | -0.92 (-3.98, 2.13)<br>p=0.5462 | -0.49 (-3.59, 2.62)<br>p=0.7530 | -0.68 (-3.66, 2.29)<br>p=0.6455 |
| VAS Nausea log(mm) | 0.0282 (-0.0517, 0.1082)<br>p=0.4800 | 0.0485 (-0.0330, 0.1301)<br>p=0.2366 | 0.0447 (-0.0359, 0.1253)<br>p=0.2697 |
| N-back mean RT 0 back (msec) | 8.2 (-18.3, 34.6)<br>p=0.5374 | 5.8 (-21.2, 32.8)<br>p=0.6665 | -21.7 (-47.5, 4.0)<br>p=0.0959 |
| N-back mean RT 1 back (msec) | **-48.5 (-91.0, -6.0)**<br>**p=0.0264** | 6.1 (-39.6, 51.8)<br>p=0.7893 | -24.4 (-67.1, 18.3)<br>p=0.2558 |
| N-back mean RT 2 back (msec) | -45.6 (-99.3, 8.2)<br>p=0.0944 | -41.1 (-99.7, 17.5)<br>p=0.1641 | **-71.4 (-126.4, -16.3)**<br>**p=0.0123** |
| N-back corr-incorr/total 0 | 0.003 (-0.023, 0.029)<br>p=0.7985 | 0.006 (-0.020, 0.032)<br>p=0.6664 | 0.007 (-0.019, 0.033)<br>p=0.5978 |
| N-back corr-incorr/total 1 | 0.026 (-0.026, 0.078)<br>p=0.3186 | 0.027 (-0.023, 0.076)<br>p=0.2811 | 0.020 (-0.029, 0.069)<br>p=0.4242 |
| N-back corr-incorr/total 2 | -0.002 (-0.068, 0.064)<br>p=0.9575 | -0.016 (-0.081, 0.049)<br>p=0.6231 | 0.036 (-0.028, 0.099)<br>p=0.2661 |
| VVLT word recall correct 1 | 0.36 (-1.31, 2.03)<br>p=0.6635 | 0.62 (-1.07, 2.32)<br>p=0.4608 | -0.12 (-1.79, 1.54)<br>p=0.8816 |
| VVLT word recall correct 2 | -0.45 (-2.44, 1.55)<br>p=0.6545 | -0.63 (-2.65, 1.40)<br>p=0.5370 | -0.01 (-2.00, 1.99)<br>p=0.9959 |
| VVLT word recall correct 3 | -0.12 (-2.42, 2.17)<br>p=0.9136 | -0.89 (-3.23, 1.44)<br>p=0.4444 | 0.99 (-1.30, 3.29)<br>p=0.3883 |
| VVLT delayed word recall correct | 0.36 (-2.02, 2.73)<br>p=0.7624 | -0.73 (-3.15, 1.70)<br>p=0.5486 | 0.48 (-1.89, 2.86)<br>p=0.6838 |
| VVLT Delayed word recognition correct | -2.60 (-6.60, 1.40)<br>p=0.1967 | -2.19 (-6.24, 1.87)<br>p=0.2825 | -0.43 (-4.43,3.57)<br>p=0.8291 |
| VVLT Delayed word recognition RT correct (msec) | -49.93 (-166.97, 67.10)<br>p=0.3944 | -30.91 (-150.24, 88.41)<br>p=0.6041 | **-120.03 (-237.07, -3.00)**<br>**p=0.0447** |

TABLE 3

| Parameter | Gln-1062 5.5 mg<br>Placebo | Gln-1062 11 mg<br>Placebo | Gln-1062 22 mg<br>Placebo |
|---|---|---|---|
| Systolic BP supine (mmHg) | 5.0 (-0.3, 10.4)<br>p=0.0644 | 1.2 (-4.1, 6.5)<br>p=0.6528 | 1.8 (-3.6, 7.2)<br>p=0.5098 |
| Diastolic BP supine (mmHg) | 2.5 (-0.6, 5.6)<br>p=0.1105 | 0.9 (-2.2, 4.1)<br>p=0.5566 | 1.2 (-1.9, 4.3)<br>p=0.4437 |
| Pulse Rate supine (bpm) | -2.5 (-6.4, 1.4)<br>p=0.2044 | -1.0 (-4.9, 2.9)<br>p=0.6030 | 0.5 (-3.5, 4.4)<br>p=0.8169 |
| EEG delta Fz-Cz closed (uV^2/Hz) | -1.8% (-30.4%, 38.5%)<br>p=0.9140 | -14.8% (-37.6%, 16.4%)<br>p=0.3023 | **-19.8% (-35.7%, -0.1%)**<br>**p=0.0495** |
| EEG delta Fz-Cz open (uV^2/Hz) | -13.6% (-35.0%, 14.8%)<br>p=0.3010 | -2.5% (-25.2%, 27.0%)<br>p=0.8435 | **-30.1% (-48.3%, -5.3%)**<br>**p=0.0235** |
| EEG delta Pz-O1 closed (uV^2/Hz) | -3.1% (-36.1%, 46.9%)<br>p=0.8765 | -12.4% (-41.5%, 31.2%)<br>p=0.5079 | **-27.4% (-46.1%, -2.1%)**<br>**p=0.0369** |
| EEG delta Pz-O1 open (uV^2/Hz) | -12.9% (-39.4%, 25.1%)<br>p=0.4391 | -5.4% (-33.6%, 34.6%)<br>p=0.7474 | **-29.1% (-45.3%, -8.0%)**<br>**p=0.0126** |
| EEG delta Pz-O2 closed (uV^2/Hz) | -13.4% (-39.2%, 23.5%)<br>p=0.4151 | -0.4% (-28.5%, 38.7%)<br>p=0.9799 | **-21.5% (-35.1%, -5.0%)**<br>**p=0.0154** |
| EEG delta Pz-O2 open (uV^2/Hz) | 3.1% (-23.8%, 39.4%)<br>p=0.8382 | 3.7% (-21.2%, 36.6%)<br>p=0.7851 | -16.5% (-33.4%, 4.8%)<br>p=0.1128 |

*Mean, confidence interval in parentheses. VAS: visual analogue scale; VVLT: visual verbal learning task; BP: blood pressure; EEG: electroencephalogram*



FIGURE 1 A and B, Plasma concentrations of galantamine cleaved from Gln-1062 on dosing days 1 and 7 after administration of Gln-1062 5.5 mg, 11 mg, or 22 mg, b.i.d. C and D, Plasma Gln-1062 concentrations on dosing days 1 and 7 after administration of Gln-1062 5.5mg, 11 mg, or 22 mg, b.i.d.

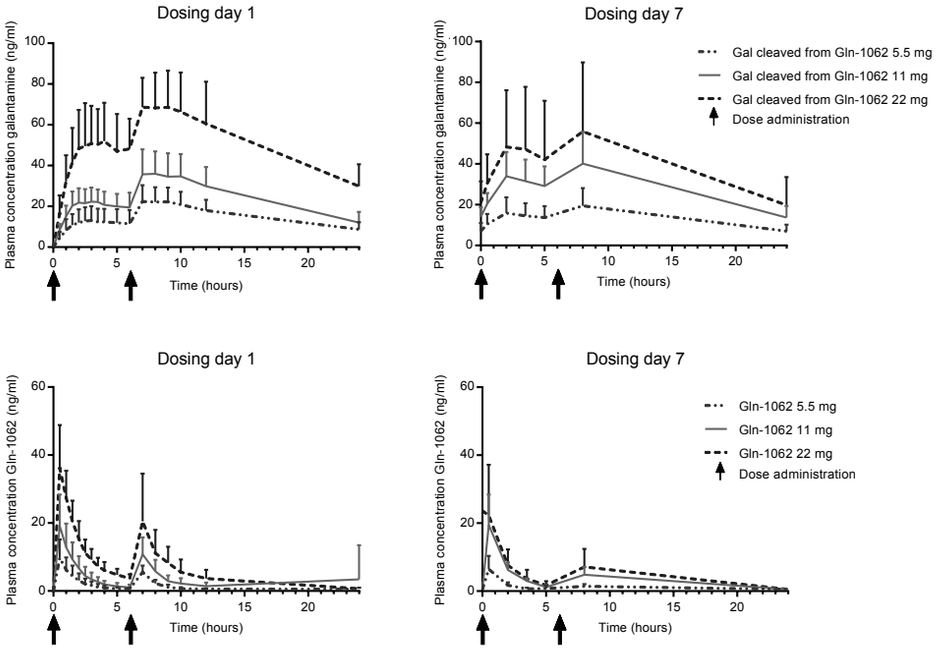


FIGURE 2 Galantamine plasma and CSF concentrations after oral galantamine, dose corrected (left) and 11 mg Gln-1062 (right). Dots/dotted line = CSF galantamine concentration. Solid line = plasma galantamine concentration

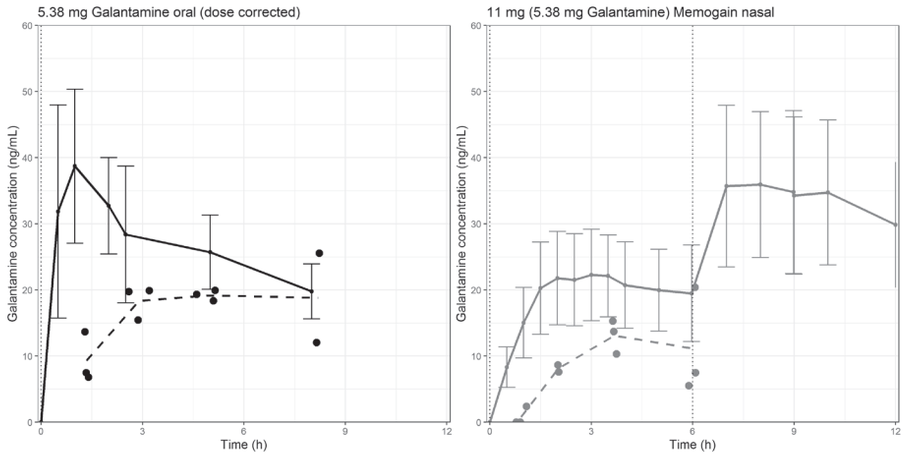
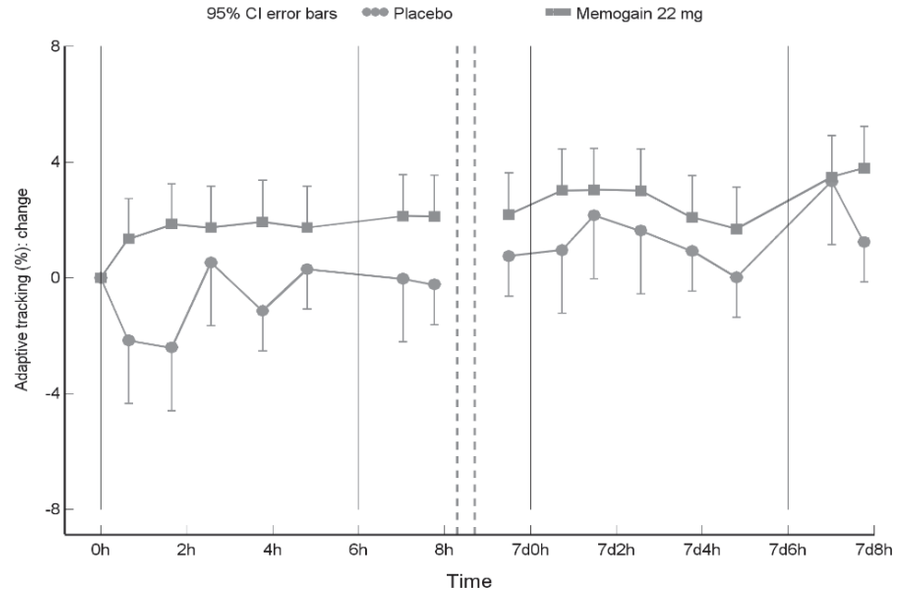
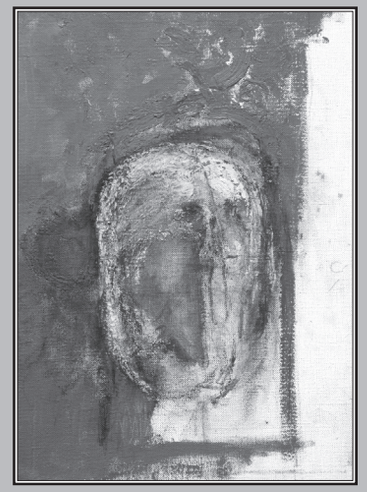



FIGURE 3 The change in adaptive tracking test performance (%-point) from baseline after 22 mg Gln-1062 on the first and seventh dosing days






CHAPTER VII



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

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## ABSTRACT

Selective $M_1$ muscarinic acetylcholine receptor (mAChR) agonists are being developed as symptomatic treatment for neurodegenerative and neuropsychiatric disorders that lead to cognitive dysfunction. Demonstrating cognition enhancing effects in early phase clinical development in healthy subjects is difficult. A challenge with the $M_1$ mAChR antagonist biperiden could be used to demonstrate procognitive and pharmacological effects of selective $M_1$ mAChR agonists. The aim of this study was to develop such a model. To this end, twelve healthy elderly subjects participated in a randomized, placebo controlled, three-way crossover study investigating tolerability, pharmacokinetic (PK) and pharmacodynamic (PD) effects of 2 and 4 mg biperiden. Repeated PD assessments were performed using neurocognitive tasks and electrophysiological measurements. A population PK-PD model was developed. Four mg biperiden showed significant impairment of sustained attention (-2.1% point in adaptive tracking (95% CI [-3.043; -1.148])), verbal memory (2-3 words fewer recalled (95% CI [-5.9; -0.2])), and working memory (up to 50 ms increase in the n-back task reaction time (95% CI [21.854; 77.882])) compared with placebo. The PK data was best fitted by a 2-compartment model and showed a high inter-occasion and inter-subject variability. Population PK-PD analysis quantified significant concentration-effect relationships for the n-back reaction times, n-back accuracies and adaptive tracking. In conclusion, biperiden caused $M_1$ mAChR related dose- and concentration-dependent temporary declines in cognitive functioning. Therefore a biperiden pharmacological challenge model can be used for proof-of-pharmacology studies and to demonstrate cognition enhancing effects of new cholinergic compounds that are being developed.




## INTRODUCTION

Acetylcholine is one of the main neurotransmitters of the central nervous system (CNS), and is involved in cognitive processes such as memory and attention[1-3]. Deficits in the cholinergic system have been found in both neuropsychiatric and neurodegenerative disorders such as Alzheimer's disease and schizophrenia. The currently mainly available (i.e. registered) therapies for the treatment of cognitive dysfunction in patients with mild to moderate Alzheimer's disease are acetylcholinesterase inhibitors (AChEIs) such as donepezil and galantamine. However, these drugs are only effective in a limited number of patients, and are associated with significant (gastro-intestinal) side-effects because the compounds are not selective for the affected parts of the central nervous system. As a consequence, the possibility of reaching effective dose levels are limited[4-6]. In response to these limitations, selective $M_1$ muscarinic acetylcholine receptor (mAChR) agonists are under development and entering early phase clinical trials. These specific muscarinic drugs are expected to cause fewer side effects than the relatively non-specifically acting cholinesterase inhibitors. The $M_1$ muscarinic acetylcholine receptor (mAChR) is an potential target of a selective muscarinic drug as this receptor plays a major role in cognitive function[7].

Several anti-cholinergic pharmacological challenge models are commonly used to investigate cognition enhancing effects in early phase clinical development, the most important of which is the scopolamine model. The idea behind an anti-cholinergic challenge is that this induces temporary (reversibly) cognitive defects, which involve the same neurobiological mechanisms as are targeted by pro-cholinergic drugs. Scopolamine is a competitive mAChR antagonist that is non-selective and thus binds to all 5 subtypes of the mAChRs. This lack of selectivity makes scopolamine a less suitable challenge agent for the investigation of new $M_1$ mAChR agonists which are currently being developed. In addition, scopolamine has been shown to induce marked sedation, which is difficult to disentangle from its cognition impairing effects[3,8].

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

biperiden (approximately 1 hour post dose); a single dose level was investigated; it was not always described whether the test battery was also performed before drug administration, to serve as baseline measurement. Also, the relation between cognitive pharmacodynamic (PD) effects and the plasma pharmacokinetics (PK) of biperiden was not investigated as in most cases the biperiden plasma concentrations were not analysed. A reliable PK-PD model provides an important indication for robust pharmacological activity, and it can be used to optimally design a future study investigating new experimental compounds by calculating the biperiden dose level, sample size and timing of PK and PD measurements. Additionally, biperiden has only been studied in few elderly subjects. Since $M_1$ MAChR agonists are under development for the treatment of Alzheimer's disease, it is useful to already know about the behaviour of the drug in elderly subjects before moving into the target patient population

The aim of this study was to develop the biperiden challenge model in healthy elderly, as a tool to prove pharmacology and to provide support for cognition enhancing effects of new $M_1$ MAChR agonists that are being developed.

## METHODS

This study was approved by the ethics committee of the Leiden University Medical Centre (Leiden, the Netherlands). Informed consent was obtained from all individual participants included in the study. It was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. The trial was registered in the Netherlands Trials Register (NL7146). A randomization code was generated SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

**TRIAL DESIGN AND SUBJECTS** This was a randomized, double-blind, placebo-controlled, three-way cross-over study in which biperiden 2 mg, 4 mg and placebo were orally administered to 12 healthy elderly subjects. Akineton® 2 mg tablets (Laboratorio Farmaceutico) and placebo tablets were over-encapsulated in Swedish orange capsules size 00 at Leiden University Medical Centre Pharmacy in accordance with local regulations. The treatment phase consisted of three identical treatment periods separated by a washout period of 1 week between the medication administrations. The tolerability of a single 4 mg dose was unknown. Therefore, subjects were randomized in such a way that biperiden 4 mg was only administered after the subjects completed the study day with the 2 mg dose. In this way, individual tolerability to 2 mg tablets would be known prior to administration of the 4 mg dose.



Before start of the study day, a light breakfast was allowed and within 30 minutes prior to dosing, subjects consumed a snack to prevent nausea.

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

**SAFETY ASSESSMENTS** During the study periods, safety was assessed using monitoring of treatment emergent adverse events (TEAEs), safety laboratory, vital signs and electrocardiogram (ECG).

**PK ASSESSMENTS** To assess the pharmacokinetic characteristics of biperiden, venous blood samples were obtained pre-dose and at 0.5, 1, 1.5, 2, 2.5, 4, 7, 10 and 22 hours post dose. Plasma concentrations of biperiden were determined by Ardena Bioanalytical Laboratory (Assen, the Netherlands). Extraction of biperiden from human K2EDTA plasma samples was performed using Liquid Liquid Extraction and followed by analysis using a Shimadzu Prominence / Nexera liquid chromatography system, equipped with a Sciex API 4000 tandem mass spectrometer (LC-MS/MS). Biperiden-$D_5$ was used as internal standard. Separation was established on a XBridge Phenyl LC column (4.6 x 100 mm, 3.5µm) using isocratic elution with 0.025% $NH_4OH$ in 67% acetonitrile at a flow of 1.0 ml/min. The mass spectrometer was equipped with a Turbo Ion Spray (TIS) probe operated in the positive Multiple Reaction Monitoring (MRM) mode. The mass transitions for biperiden was 312 à 143 (m/z) and 317 à 148 for the internal standard. The analytical range of the assay was 0.100–10.0 ng/mL. The accuracy and precision of the assay were monitored during all analysis runs using Quality Control samples (QCs) at the levels Low (0.300 ng/mL), Medium (1.50 ng/mL) and High (8.00 ng/mL). The overall accuracy was 100.8% (level Low), 99.2% (level Medium) and 102.1% (level High). The between-day variability, expressed as CV% was 6.5% (level Low), 3.1% (level Medium) and 2.1% (level High). Non-compartmental analysis was performed in R, version 2.12.0 for Windows (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria, 2010).

**PD ASSESSMENTS** To assess the effects of biperiden on central nervous system (CNS) functioning, PD tests were performed repeatedly using the NeuroCart, a battery of neuropsychological and neurophysiological tests that can be used to examine the effects of CNS active drugs on a wide range of CNS domains[17]. A customized set

of tasks to detect PD effects to be expected of cholinergic drugs was performed twice immediately prior to dosing and at 1, 2.5, 4, 7 and 22 hours post dose. The duration of one PD testing round was 1 hour. The visual verbal learning test (VVLT) immediate part was only performed 1.5 hour post dose and the delayed recall/recognition condition was performed 40 minutes thereafter. Timing of the PD tests was based on the PK characteristics described in the summary of product characteristics: $T_{max}$ between 1 and 1.5 hour after administration and mean half-life of 24-37 hours after administration of a single dose of 4 mg in elderly subjects[18].

- **Adaptive tracking test**

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

- **N-back task**

The N-back test was used to evaluate working memory[24-26]. Per condition, 24 letters were presented consecutively on the screen with a speed of 30 letters per minute. The target:non-target rate was 1:3. Subjects were required to press a key for both targets and non-targets. In the 0-back condition subjects had to indicate whether the letter on the screen was identical to the target letter. In the 1-back condition, subjects indicated whether the letter seen was identical to the previous letter. In the 2-back condition, subjects were asked to indicate whether the letter was identical to 2 letters before the letter seen. The outcome parameters are 'correct responses –incorrect responses/total responses' (accuracy measure) and reaction time[25].

- **Visual verbal learning test**

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

- **Pupillometry**

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



- **Body sway**

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

- **Saccadic and smooth pursuit eye movements**

Saccadic eye movements and smooth pursuit are sensitive parameters for sedation[29,30]. The use of a computer for the measurements have been described elsewhere[31,30,23]. The subject was requested to follow a horizontally moving target on a screen at 58 cm distance. The target moved continuously for measurement of smooth pursuit and jumps from side to side for measurement of saccadic eye movements.

- **Resting-state-electroencephalography**

Resting-state-electroencephalography (EEG) is very sensitive to central actions of pharmacological substances. EEG recordings were performed with open and closed eyes for 5 min in each eye state[32]. Each recording employs alternating periods with eyes open and closed with a duration of 64-seconds for each period. EEG was continuously recorded using a 40-channel recording system (Refa-40, TMSi B.V., the Netherlands). Twenty-one electrodes were placed according to the international 10-20 system (32-lead cap, TMSi B.V.), but replacing electrodes placed at the earlobes (i.e., A1 and A2) with electrodes placed at the mastoids (i.e., $M_1$ and $M_2$). The scalp electrode impedance was kept below 5kΩ. The ground electrode was placed at AFz. Additionally, to detect ocular artefacts, vertical and horizontal EOG were also recorded. Two Ag/AgCl electrodes were placed at the outer canthi of both eyes, and two Ag/AgCl electrodes are placed approximately 2 cm above and below the right eye. All signals were sampled at a sampling rate of 1024 Hz and were filtered prior to storage using a first order recursive high-pass filter with a cut-off frequency at 0.1 Hz. Digital markers were recorded by the amplifier indicating the start and end of each eye state. The electrodes of interest were Fz-Cz, Pz-O1, and Pz-O2. Changes in the amplitude of the following frequency bands were quantified by spectrum-analysis (*i.e.*, fast Fourier transformation): ß-band (12.5-30 Hz), γ-band (30-40 Hz), α-band (8.5-12.5 Hz), and θ-bands (6.0-85 Hz) and δ-bands (1.5-6.0 Hz).

- **Mismatch Negativity**

The mismatch negativity (MMN) auditory event-related potential (ERP) is a method that is proposed as an index of auditory sensory memory[33]. During an auditory passive oddball task, subjects were watching a silent movie while being presented auditory tones. A total of 750 tones were presented of which 600 were presented as

frequent stimuli and 150 as deviant/infrequent stimuli. The frequent and infrequent tones were 150 ms at a sound pressure level of 75 dB. All tones had a 5 ms rise and fall time. Tones were presented at a fixed rate of 2 Hz.

- **Visual analogue scales**

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

- **Tapping test**

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

**STATISTICS** Usually experimental drugs are investigated in small groups to minimize the exposure of human subjects to a new chemical entity. As this biperiden model might be used to further investigate new drugs, a small sample size has to be sufficient.

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

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

### POPULATION PK-PD ANALYSIS

- **Population PK-PD model development**

To investigate the relationships between biperiden plasma concentrations and PD parameters, a population PK-PD model was developed using non-linear mixed effect modelling (NONMEM V7.3)[39].



For the PK model, one- two- and three compartmental models, with and without lag time on the absorption of biperiden and transit compartments, were explored. Inter-individual variability and between-occasion variability was included on the model parameters following a bottom-up inclusion procedure and were included if a significant ($p < 0.01$) improvement in model fit was obtained. The empirical Bayes estimates were fixed for the development of the PD models. The existence of a learning/placebo effect over time was explored using a linear or Bateman function on data from the placebo occasion only. In order to capture the concentration-effect relationship, linear, EMAX and sigmoidal EMAX relationships were explored.

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

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

- **Simulation of statistical power**

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

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

## RESULTS

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

**PHARMACOKINETICS** The PK of biperiden showed high variability between occasions and high inter-subject variability after 2 mg and 4 mg dosing (Figure 1). The median $T_{max}$ of the plasma concentration is at 2 hours post dose (range 1-4 hours). The mean $c_{max}$ is 3.51 ng/ml (range 0.50-7.40 ng/ml, CV 56.7%) after the 2 mg dose and 7.45 ng/ml (range 0.72-22.30 ng/ml, CV 80.4%) after the 4 mg dose. The $AUC_{(0\text{-last})}$ was 18.4 ng*h/ml (range 1.64-35.16 ng*h/ml) following 2 mg and 39.47 ng*h/ml (range 3.36-79.7 ng*h/ml) following 4 mg biperiden.

**PHARMACODYNAMIC EFFECTS**

- **Adaptive tracking test**

A significant and dose related decrease in mean adaptive tracking test performance of 1.36% point was observed after 2 mg biperiden (95% CI [-2.31; -0.42], p= 0.0075) and of 2.10% point after 4 mg biperiden (95% CI [-3.04; -1.15], p=0.0002) (Figure 2).

- **N-back task**

Visual inspection of n-back the graphs indicated a dose related increase in reaction time in all 3 conditions of the task, however only the mean reaction time following 4 mg biperiden was significantly different compared with placebo for the 0-back condition (mean difference 37.2 ms, 95% CI [6.40; 68.0], p=0.0212) and 1-back condition (mean difference 49.9 ms, 95% CI [21.9; 77.9], p=0.0016). The accuracy was slightly but significantly decreased with 0.06 (95% CI[-0.12; -0.01], p=0.0209) after 4 mg biperiden in the 2-back condition compared with placebo (Figure 2). No significant change in reaction time and accuracy was observed following 2 mg biperiden.

- **Visual verbal learning test**

Visual inspection of the VVLT graphs showed a dose related decrease in performance of all parts of the memory test. Only the effects following 4 mg biperiden were significantly different from placebo on all parameters except for the first immediate recall round. During the second immediate recall round 2.5 (95% CI [-4.9; -0.1], p=0.0387) fewer words were recalled; during third immediate recall round 2.9 (95% CI [-5.8; -0.1], p=0.0453) fewer words were recalled; 3.1 (95% CI [-5.9; -0.2], p=0.0344) fewer words were recalled after a delay of 30 minutes and 6.5 (95% CI [-10.8; -2.2], p=0.0053) fewer words were recognized after a delay while the reaction time was 92.2 ms (95% CI [5.1; 179.3], p=0.0390) longer.

- **N-back task**

Inspection of the pupil/iris ratio graphs showed a dose related increase in pupil size in both eyes with only the change following 4 mg biperiden being significantly different from placebo (right eye: mean difference 0.07341, 95% CI [0.02957; 0.11725], p=0.0033; left eye: mean difference 0.065, 95% CI [0.02789; 0.10211], p=0.0028)). Following the maximum mean change, the pupil/iris ratio in both eyes decreased, however it was not normalized at 22 hours post dose (Figure 2).

- **Body sway**

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

- **Saccadic and smooth pursuit eye movements**

Smooth pursuit decreased with 3.55% point following 4 mg biperiden compared with placebo (95% CI [-5.58; -1.53], p= 0.0016). No significant effect was observed after 2 mg biperiden. No significant effects were observed on saccadic inaccuracy, peak velocity or reaction time for both dosages compared to placebo.

- **Resting-state-electroencephalography**

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

- **Mismatch Negativity**

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

- **Visual analogue scales and Tapping test**

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

### POPULATION PK-PD ANALYSIS

- **Population PK-PD model development**

The PK data was best fit by a 2-compartment model with linear elimination. Inclusion of a lag time and transit compartment were required to correctly capture the absorption phase of biperiden. Significant variability was estimated on the absorption parameters, the volume of distribution and the clearance of biperiden (table 1). No covariates were identified. The model-derived terminal half-life is 29.5 hour.

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

- **Simulation of statistical power**

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

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

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

## DISCUSSION

This study was performed to develop a biperiden challenge model as a tool to prove pharmacology and to provide support for cognition enhancing effects of new $M_1$ MAChR agonists in future studies. Previous studies investigated the effects of biperiden on cognitive functioning mainly in young subjects with only one session of testing post dose, in most cases around the expected $T_{max}$ of biperiden, although no PK was measured. Furthermore only a single dose level was investigated in these studies. We investigated the PK and PD effects of both 2 mg and 4 mg of the competitive $M_1$ MAChR antagonist biperiden on frequently repeated cognitive and neurophysiological tests in healthy elderly. Biperiden plasma concentrations were measured and the relationship between the PK and PD were modelled in a two-compartment population PK-PD model with linear elimination and corresponding concentration-effect relationships. This population PK-PD model was used to inform on the design of future studies regarding sample size and can be further extended with the biperiden dose level and timing of PK and PD measurements.

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

The PK of biperiden was well characterized in this study even though high levels of variability were present. The median $T_{max}$ is comparable with previously reported $T_{max}$[42,43] suggesting no relevant effect of the over-encapsulation. In the population PK model, the IIV of the central volume of distribution (79.5%) and clearance (172%) were high in comparison with results of previous studies[44,43]. However, the quantified level of variability most likely partially originated from variability in the bioavailability after oral administration. In our population PK model, no information on this bioavailability could be quantified since no intravenous PK data was available.

The variability in these structural model parameters may therefore be over-predicted. The model, including the identified IIV and BOV, can be used for simulations of oral administration but should be adapted when simulating intravenous administration of biperiden.

The results indicate that the majority of the variability originates from the PK (CV%'s ranging from 12% to 172%), with only low to moderate CV% present on the studied PD effects (CV%'s up to 76.4%). Therefore, in order to improve the statistical power of a challenge study with biperiden, this variability could be reduced by intravenous dosing of biperiden. With an assumed bioavailability of approximately 33%[18], an intravenous dose of 1.25-1.5 mg would reach similar peak concentrations. The exact intravenous dose required in this population should be investigated in future research. However, even though high variability was present in this population, sufficient (80%+) statistical power could already be obtained with moderate sample sizes after oral administration of 4 mg biperiden.

In order to optimize the quantification of the reversal of biperiden-induced effect, the maximum PD effect of biperiden should occur at around the same time as the maximum PD effect of the experimental compound, which requires accurate planning of dosing at the study day. This timing might be improved by administering the experimental drugs when biperiden is at steady state. This could lead to stable PD-effects throughout the challenge experiment, which would simplify the interpretation of antagonistic effects of a concomitantly administered $M_1$ MAChR agonist. Continuous or repeated administration could raise the possibility of tolerance[45]. In the current cross-over study there were no evidence of tolerance after the wash-out period of 1 week.

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

The observed effects on n-back, VVLT and adaptive tracking can be explained by the pharmacological mechanism of biperiden, since the brain areas involved in these



tests comprise a high density of $M_1$ MAChRs. The n-back test is a working memory task associated with prefrontal function[47,48], the VVLT is associated with hippocampus (right anterior), prefrontal cortex (right dorsolateral), left medial temporal lobe activity[49], and sustained attention measured by the adaptive tracking test is associated with basal forebrain, prefrontal cortex, and parietal cortical regions activity[50]. Thus in these tests the prefrontal cortex or hippocampus play an important role. The $M_1$ MAChR is the most abundant receptor of all MAChRs in the hippocampus (47-60%) and in the cortex (34-55%)[51,52], and antagonizing the $M_1$ MAChR will hamper cortical and hippocampal functioning. Dilatation of the pupil is caused by blocking parasympathetic contraction of the iris sphincter muscle. In the human iris, the $M_3$ MAChR is the most expressed receptor. The $M_1$ MAChR only comprises 7% of the total number of expressed MAChRs[53] which may explain why only a relatively small effect on pupil size is observed.

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

The body sway was not normalized at 22 hours post dose. A delayed recovery of the balance could be due to binding to the $M_1$ MAChRs in the vestibular system, where the clearance might be slower than clearance from the plasma[55]. Just like the disturbed body balance, the pupil enlargement was still present 22 hours after 4 mg biperiden administration. It could be that clearance of biperiden from the peripheral $M_1$ MAChRs in the iris and ciliary body is slower than from the plasma, although it has been assumed that clearance from the vitreous is similar to plasma[56]. A long duration of pupillary dilation has also been observed with scopolamine[57].

When comparing the biperiden effects observed in the current study to scopolamine effects described in literature, the biperiden effects seem smaller. For example the decrease in adaptive tracking in the current study was 2.1%-point, compared to 9-10%-point after scopolamine[57-60]. The impairment in verbal memory (2-3 fewer words correctly recalled) was also smaller than the effects of scopolamine (2-7 words fewer recalled) [61, 57, 58, 60, 3]. It could be that the dose level of biperiden is relatively lower than the used scopolamine dose levels or due to difference in pharmacological targets of both compounds. It is also possible that different MAChR-subtypes contribute to the functional domains that were tested in this study. Scopolamine antagonizes $M_1$-$M_5$ MAChRs, whereas biperiden is a relatively specific $M_1$ MAChR antagonist. The $M_1$ MAChR plays a major role in cognitive function[7] and represent

35-60% of the total mAChRs in areas related to cognitive function: the neocortex and the hippocampus [51, 62, 52]. However, the $M_1$ mAChR is not associated with all hippocampus dependent learning tasks[7] and the remaining 40-65% of the total mAChRs consists of $M_2$-$M_5$ mAChRs . These other mAChRs are also involved in learning and memory[63-68], although the role of the $M_3$ mAChR in cognitive function could not be demonstrated in humans[69]. Body sway was increased into a greater extent after scopolamine (increase of 150-162 mm[58,60]) than after biperiden administration (increase of 79.7 mm after 4 mg biperiden). Besides the $M_1$ mAChR, the $M_2$ and $M_5$ mAChRs are expressed in the afferent vestibular ganglia and the vestibular end-organs[70]. Consequently, antagonism of $M_2$ and $M_5$ mAChRs can contribute to a disturbed balance. Also the $M_3$ mAChR antagonist darifenacin has been shown to increase body sway[69].

In addition to antagonism of the $M_2$-$M_5$ mAChRs in the brain structures involved in cognition, also the sedative effect of scopolamine might have contributed to the impaired performance of PD tests. The saccadic eye movements are a very sensitive marker for sedative effects[29]. Changes in saccadic eye movements are often attributed to suppression of the brainstem reticular formation by stimulation of gamma-aminobutyric acid (GABA) type A receptors with subunit $\alpha 1$[71,72]. Nonetheless, a concentration-related decrease in peak saccadic velocity was also observed after scopolamine [60, 58, 59, 57], suggesting a role for mAChRs in sedation. An interaction between mAChRs and GABA receptors has been described[73], however, the exact contribution of each type of mAChR to sedative effects has not been well established. In the brainstem, the $M_2$ mAChR represents 80% of all mAChRs[52] and GABAergic neurons in the reticular formation also contain $M_2$ mAChRs[74]. In other brain areas, the activation of the $M_2$ and $M_4$ mAChRs decreased the release of GABA[73,75]. The latter might suggest that inhibition of the $M_2$ mAChRs result in an increase of GABA and consequently a sedative effect. As the $M_1$ mAChR is barely present in the brain stem and the sedative effect of mAChR stimulation seems to be mediated by agonism of the $M_2$ mAChR, the saccadic peak velocity was not decreased and the score on the VAS measuring alertness did not change after biperiden administration in this study, we feel it is safe to conclude that scopolamine has a larger sedative effect than biperiden.

Due to the effects of $M_2$-$M_5$ antagonism by scopolamine on cognitive performance and sedation, it is expected that an $M_1$ mAChR agonist can reverse the effects only to a limited extent. As a consequence the reversal might get lost in the margins of variability and therefore the biperiden challenge model seems favorable over the scopolamine model to demonstrate effects of selective $M_1$ receptor agonists.



## CONCLUSIONS

Biperiden doses of 2 mg and 4 mg were very well tolerated and especially 4 mg biperiden caused clear temporary PD effects in different CNS domains, including decline in cognitive function. The PD effects are concentration-related and are therefore explained by the pharmacological mechanism of biperiden, making this model a tool to proof pharmacology and a tool to provide support for cognitive enhancing effects of $M_1$ mAChR agonist.

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

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

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

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

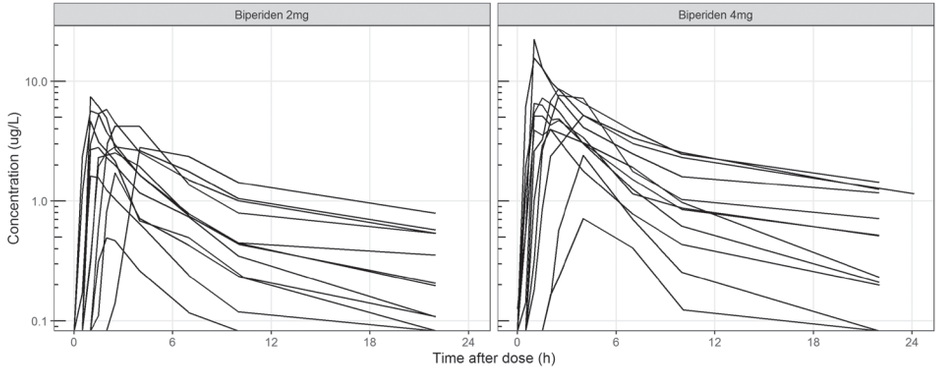





FIGURE 2 **Pharmacodynamic effects on adaptive tracking, n-back test, body sway and pupil size presented as change from baseline (mean, 95% CI error bars)** – *see inside back cover for these images in full color.*

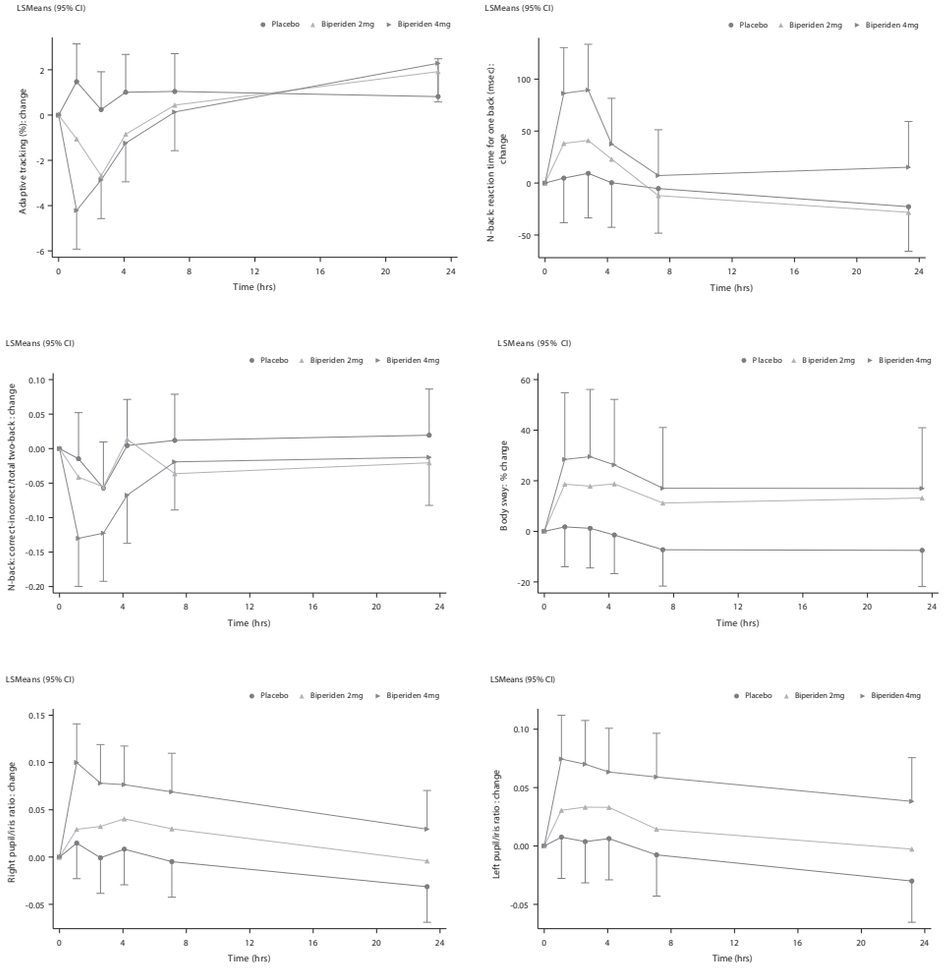


FIGURE 3A Heatplots showing the effects of 4 mg biperiden on EEG eyes closed condition. For each frequency band and each electrode (representing a cortical area) the % of change in power compared with placebo is shown. * = p<0.05; **=p<0.01 – *see inside back cover for these images in full color.*

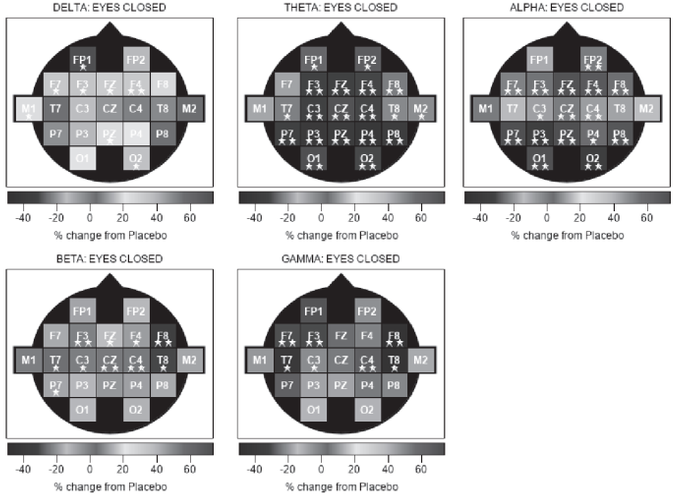



FIGURE 3B Heatplots showing the effects of 4 mg biperiden on EEG eyes open condition. For each frequency band and each electrode (representing a cortical area) the% of change in power compared with placebo is shown. * = p<0.05; **=p<0.01 – *see inside back cover for these images in full color.*

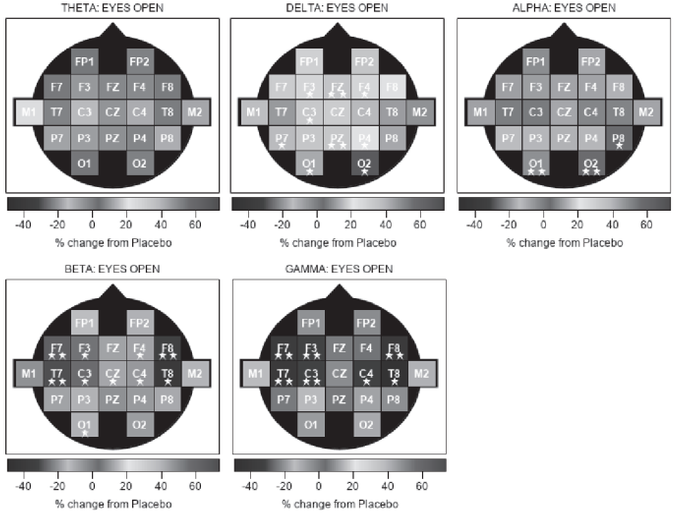



FIGURE 4 Visualization of the typical concentration-effect relationships for the n-back (A) and the adaptive tracking task (B).

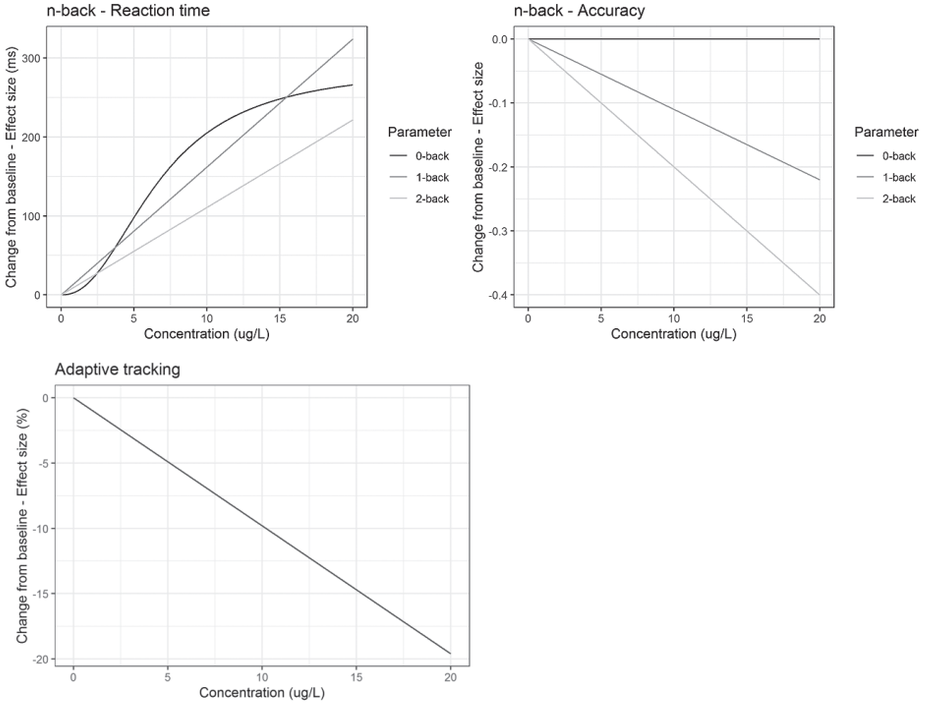


FIGURE 5A Simulated (n=1000) PK profiles after 4 mg oral administration of biperiden hydrochloride. Solid black line = median prediction, grey ribbon = 90% prediction interval.

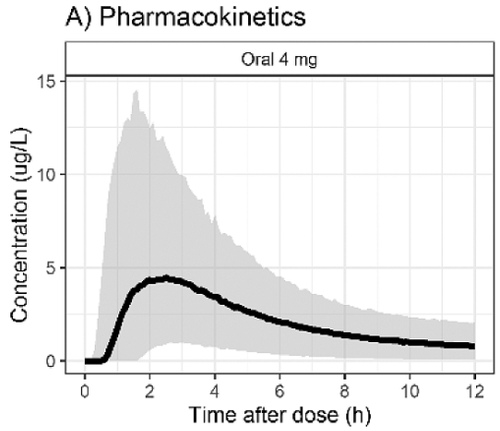
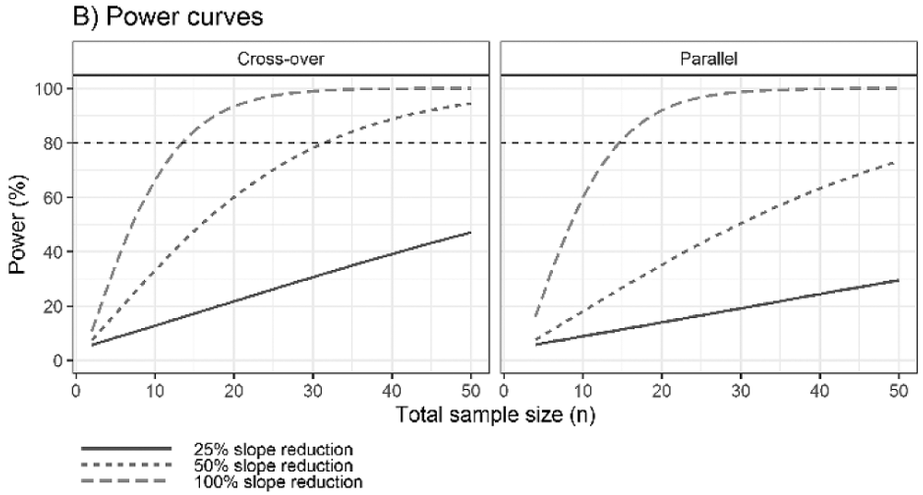



FIGURE 5B Model-derived statistical power versus total sample size to detect a 25%, 50%, or 100% reduction of the estimated concentration-effect relationship on the adaptive tracking task in a cross-over and parallel study design.






CHAPTER VIII

# BIOMARKERS FOR THE EFFECTS OF CHOLINERGIC DRUGS IN THE CENTRAL NERVOUS SYSTEM IN HEALTHY SUBJECTS

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## ABSTRACT

Novel therapeutic agents targeting the central cholinergic system are under development. In early phase development studies in healthy volunteers biomarkers are used to proof pharmacology and determine the optimal dose level for further development. There is no consensus, however, on which biomarkers are most useful. This review provided an overview of biomarkers used to investigate effects of pro- and anticholinergic drugs in healthy subjects and their ability to detect drug effects was evaluated. In total 132 useful articles were included, comprising 223 individual tests. The most prominent effects were found for muscarinic receptor antagonists, which produced consistent deteriorations in learning and memory tests in 69% to 79% of the cases, in general dose related, and less consistent reductions in alertness (56% of the cases). Fewer tests were able to demonstrate effects of nicotinic receptor antagonists on learning and memory (36% to 50% of the cases). Nicotinic receptor agonist produced moderate improvements (up to 32% of the cases). By themselves, cholinesterase inhibitors did not produce reliable effects on any test in healthy volunteers. However, the well measurable temporary effects of anti-cholinergic drugs could be used as pharmacological challenge in healthy subjects, in order to demonstrate pharmacological activity of pro-cholinergic drugs.




## INTRODUCTION

The cholinergic system is involved in a wide range of central nervous system (CNS) activities. It comprises neurons that are activated by or contain and release the neurotransmitter acetylcholine. Acetylcholine is produced by neurons in the synaptic bud and released from vesicles into the synaptic cleft where it binds to acetylcholine receptors. These receptors can be divided into two classes: the nicotinic acetylcholine receptors and the muscarinic acetylcholine receptors. The nicotinic receptor consists of 5 subunits that can be classified as α (α2-α7, α9 and α10) or β (β2-β4), which can be combined in a heteromeric and homomeric way. The nicotinic receptors that are most present in the brain are $\alpha4\beta2$ and the α7 subunit combinations[1]. The $\alpha4\beta2$ receptors are widely distributed throughout the brain, however the highest density is in the thalamus, intermediate density in the basal ganglia and brain stem, and are slightly lower in the cortical regions. Also the α7 receptor subunits are widely distributed in all brain areas, although a higher concentration is found in the cerebral cortex and putamen and a lower concentration in the caudate and cerebellum[1]. The muscarinic receptors can be divided in five subtypes, $M_1$-$M_5$. The $M_1$ receptor is the predominant muscarinic receptor in the brain with a high density in the hippocampus and cortex[2,3]. These brain structures are involved in memory and learning[4,5]. $M_2$ receptors are mainly expressed in the occipital cortex, dorsal side of the caudate nucleus, putamen and brain stem[2,3]. The expression of the $M_3$ receptors in the brain is low, this subtype is mainly present in the peripheral autonomic nervous system[3]. The $M_4$ receptor is highly expressed in neocortex and in the striatum where it modulates dopaminergic neurotransmission and to a lower extent in the occipital region of the cortex [2, 3, 6, 7]. $M_5$ receptors are present at a low level in the outermost layer of the cortex, hippocampus, striatum and superior and inferior colliculi. Their presence on the dopaminergic neurons of the ventral tegmental area mediates a key role in the mesolimbic reward pathway[8].

Acetylcholine is removed from the synaptic cleft in less than a millisecond through diffusion and degradation by the enzyme acetylcholinesterase[9]. Inhibition of cholinesterase increases the availability of the neurotransmitter in the synaptic cleft and consequently the duration of transmitter action.

Disturbance of the cholinergic system have been found in a.o. Alzheimer's disease, Lewy body disease (including Parkinson's disease, dementia with Lewy bodies and Parkinson's disease dementia), and schizophrenia. In these diseases, cognitive dysfunction due to cholinergic deficits is an important symptom starting either in early or later phase of the disease. The current treatment to improve the cholinergic balance is only symptomatic. In Alzheimers disease, dementia with Lewy bodies

and Parkinson's disease dementia cholinesterase inhibitors galantamine, rivistigmine and donepezil are prescribed. The efficacy of these drugs is limited and therefore there is room for improvement. Multiple new pro-cholinergic compounds are under development, targeting acetylcholinesterase, muscarinic receptors (mainly selective for the $M_1$ and/or $M_4$ subtypes) and nicotinic receptors (mainly selective for the $\alpha 7$ and $\alpha 4\beta 2$ subtypes)[10-12]. In patients with schizophrenia, treatment with cholinesterase inhibitors donepezil and rivastigmine showed no significant improvement in cognition[13,14] and galantamine treatment resulted only in temporary improvement of social memory[15]. Therefore treatment affecting the cholinergic system does not belong to the standard of care for schizophrenics, however, development of new therapeutics for this disease targeting the cholinergic system is ongoing[16].

Development of new medicines targeting the central nervous system is a long and expensive trajectory with high failure rates, of which 30% is caused by a lack of efficacy[17]. To reduce attrition rates, there is need to carry out proof-of-concept clinical trials in early phase of development. In these trials, biomarkers are used to demonstrate acute drug effects and dose/concentration-effect relationships that can support the proof of pharmacology. Considering the widespread distribution of nicotinic and cholinergic targets in different CNS-networks, a large variety of functional test can be used to demonstrate effects of cholinergic agonists or antagonists. This large choice complicates the selection of useful tests in early development studies.

The current review aims to identify the most useful types of tests, by providing an overview and an evaluation of the extensive literature that described the effects of biomarkers for CNS-active pro- and anti-cholinergic drugs in healthy subjects.

## METHODS

**STRUCTURED LITERATURE EVALUATION** An overview of registered drugs affecting the cholinergic system was found on drugbank.ca. Only compounds approved by the regulatory agencies that are able to pass the blood brain barrier and thus can affect the cholinergic system in the central nervous system were selected. As it has to be certain that the compound is effective in order to be able to assess the effectiveness of a biomarker, no experimental compounds were included in this review. An overview is shown in Table 1. The compounds were grouped based on target receptor or enzyme.

To date, there are no approved drugs that selectively stimulate or positively modulate muscarinic receptors. To our best knowledge, the results of seven muscarinic agonists/positive allosteric modulators (PAMS) investigating trials in healthy humans have been published in full text or abstract form. No PD effects were investigated or observed, or no PD effects have been published in three of these compounds (NGX267, VU319, TAK-071). PD effects of GSK1034702 were only demonstrated in a challenge model. The remaining three compounds (xanomeline, MK-7622, HTL0018318) showed PD effects in healthy subjects, however, in addition to our requirement that a drug has to be approved, not enough data were available to draw a conclusion on the effect of muscarinic agonists on biomarkers. Therefore these were not included in this formal review.

The literature search was performed in PubMed up to 15 January 2020 using the following keywords: '[name of cholinergic drug] healthy' All searches were limited to humans, and in case of more than 1000 results also limited to clinical studies (article type). The results were manually scanned for:

- Administration of compounds in healthy subjects
- Administration of a known dose
- Being an original investigation
- Measurement of pharmacodynamics effects

Both studies investigating single doses and multiple doses were included. Specific interactions of compounds, in particular with age, personality features, challenge models, other drugs or nicotine addiction were not considered in this review, and MRI-studies or studies in animals were excluded.

The study characteristics and each individual test result were put into a database (Microsoft Excel). The following items were recorded: number of subjects exposed to the compound and included in the analyses of acute effects, sex (male; female), age, blinding (double blind; single blind; open; unknown), design (crossover; parallel; unknown), drug name, dose, route of administration and test name, as well as test cluster and functional domain as explained below. The subdivision of tests and effect scores were initially performed by one author and 10% of the manuscripts was checked by another author. The total number of evaluated tests (cases) was a product of the number of articles, drugs, doses and tests.

**INDIVIDUAL TEST RESULTS** The actual results of tests could not be recorded quantitatively, due to large the diversity of methods, outcome variables and treatments. Therefore, the results were scored as + (significant improvement/increase),=(no significant effect) or – (significant impairment/decrease) per outcome variable, compared with placebo or baseline. Although statistical significance is dependent on several factors such as test variability and group size, this approach at least allows an evaluation of the applicability of a test as a biomarker. No efforts were made to further quantify the overall level of statistical significance. The

different outcome variables of a single test were grouped together, if they provided information on the same cluster. When multiple dose levels were tested within a single study, and the test outcome of the dose levels showed conflicting but statistically significant responses, the items were separately scored for each dose level. When a certain outcome variable in a task from one cluster improved, while another outcome variable within the same task deteriorated, both items were scored separately within the different clusters. If studies described tests in the methods sections, but the results were not presented without a clear reason (*eg* publication elsewhere), we included these tests and assumed that they had shown no significant effects.

**CLUSTERING OF INDIVIDUAL TEST RESULTS** Since this review intended to identify generally applicable biomarkers, results from tests that were used only once or by one research group were not individually analysed. Such tests were grouped ('clustered') with other comparable tests. The first step in this process included grouping of tests that could be regarded as variants from a basic form into a single cluster, using compendiums of neuropsychological tests (ref). Single tests could include different outcome variables that measure various functions (e.g. memory, executive function) and can therefore provide information on different clusters. Subsequently, tests and clusters were grouped into domains.

**TEST CRITERIA** Ideally, a good biomarker for activity of a drug class should meet the following criteria:

- a clear, consistent response across studies and drugs from the same class;
- a clear response of the biomarker to therapeutic doses;
- a dose (concentration)–response relationship; and
- a plausible relationship between the function addressed with the biomarker, the pharmacological activity of the drug class and the pathogenesis of the therapeutic area.

Previously, these criteria were used to evaluate the usefulness of biomarkers for the effects of antipsychotic drugs, benzodiazepines, selective serotonin reuptake inhibitors, and 3,4-methylene-dioxy-methamphetamine (ecstasy)[18-21]. These criteria are also applied in the current review to evaluate the biomarkers.

**DOSE-EFFECT RELATIONSHIPS** A clear increase of an effect with dose provides strong support for the usefulness of a test as a biomarker of pharmacological activity. To investigate this , for the most frequently used tests and drug dosages it was determined whether the number of statistically significant results increased with dose. To this end, drug doses were pooled into 'lower', 'medium' and higher' dosages (Table 2). The 'medium' dose was determined as the range between the lowest recommended therapeutic starting dose and halfway the highest recommended clinical maintenance dose[22]. The 'lower' and 'higher' doses were all dosages below or above this level.

**STATISTICAL EVALUATION** All data processing steps and calculations were performed using R software for Statistical Computing (version R 4.0.3). In order to calculate the average responses with confidence intervals for binomial proportions, responses were coded as follows: Impairment/decrease was coded as 0, no change was coded as 0.5 and improvement/increase was coded as 1. A cumulated response code was calculated by multiplying the number of occurrences for each response by the coding and adding this over the three responses. A proportion was calculated by dividing the cumulated response code by the total number of responses. This resulted in an average response between 0 (impairment/decrease) and 1 (improvement/increase) for which two-sided 95% exact (Clopper-Pearson) confidence intervals for binomial proportions were calculated.

## RESULTS

**LITERATURE** In total 132 studies were included; 38 trials investigated cholinesterase inhibitors[23-60], 41 trials studied nicotinic receptor agonists[61-101], 13 studies used nicotinic receptor antagonists[88,102-113] and 54 trials investigated muscarinic receptor antagonists[23-25,56,102-105,108,110,111,113-155]. In 13 studies more than one drug class was investigated and in 13 studies more than one dose was administered. Characteristics of these studies are provided in Table 3. Across all studies 16 different study designs were used.

In total 223 tests were used, which were grouped into 69 clusters. Subsequently the tests and clusters were grouped into 9 domains (Table 4). An overview of the effects on each individual test in each study is shown in Suppl table S1a-d.

**TESTS** In the 38 studies investigating cholinesterase inhibitors, 99 unique tests were used. Of these, 11 tests were used more than 5 times (Table S2). Only the verbal learning task was used more than 10 times and showed an improvement in 2 cases, no significant effect in 7 cases and an impairment in 1 case (Table S2).

Nicotinic receptor agonists were investigated using 77 individual measurements of which the saccadic and anti-saccadic eye movements were used the most (both n=5). The anti-saccadic eye movements were improved in 3 cases, the saccadic eye movements was improved in 1 case Impairments were not observed.

In the 13 studies investigating nicotinic receptor antagonists 50 individual tests were used. The n-back test (n=6) and pupil size (n=5) were used most frequently. In the majority of the cases no effect on these test could be demonstrated. The n-back tests was impaired once and the pupil size increased once.

Also in the 54 papers studying muscarinic receptor antagonists many different tests were used (n=117). However, there seemed to be less variety as 18 tests were used more than 5 times and of these 6 tests were used more than 10 times (Table S3). These 6 tests (Simple reaction time, Digit span, N-back, Critical flicker fusion test, Verbal learning task and visual analogue scale (VAS) according to Bond and Lader) were able to show an impairment in 18% (Digit span) to 90% (Verbal learning task) of the cases. An improvement was only observed once (n-back test) and in the other cases, no effect was observed.

**CLUSTERS** Although many different tests were used to evaluate the effect of each drug class, most tests were not used frequently enough for further analysis. Therefore, the tests were grouped into clusters. In table 5, 14 clusters are presented which were used most frequently across all drug classes.

- **Cholinesterase inhibitors**

In the majority of the clusters, (71-100% of the cases) no effect was observed. The improvements and impairments that were shown occurred in a maximum of 18% of the cases and were inconsistent within almost each cluster (Tabel 5, Figure S4). A higher percentages of improvements (27%) and deteriorations (18%) were observed within the cluster Focused/selective attention, however, these were inconsistent.

- **Nicotinic receptor agonists**

Inhibition was improved in 32% of the cases, Sustained attention showed an improved in 30% of the cases and Scale alertness was impaired in 33% of the cases. In the other clusters an effect of this drug class was demonstrated in a maximum of 18% of the cases (Table 5, Figure S5). The high percentages showing an effect on Focused/selective attention (33%) and delayed recall of the auditory/verbal memory (50%) can be attributed to the low frequency of these clusters.

- **Nicotinic receptor antagonists**

In the domain Memory, an impairment of the Learning (50%), Auditory/verbal memory: immediate recall (38%) and delayed recall (36%) clusters was demonstrated (Table 5, Figure S6). The high percentages showing an effect on Inhibition and Motor control can be attributed to the low frequency of these clusters. In the remaining clusters, there was no clear effect of nicotinic antagonists.



- **Muscarinic receptor antagonist**

Impairments were demonstrated repeatedly in many clusters (table 5). In most of these clusters, there was still a lack of effect in at least 50% of the cases. Only in the clusters Learning, Auditory/verbal memory: immediate recall, delayed recall, delayed recognition and Scale alertness an impairment was observed more often than a lack of effect. Visualizing the data in a forest plot (Figure S7) shows a fairly consistent impairment within the clusters Working memory, Auditory/verbal memory: immediate recall and delayed recall and Scale alertness.

The effects of all four drug classes on clusters are presented in a spider plot (Figure 1). Impairments were clearer following muscarinic receptor antagonist than after nicotinic receptor antagonist.

**DOSE-RESPONSE RELATIONSHIPS** The potential relationships between the dose levels of each drug class and the effects on the 14 clusters were investigated (Table S8, Figure S7)). There were no clear dose related effects after administration of cholinesterase inhibitors, nicotinic receptor agonists and nicotinic receptor antagonists. From the studies investigating muscarinic receptor antagonists there appeared to be a relationship between the effects on clusters Scale alertness and Auditory/verbal memory: immediate recall and delayed recall (Figure S9). Following a low dose, an impairment on Scale alertness was observed in 33% of the cases which is less frequently than after a medium dose (42%) and a high dose (80%). The cluster Auditory/verbal memory immediate recall showed no effect after a low dose (only one case present) and deterioration after a medium dose in 77% of the cases and after a high dose in 83% of the cases. Impairment increased with dose for the cluster Auditory/verbal memory delayed recall from 50% in the lowest dose group (2 cases present at this dose level) to 85% in the highest.

## DISCUSSION

In this review we aimed to provide an overview and evaluation of biomarkers that were used to detect acute drug effects of cholinergic drugs acting on the central nervous system in healthy subjects. The biomarkers were evaluated for the drug classes cholinesterase inhibitors, nicotinic receptor agonist, nicotinic receptor antagonists and muscarinic receptor antagonists separately. No studies with (subtype) selective muscarinic receptor agonists were included, these drugs are not (yet) used in clinical practice, and experimental compounds were excluded. A large number of 223 tests were described in 132 publications, the majority of which were used infrequently. This huge variability is comparable to the results of similar reviews of biomarkers used to investigate CNS-active drugs in healthy subjects [156, 20, 21, 19, 18, 157]. In

each of the reviews, a call has been made for a harmonisation and standardisation of tests in drug development, in order to facilitate selection of methods, comparisons of compounds and functional interpretations of effects. Although some tests seem to be sensitive to drug effects such as the anti-saccadic eye movements after nicotinic receptor agonists (improved in 3/5 cases), and digit span (improved in 2/6 cases) and EEG alpha (decreased in 2/5 cases) after cholinesterase inhibitors, no conclusions about individual test used to evaluated the effect of cholinesterase inhibitors, nicotinic receptor agonists and antagonists can be drawn due to this low frequency. The tests used for muscarinic receptor antagonists show a more consistent effect (mainly impairment), but also in this drug class, there was a lack of effect in more than 50% of the cases. Because of the wide variety of tests and their low frequency, we have grouped these test in clusters of tests that measure similar CNS-functions. Grouping these tests in clusters might obscure information: the 'perfect' biomarker could be masked by nonresponsive tests in the same cluster. Additionally test variants and differences among research groups were bypassed. However, excluding tests based on their limited application could have resulted in missing possibly valuable information.

Analysis of the clusters showed moderate effects of nicotinic receptor agonists (improvement in up to 30% of the cases on inhibition and sustained attention) and a lack of clear effects after cholinesterase inhibitors. As most of the clusters represent a cognitive function, these lack of effects and moderate cholinergic-induced improvements could reflect the challenge of investigating cognitive improvement in healthy subjects: most tests in this review have ceiling effects in healthy optimally functioning subjects.

Ceiling effects are also suggested by the contrast between the limited results of the pro-cholinergic drugs, with the clearer impairments observed with anticholinergic compounds. Muscarinic receptor antagonists, for instance, showed deteriorations in 58-79% of memory tests.

The effects of nicotinic receptor antagonists were more limited, but this seems to be at least partly related to the low numbers of studies (n=13) included in this review. In several specifically designed human pharmacological studies, evident dose- and concentration-response relationships found on a number of sensitive tests [108, 158, 107]. However, these methods were all from the same centre, and not used often enough by other groups to be analysed in this review. The same investigators showed a different pharmacodynamic profile of a nicotinic receptor antagonist (mecamylamine) compared with the (more pronounced) effects of a muscarinic receptor antagonist (scopolamine)[108].



A consistent impaired effect on multiple clusters was shown after muscarinic receptor antagonist. Data of muscarinic receptor agonists were not included in this review, as these drugs are not approved (yet), The few clinical studies investigating the experimental muscarinic receptor agonists/PAMS in healthy subjects that were published showed a reduction in 2nd REM latency on a sleep EEG after xanomeline[159], an increase in pupil size after single doses of HTL0018318[160], and increases in sigma, delta and theta EEG frequency bands after multiple doses of MK-7622[161]. EEG delta and theta were also increased after muscarinic receptor antagonists thus no opposite effects were observed. Sleep EEG, pupil size, and EEG sigma were not used frequently enough after muscarinic receptor antagonists to compare with agonists/PAMS.

Analysing a dose-response relationship of the clusters revealed a relationship between the muscarinic receptor antagonists and the clusters Scale alertness and Auditory/verbal memory: immediate recall and delayed recall. These three relationships can be explained by the pharmacology of the drug, as the muscarinic receptors are highly prevalent in the hippocampus, a brain structure involved in memory[4,5] and in the brain stem and thalamus[3] which are involved in alertness[162]. In the remaining clusters, the low number of cases per dose level could have masked potential dose-effect relationships easily.

Given the effects on the tests, clusters and the dose-relationship in this review, there are only a limited number of clusters that meet the criteria of a good biomarker as defined in the method section. This does not exclude the existence of other good biomarkers. The success of a biomarker depends on multiple factors such as sample size and characteristics of the study population, study design and timing of the application, which were not taken in account in our analysis. Additionally, as mentioned before, grouping the tests into clusters could have masked good biomarkers. It was also mentioned that studies that are specifically designed to detect concentration-effect relationships (by employing different doses and frequent measurements of concomitant drug concentrations and effects) can provide unequivocal evidence for the suitability of a test as a pharmacological biomarker, even in a single study. An example of a good biomarker included in this review is the adaptive tracking test, a measure for attention[163-165]. This test was used to measure effects of cholinesterase inhibitors donepezil and an experimental CNS-penetrating prodrug of galantamine[54,52]. This example is encouraging to further evaluate and validate the existing biomarkers, because the reliability of biomarkers can be more carefully assessed when more data is available. Because of this example and the effects of cholinesterase inhibitors on individual tests such as digit span and EEG alpha we also strongly recommend to keep using biomarkers in experimental studies in healthy subjects for

the investigation of pro-cholinergic drugs, as is recommended by the guideline of the EMA[166]. If test improvements or impairments are observed in early phase clinical trials, these can be further investigated by analysing the concentration-response relationship in order to avoid a type 1 error. Additionally, to avoid the ceiling effects of biomarkers, challenge situations can be applied such as the scopolamine, mecamylamine or biperiden challenge models, sleep deprivation challenge or inclusion of elderly subjects. Scopolamine, mecamylamine and biperiden temporary induce cognitive deficits and neurophysiological effects [108, 158, 167], which create the possibility to improve cognition in healthy subjects. Co-administration of the pro-cholinergic compound can then (partially) reverse these effects, and elucidate drug effects which cannot be demonstrated in unchallenged optimally functioning individuals[107]. Cholinesterase inhibitors have been investigated in scopolamine challenge models. These ameliorated the magnitude of the scopolamine-induced effects on learning efficiency of the Groton maze learning test[56] and power and continuity of attention and quality of working memory, measured as a combination of multiple tests[168]. As these tests are sensitive to the effects of cholinesterase inhibitors, they it be considered to also use them in early phase drug clinical studies.

In conclusion, an excessive number of tests has been used to evaluate the effects of pro-cholinergic and anti-cholinergic drugs in healthy subjects. This huge variability is detrimental to the proper use of biomarkers in early drug development. From this review, no single test could be identified that was able to demonstrate pro-cholinergic effects consistently, although there are tests that are able to detect dose dependent effects of pro-cholinergic drugs in healthy subjects, such as the adaptive tracking test. Therefore further evaluation and validation of the the potential pro-cholinergic functional biomarkers is recommended. Effects of nicotinic and muscarinic receptor antagonists could be demonstrated more consistently. These well measurable temporary anti-cholinergic effects can be used ins pharmacological challenge experiments in healthy subjects, in order to allow detection of the effects of pro-cholinergic drugs.



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TABLE 1 **Cholinergic drugs included in this review.**

| Drug class | Drugs |
|---|---|
| Cholinesterase inhibitor | Galantamine, rivastigmine, donepezil, physostigmine, tacrine |
| Nicotinic receptor agonists | Nicotine, varenicline |
| Nicotinic receptor antagonist | Mecamylamine |
| Muscarinic receptor antagonist | Scopolamine, biperiden, atropine, procyclidine |

TABLE 2 **Classification of dose levels per drug.**

| | Low dose level | Medium dose level | High dose level |
|---|---|---|---|
| **Nicotine** | | | |
| Chewing gum | <2 mg | 2 mg | >2 mg |
| Plaster (transdermal) | <14 mg/24h | 14 -20 mg/24h | >20 mg/24h |
| Tablet | <2 mg | 2-4 mg | >4 mg |
| Intranasal | | 1 mg | |
| Mouth spray | <1 mg | 1-2 mg | >2 mg |
| Subcutaneous | | 6 ug/kg | 12ug/kg, 1 mg |
| Donepezil (oral) | <5mg | 5-7.5mg | >7.5mg |
| Galantamine (oral) | <8 mg | 8 mg | 16 mg |
| **Rivastigmine** | | | |
| Capsule | <3 mg single dose | 3-5mg single dose | >5 mg single dose |
| Plaster (transdermal) | 4.6 mg/24h | 9.5 mg/24h | |
| Mecamylamine (oral) | <5 mg/day | 5-20 mg/day | > 20 mg/day |
| **Biperiden** | | | |
| Oral | <1 mg single dose | 1-3 mg single dose | >3 mg single dose |
| Intraveneous | <2.5 mg | 2.5 mg-4 mg | >4 mg |
| **Scopolamine** | | | |
| Transdermal | <1.0 mg | 1-1.5 mg | >1.5 mg |
| Intramuscular | <0.3 mg single dose | 0.3-0.5 mg single dose | >0.6 mg single dose |
| Intravenous | <0.3 mg single dose | 0.3-0.5 mg single dose | >0.6 mg single dose |
| Oral | <0.4 mg | 0.4-08 mg | >0.8 |
| Procyclidine (oral) | 2.5 mg single dose | 0.5 mg-5 mg single dose | >5 mg single |
| Varenicline (oral) | <0.5 mg single dose | 0.5-1 mg single dose | 2 mg single dose |
| Tacrine (oral) | low <20mg per dose | medium 20-60mg per dose | >60mg per dose |
| Physostigmine (IM, IV) | | 0.5 to 2 mg | |



TABLE 3 **Characteristics of the studies included in this review. One study consisted of two differently designed study parts resulting in n=133 for the design related columns.**

| Randomization (total n=133) | Blinding (total n=133) | Design (total n=133) | Control (total n=133) | Age (total n=132) | Number of subjects included (total n=132) | Sex of subjects (total n=132) |
|---|---|---|---|---|---|---|
| Randomized n= 112 (84%) | Double-blind n= 111 (83%) | Cross-over n= 97 (73%) | Placebo-controlled n= 122 (92%) | Mean (range) age 29.2 (21-73.10) | Mean (range) 23.5 (6-116) | Only males n=40 (30%) |
| Pseudo-randomized n= 3 (2%) | Single-blind n= 9 (7%) | Parallel n= 31 (23%) | Not placebo-controlled n= 11 (8%) | | | Only females n=3 (3%) |
| Non-randomized n= 12 (9%) | Open label n= 12 (9%) | Unknown n= 5 (4%) | | | | Both males and females n=86 (n=64%) |
| Unknown n= 6 (5%) | Unknown N=1 (1%) | | | | | Unknown n=3 (3%) |

*n=number of studies*

TABLE 4 **Overview of all the tests included in this review and the grouping in clusters and domains.**

| Test | Cluster | Domain |
|---|---|---|
| Divided attention, Paced Auditory Serial Addition Test | Divided attention | Attention |
| Digit symbol substitution test, Symbol digit substitution test | DSST-like | |
| Attention Network Test | Executive control | |
| Critical flicker fusion task | Flicker discrimination | |
| Attention Network Test | Orienting | |
| 5-choice reaction time, Choice Reaction Time task, Detection task, Faces Dot Probe Task, Go no go paradigm, Incongruent choice reaction time task, Multiple choice reaction time test, Psychomotor Vigilance Task, Rapid visual information processing, Self paced and externally triggered reaction times, Simple reaction time test, Spatial attentional resource allocation task, Sustained attention to response task, Visual choice reaction oddball task | Reaction time | |
| 3-D multiple object tracking, Adaptive tracking, Attention Network Test, Choice Reaction Time task, Continuous performance test, Continuous performance test – identical pairs version, Digit vigilance, Identify task, Multiple choice reaction time test, Paced Auditory Serial Addition Test, Psychomotor Vigilance Task, Rapid visual information processing, Span of apprehension test, Sustained attention to response task, Unstable tracking, Visual choice reaction oddball task, Wilkins counting test | Sustained attention (vigilance) | |
| Attentional blink test, Posner cueing task, D2 concentration test, Digit span, Inspection time task, Shape matching task, Simple two-choice visual discrimination task, Spatial attentional resource allocation task, Spatial span, Visual pop out search, Visual scanning test, Visual selective attention, Visuospatial cueing task | Focused/selective attention | |
| ECG | ECG | Autonomic |
| Pupil size | Pupil size | |
| Visual accommodation and acuity | Visual acuity | |
| Blood pressure, Oral temperature, Pulse rate | Vital signs | |
| Emotion-potentiated startle task, Flanker task, Go no go paradigm, Magnetoencephalography (pre pulse inhibition), Prepulse inhibition of the acoustic startle reflex, Simon task, Stop signal task, Stroop Test, Three card stroop task | Inhibition | Executive |
| Inspection time task | Judgement | |
| Controlled Oral Word Association Test, Oral language, Phonemic letter fluency, Reading, Regensburger Wortfluessigkeitstes, Spelling | Language | |
| Tower of London task, Zoo map test | Planning | |
| Emotion recognition task, Emotion recognition/matching, Facial expression recognition task, Mathematical processing, Emotional Categorisation Task, Word categorization and memory task | Reasoning/association | |
| Balloon Analogue Risk Task, Signal detection task | Reward | |
| Dual task paradigm, Intra/extradimensional shift, Plus-minus task, Trail Making Test, Wisconsin card sorting test | Shifting | |
| Little man test, Manikin task, Stockings of Cambrigde | Spatial orientation | |
| Time wall | Time estimation | |
| Speed anticipation test | Time-distance estimation | |
| Corsi block test, Counting span, Digit span, Immediate memory task, Letter-number sequencing, Letter memory task, Match to sample, Maze learning task, N-back, Non-spatial working memory, Paced Auditory Serial Addition Test, Short Blessed Test, Spatial information processing, Spatial recognition memory, Spatial span, Spatial working memory, Sternberg working memory task, Symbol digit recall test, The arena, Visual spatial working memory test | Working memory | |



| | | |
|---|---|---|
| Buschke Selective Reminding Test , California verbal learning test, Emotional Recall Task, Levels of processing, Logical memory test, Word categorization and memory task, Paired associate learning, Rey Auditory Verbal Learning Test, Selective reminding task, Verbal learning task | Auditory/verbal memory: immediate recall | Memory |
| Buschke Selective Reminding Test, California verbal learning test, Cued recall task, Free recall test, Hi-lo imaginary test, Hopkins Verbal Learning Test-Revised, Levels of processing, Logical memory test, Memory task of Jacoby (adjusted version), Paired associate learning, Repeated Acquisition Task, Rey Auditory Verbal Learning Test, Selective reminding task, Verbal learning task | Auditory/verbal memory: delayed recall | |
| California verbal learning test, Emotional Recognition Memory Task, Word categorization and memory task, Rey Auditory Verbal Learning Test, Verbal learning task, Verbal recognition task, Word completion task | Auditory/verbal memory: delayed recognition | |
| Continuous recognition memory test, Episodic memory paradigm, Running word recognition, Hi-lo imaginary test | Auditory/verbal memory: immediate recognition | |
| Continuous Visual Recognition, Object relocation task, Rivermead Behavioral Memory Task, Spatial free recall: selective reminding, Spatial memory task, Visual episodic memory, Visual reproduction | Visual/spatial memory: immediate recall | |
| Maze learning task, Memory task of Jacoby (adjusted version), Object relocation task, Rivermead Behavioral Memory Task, Self-paced subsequent recognition memory task, Spatial memory task, Visual episodic memory, Visual memory task, Visual reproduction | Visual/spatial memory: delayed recall | |
| Benton Visual Retention Test, Delayed picture recognition, Face recognition, Novelty test, Object recognition, Pattern recognition memory, Processing depth, Spatial memory task, Spatial recognition memory, Sternberg working memory task | Visual/spatial memory: delayed recognition | |
| Change detection task, Continuous recognition memory test, Pattern recognition memory, Picture memory test, Running picture recognition, Spatial memory task, Spatial recognition memory, Visual working memory task, Word categorization and memory task | Visual/spatial memory: immediate recognition | |
| 10-response sequences task, Buschke Selective Reminding Test, California verbal learning test, Continuous paired associate learning task, Face encoding recognition task, Hi-lo imaginary test, Learned irrelevance task, Levels of processing, Motion direction discrimination task, One Card learning test, Paired associate learning, Repeated Acquisition Task, Rey Auditory Verbal Learning Test, Selective reminding task, Spatial memory task, Symbol digit recall test | Learning | |
| Automatic task, Controlled task, Priming task | Priming | |
| Prospective memory task | Prospective memory | |
| Circular lights task, Finger tapping task, Finger tapping task (auditory paced), Hand cooperation test, Pointing task | Motor control | Motor |
| Detect task | Reaction time | |
| Adaptive tracking, Compensatory tracking task, Handwriting test, Line copying task, Maze learning task, Tangle, Tangle task, The grooved pegboard, Trail Making Test | Visuo-motor control | |
| Brain-derived neurotrophic factor | Brain-derived neurotrophic factor | (Neuro) endocrine |
| Acetylcholinesterase activity, Butyrylcholinesterase activity | Cholinesterase | |
| Cortisol | Cortisol | |
| Follicle-stimulating hormone | FSH | |
| Ghrelin | Ghrelin | |
| Luteinizing Hormone | LH | |
| Prolactin | Prolactin | |

| | | |
|---|---|---|
| EEG delta | EEG delta | Neuro-physio-logical |
| EEG theta | EEG theta | |
| EEG alpha | EEG alpha | |
| EEG beta | EEG beta | |
| EEG gamma | EEG gamma | |
| EEG sigma | EEG sigma | |
| EEG (novelty oddball paradigm), ERP (3 types of auditory stimuli paradigm), ERP (attentional blink test), ERP (during rapid visual information processing, ERP (learned irrelevance paradigm), ERP (match to sample probe matching cue), ERP (MMN paradigm), ERP (Novelty oddball paradigm), ERP (P50 paradigm), ERP (Paired-click paradigm), ERP (Verbal learning test), ERP (visual memory task), High frequency oscillations, Magnetoencephalography (relevance modulation task), Magnetoencephalography (somatosensory stimuli (electrical pulses)), Magnetoencephalography (standard and deviate auditory stimulus), Magnetoencephalography (visuomotor task), Somatosensory evoked potentials | Evoked potential | |
| Smooth pursuit eye movements | Eye movements – pursuit | |
| Anti-saccadic eye movement, Saccadic eye movements | Eye movements – saccadic | |
| Body sway | Postural stability | |
| TMS | TMS | |
| Object relocation task | Visual perception | Perception |
| Profile of Mood States, VAS mood scale | Scale aggression | Subjective experience |
| Eigenschaftswörterliste, Profile of Mood States, Subjective well-being, VAS Bond and Lader, VAS Norris, Visual analogue scale | Scale alertness | |
| Brief Psychiatric Rating Scale, Positive and negative affective schedule, Profile of Mood States, State trait anxiety inventory, Visual analogue scale | Scale anxiety | |
| Profile of Mood States, Subjective well-being, VAS Bond and Lader | Scale calmness | |
| Addiction Research Center Inventory, Brief Questionnaire of Smoking Urges, Drug Effects Questionnaire | Scale craving | |
| Side effects, Visual analogue scale | Scale dizziness | |
| Addiction Research Center Inventory, Drug Effects Questionnaire | Scale drug effect | |
| Positive and negative affective schedule, Profile of Mood States, Side effects | Scale fatigue | |
| Addiction Research Center Inventory, VAS Bowdle | Scale high | |
| Addiction Research Center Inventory, Beck depression inventory, Befindlichkeits scale, Brief Psychiatric Rating Scale, Positive and negative affective schedule, Profile of Mood States, Subjective well-being, VAS Bond and Lader, VAS mood scale, Visual analogue scale | Scale mood | |
| Subjective well-being, VAS Bond and Lader | Scale performance | |
| Brief Psychiatric Rating Scale, Positive and Negative Symptoms Scale, VAS Bowdle, VAS Norris | Scale psychotomimetic | |
| Visual analogue scale | Scale satiety | |
| Biphasic Alcohol Effects Questionnaire, Bodily symptoms, Neurovegetative effects, Side effects, VAS nausea, Visual analogue scale | Scale symptoms | |

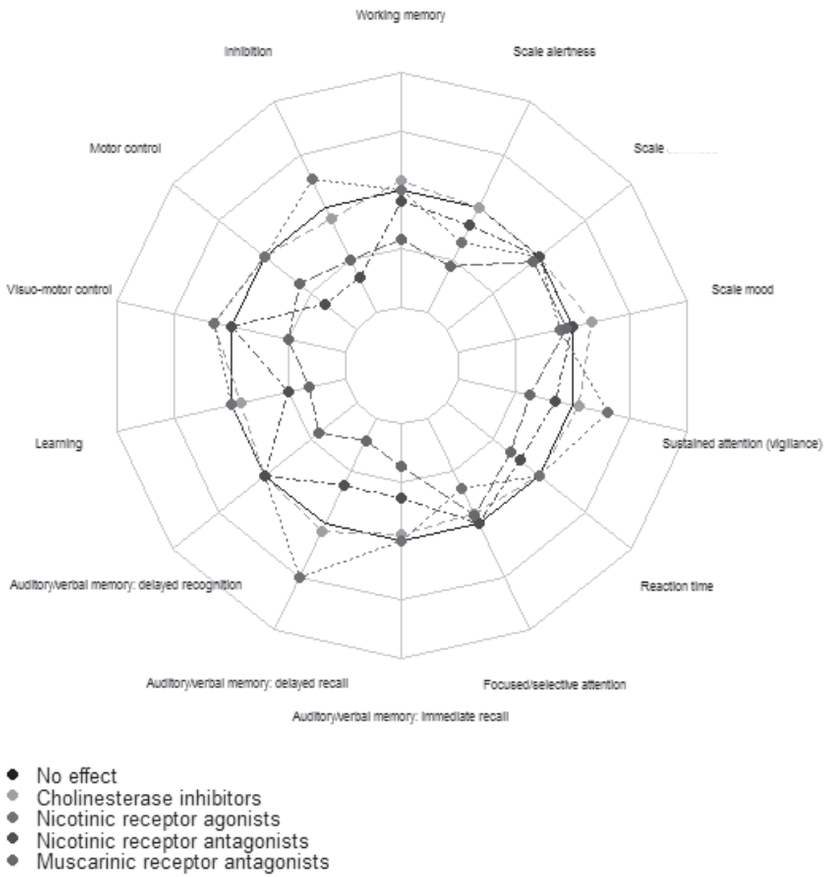


TABLE 5 **Effect of drug classes on clusters.**

| | | Pro-cholinergic drugs | | | | | | | | Anti-cholinergic drugs | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Domain** | | **Cholinesterase inhibitors** | | | | **Nicotinic receptor agonists** | | | | **Nicotinic receptors antagonists** | | | | **Muscarinic receptor antagonists** | | | |
| | Cluster | n | – | = | + | n | – | = | + | n | – | = | + | n | – | = | + |
| Executive | Working memory | 35 | 1 (2.9%) | 30 (85.7%) | 4 (11.4%) | 11 | 1 (9.1%) | 9 (81.8%) | 1 (9.1%) | 11 | 1 (9.1%) | 10 (90.9%) | 0 (0.0%) | 41 | 18 (43.9%) | 22 (53.7%) | 1 (2.4%) |
| | Inhibition | 9 | 1 (11.1%) | 8 (88.9%) | 0 (0.0%) | 22 | 1 (4.5%) | 14 (63.6%) | 7 (31.8%) | 3 | 2 (66.7%) | 1 (33.3%) | 0 (0.0%) | 6 | 3 (50.0%) | 3 (50.0%) | 0 (0.0% |
| Motor | Motor control | 2 | 0 (0.0%) | 2 (100.0%) | 0 (0.0%) | 3 | 0 (0.0%) | 3 (100.0%) | 0 (0.0%) | 3 | 2 (66.7%) | 1 (33.3%) | 0 (0.0%) | 13 | 5 (38.5%) | 8 (61.5%) | 0 (0.0%) |
| | Visuo-motor control | 7 | 0 (0.0%) | 6 (85.7%) | 1 (14.3%) | 7 | 0 (0.0%) | 6 (85.7%) | 1 (14.3%) | 1 | 0 (0.0%) | 1 (100.0%) | 0 (0.0%) | 12 | 6 (50.0%) | 6 (50.0%) | 0 (0.0%) |
| Memory | Learning | 12 | 2 (16.7%) | 9 (75.0%) | 1 (8.3%) | 1 | 0 (0.0%) | 1 (100.0%) | 0 (0.0%) | 6 | 3 (50.0%) | 3 (50.0%) | 0 (0.0%) | 19 | 13 (68.4%) | 6 (31.6%) | 0 (0.0%) |
| | Auditory/ verbal memory: immediate recall | 18 | 1 (5.6%) | 17 (94.4%) | 0 (0.0%) | 3 | 0 (0.0%) | 3 (100.0%) | 0 (0.0%) | 8 | 3 (37.5%) | 5 (62.5%) | 0 (0.0%) | 28 | 20 (71.4%) | 6 (21.4%) | 2 (7.1%) |
| | Auditory/verbal memory: delayed recall | 13 | 1 (7.7%) | 10 (76.9%) | 2 (15.4%) | 2 | 0 (0.0%) | 1 (50.0%) | 1 (50.0%) | 11 | 4 (36.4%) | 7 (63.6%) | 0 (0.0%) | 29 | 23 (79.3%) | 6 (20.7%) | 0 (0.0%) |
| | Auditory/verbal memory:delayed recognition | 7 | 1 (14.3%) | 5 (71.4%) | 1 (14.3%) | 3 | 0 (0.0%) | 3 (100.0%) | 0 (0.0%) | 5 | 0 (0.0%) | 5 (100.0%) | 0 (0.0%) | 12 | 7 (58.3%) | 5 (41.7%) | 0 (0.0%) |
| Attention | Sustained attention (vigilance) | 21 | 0 (0.0%) | 20 (95.2%) | 1 (4.8%) | 10 | 0 (0.0%) | 7 (70.0%) | 3 (30.0%) | 13 | 2 (15.4%) | 11 (84.6%) | 0 (0.0%) | 29 | 11 (37.9%) | 18 (62.1%) | 0 (0.0%) |
| | Focused/selective attention | 11 | 3 (27.3%) | 6 (54.5%) | 2 (18.2%) | 3 | 1 (33.3%) | 2 (66.7%) | 0 (0.0%) | 5 | 0 (0.0%) | 5 (100.0%) | 0 (0.0%) | 23 | 2 (8.7%) | 21 (91.3%) | 0 (0.0%) |
| | Reaction time | 17 | 0 (0.0%) | 17 (100.0%) | 0 (0.0%) | 6 | 0 (0.0%) | 6 (100.0%) | 0 (0.0%) | 14 | 3 (21.4%) | 11 (78.6%) | 0 (0.0%) | 32 | 10 (31.2%) | 22 (68.8%) | 0 (0.0%) |
| Subjective experience | Scale mood | 12 | 0 (0.0%) | 10 (83.3%) | 2 (16.7%) | 9 | 1 (11.1%) | 8 (88.9%) | 0 (0.0%) | 10 | 0 (0.0%) | 10 (100.0%) | 0 (0.0%) | 17 | 2 (11.8%) | 14 (82.4%) | 1 (5.9%) |
| | Scale alertness | 9 | 1 (11.1%) | 7 (77.8%) | 1 (11.1%) | 6 | 2 (33.3%) | 4 (66.7%) | 0 (0.0%) | 6 | 1 (16.7%) | 5 (83.3%) | 0 (0.0%) | 25 | 14 (56.0%) | 11 (44.0%) | 0 (0.0%) |
| | Scale calmness | 7 | 0 (0.0%) | 7 (100.0% | 0 (0.0%) | 3 | 0 (0.0%) | 3 (100.0%) | 0 (0.0%) | 3 | 0 (0.0%) | 3 (100.0%) | 0 (0.0%) | 15 | 3 (20.0%) | 10 (66.7%) | 2 (13.3%) |

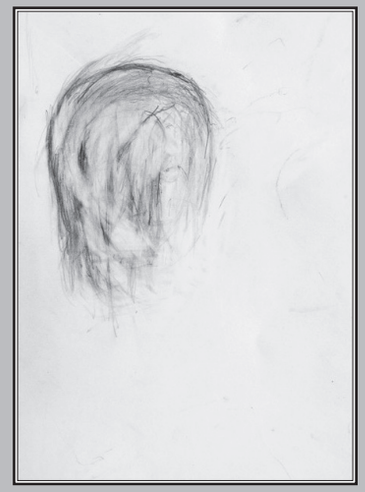
*n= The frequency of the cluster used (cases) as a product of the number of articles, drugs, doses and tests; '–' is impairment; '=' is no effect; '+' is improvement*

FIGURE 1 Effect of all drug classes on the 14 most investigated clusters. The line moving towards the centre of the spider plot represents an impairment. The line moving towards the edge of the spider plot represents an improvement.






CHAPTER IX



# SUMMARY AND DISCUSSION

## IN THIS DISSERTATION

As discussed in the introduction (Chapter I), the cholinergic system comprises neurons that release or respond to the neurotransmitter acetylcholine and controls crucial functions in both the central and peripheral nervous system. In the central nervous system (CNS), the main cholinergic projection systems include the nucleus basalis (of Meynert), projecting to the cerebral cortex and hippocampus, the pedunculopontine nucleus and laterodorsal tegmental nucleus, providing innervation for the thalamic nuclei, and the cholinergic neurons intrinsic to the striatum[1]. Because the projection areas (cerebral cortex and hippocampus) are involved in cognitive functions such as memory, learning and attention[2-4], the cholinergic neurons originating from the nucleus basalis are involved in these cognitive functions as well. The neurons intrinsic to the striatum contribute to the balance of dopamine and acetylcholine. Dopamine, and more specifically the balance between dopamine and acetylcholine, plays an important role in motor skills. Therefore cholinergic neurons within the striatum are indirectly involved in motor skills. In the peripheral system the cholinergic neurons mediate parasympathetic activities such as blood pressure regulation, and smooth muscle contraction in heart, bladder and the gastrointestinal system[5,6] as well as sympathetic innervation of sweat glands.

Dysfunction of the cholinergic system in the CNS plays a key role in the neurodegenerative diseases Alzheimer's disease, Lewy bodies dementia and Parkinson's disease dementia, and in the psychiatric disease schizophrenia. Currently, there are only drugs approved that treat the cognitive deficits of these diseases symptomatically. These drugs, cholinesterase inhibitors rivastigmine, donepezil and galantamine, increase acetylcholine in the synaptic cleft of cholinergic neurons. Their beneficial effects on cognition are modest and a high number of patients experience peripheral side effects such as nausea, vomiting and diarrhoea[7-9]. These side effects are a consequence of the drug's non-selective nature, leading to an increase of the acetylcholine throughout the body, activating all acetylcholine receptors. Improvement in these symptomatic treatments is highly needed. Therefore, new drugs that selectively target subtypes of acetylcholine receptors or improved formulations of current drugs are being investigated. Interesting targets for the treatment of cognitive dysfunction are the nicotinic $\alpha 7$ receptor and the $M_1$ muscarinic receptor. These receptors are relatively well preserved in patients with Alzheimer's disease[10-12] and are located in brain areas that are involved in memory, learning and attention[2-4]. The $M_4$ receptor is a potential target to restore the striatal dopamine imbalance in patients with Lewy bodies disease as this receptor is involved in modulation of dopaminergic activity[13,14]. Additionally this receptor is associated with psychotic symptoms by modulating the dopamine activity[15,16] and may therefore be a promising target for the treatment of psychotic symptoms in patients with dementia or schizophrenia[17].

**AGONISTS SELECTIVE FOR THE $M_1$ RECEPTOR** In this dissertation, two agonists selective for the $M_1$ receptor were investigated: HTL0009936 and HTL0018318.

A study of the $M_1$ receptor agonist HTL0009936 is described in Chapter II (HTL0009936). We investigated safety, tolerability, pharmacokinetics and pharmacodynamics of HTL0009936 in healthy elderly subjects with below-average cognitive function. Infusion of HTL0009936 consisted of a loading dose to reach the target steady-state plasma concentration. This was followed by a maintenance dose designed to maintain the target steady-state concentration and ensure sustained exposure within the CNS over the period of cognitive testing. Results were compared with placebo and the comparator cholinesterase inhibitor physostigmine. Key findings were (I) an acceptable safety profile, and (II) an overall lack of positive pharmacodynamics effects, except for a selective effect on the P300 amplitude suggesting an improvement in early attentional processing following the administration of HTL0018318 and (III) an improved performance in the adaptive tracking test after administration of physostigmine representing an improvement in sustained attention.

Chapter III describes the results of a study investigating safety, tolerability, pharmacokinetics and pharmacodynamics of HTL0018318, a partial agonist selective for the $M_1$ receptor. Single doses of HTL0018318 at different dose levels were tested in healthy young adult subjects and healthy elderly subjects. In this study, pharmacokinetics of HTL0018318 were well-characterized and we found thatsingle doses of HTL0018318 were associated with dose-related adverse events of low incidence in both younger adult and elderly subjects. Mild increases in blood pressure were observed. There were no statistically significant effects observed on cognitive function.

In Chapter IV, we conducted a trial with multiple doses of HTL0018318 in healthy young adult subjects and healthy elderly subjects. Like in Chapter III, safety, pharmacokinetics and pharmacodynamics were investigated. The safety profile observed in this study was in line with the profile seen in the single ascending dose study (Chapter III). Besides, improvements on the n-back test performance (working memory) and Milner maze test (learning and memory) were observed.

In Chapter V, we explored the interaction between HTL0018318 and acetylcholinesterase inhibitor donepezil. As a treatment for Alzheimer's disease, HTL0018318 will very likely be given in combination with acetylcholinesterase inhibitors (standard of care). Both HTL0018318 and cholinesterase inhibitors increase cholinergic

activity, and therefore the aim of this study was to investigate whether HTL0018318 can be safely co-administered in combination with donepezil. Additionally, the effect of HTL0018318 and donepezil on each other's pharmacokinetics was analysed. We found that HTL0018318 given in combination with donepezil to elderly healthy subjects was generally well tolerated, did not lead to clinical, safety or pharmacokinetic concerns. Pharmacodynamics were not investigated.

**INHIBITION OF ACETYLCHOLINESTERASE** In addition to the study of agonists selective for the $M_1$ receptor, we also studied a prodrug of the acetylcholinesterase inhibitor galantamine: Gln-1062.

In Chapter VI, we describe the study of an augmented form of acetylcholinesterase inhibitor galantamine, Gln-1062. This is an inactive prodrug of galantamine that is cleaved into active galantamine by a carboxy-esterase and butyrylcholinesterase. We investigated safety, tolerability, pharmacokinetics and pharmacodynamics. An important finding was the improvement of adaptive tracking test performance (sustained attention) after Gln-1062 administration in healthy elderly subjects compared with placebo. We also found that fewer cholinergic related side effects were experienced after Gln-1062 compared with the parent drug galantamine. Nasal symptoms, however, were reported at a higher frequency after Gln-1062 compared with galantamine.

**IMPROVING MEASUREMENT OF PHARMACODYNAMIC EFFECTS** As described in Chapter I, using biomarkers in drug development is essential to demonstrate pharmacological effects and to determine the therapeutic window. This therapeutic window is the range between the dose level at which pharmacological effects are observed and the dose at which limiting side effects are observed. Based on this window, the optimal dose level for treatment of patients can be selected. During the conduct of the studies described in chapter II-IV and VI, dose decisions were made using safety, pharmacokinetic and pharmacodynamic data. In these studies, neuropsychological and neurophysiological tests, which are part of the Neurocart test battery, were used to measure pharmacodynamics effects. The sets of tests were customized to detect effects that can be expected with drugs modulating the cholinergic system, including effects on sustained attention (adaptive tracking test), working memory (n-back test) and memory (Milner maze test and/or visual verbal learning test).

Each of these studies demonstrated the value and/or associated challenges of measuring pharmacological effects in healthy subjects. In Chapter III, no consistent pharmacodynamic effects were observed after single doses of HTL0018318, however, effects were detected after multiple doses. It was challenging to investigate pharmacodynamics in healthy subjects because of the ceiling effects of the tests, however in the multiple dose study these pharmacodynamic data guided us when making a decision on the dose level to be investigated. In Chapter II we tried to avoid the ceiling effect of tests in healthy subjects by investigating the drug in healthy elderly with below average cognitive function. This study population should not be confused with patients with mild cognitive impairment. To the best of our knowledge, it was the first time such a study population was selected for a trial investigating drug induced effects on cognitive function. The ceiling effects of the tests in this study population were unknown, as it was uncertain how much room there would be to improve cognition. After all, these subjects had no evidence of cognitive dysfunction caused by cholinergic deficiency. Therefore the possible ceiling effects were still a challenge. An increase in P300 amplitude was shown after 13.5 mg HTL0009936 and an improvement on adaptive tracking test performance was demonstrated after physostigmine. The pharmacodynamic data confirmed the effectiveness of the comparator physostigmine, which can be considered as an added value of the use of biomarkers. As described in Chapter VI, Gln-1062 showed dose related effects on the adaptive tracking test, which is confirmed by PK-PD analysis. This finding encouraged the developer to continue the development of this product.

Although demonstrating improvement in cognitive function in healthy subjects is challenging, using biomarkers in early phase drug development to detect pharmacodynamic changes is valuable. The studies described in Chapter VII and 8 contribute to the improvement of the investigation of pharmacodynamics effects.

In Chapter VII we investigated a pharmacological challenge model using biperiden, an $M_1$ receptor antagonist, in healthy elderly subjects. Biperiden induced dose-related temporary cognitive deficits; impairments of sustained attention, verbal memory and working memory were observed. Because of these drug induced cognitive impairments, there is room for cognitive improvement. When investigating a new experimental product, for example an $M_1$ receptor agonist, no or fewer ceiling effects of tests are to be expected. This model can be used for proof-of-pharmacology studies and to demonstrate cognition enhancing effects of new cholinergic compounds.

The review described in Chapter VIII provides an overview of biomarkers used to investigate effects of pro- and anticholinergic drugs in healthy subjects. In addition, their ability to detect drugs effects was evaluated. In total, 132 relevant articles were included, comprising 223 individual tests. The most prominent effects were found for muscarinic receptor antagonists, which produced consistent deteriorations in learning and memory tests. Fewer tests were able to demonstrate effects of nicotinic recep-

tor antagonists on learning and memory. Nicotinic receptor agonist produced moderate improvements of cognitive functions. By themselves, cholinesterase inhibitors did not produce consistent and reliable effects on any test in healthy volunteers.

## Current status of muscarinic compounds

At the time of writing, three trials have been conducted with HTL0009936, of which the trial described in Chapter II was the last one. All three represent phase I studies of drug development. No official decision has been made on the continuation of the development programme. After the trials described in Chapter III-V, HTL0018318 has been investigated in patients with Alzheimer's disease (phase 1b and phase 2 trials). Further development was halted based on new results from a toxicology study in non-human primates. In this toxicology study a rare tumor was observed at doses and durations exceeding those used clinically in humans to date. This toxicology finding is being investigated to understand the relevant mechanism and to enable the human clinical development program with HTL0018318 to continue. Gln-1062 has been further developed as an enteric-coated tablet instead of nasal spray. This formulation has been investigated in a phase 2 trial and is expected to be studied in a pivotal phase 3 trial starting in Q3 of 2021.

Other compounds targeting the $M_1$ receptor have entered the clinical development phase as well. TAK-071 is an orally administered positive allosteric modulator (PAM) investigated in healthy subjects and patients with mild cognitive impairment (NCT02769065). Results of this study remain to be published. PAM VU319 was tested in a phase I single dose trial[18]. The published abstract states that no dose limiting side effects were observed but detailed information was not provided. Pharmacodynamics were not investigated. A phase 2 study with VU319 in patients with mild cognitive impairment is being planned. The $M_1$ receptor agonists NGX267 was studied in a single ascending dose study to estimate the maximally tolerated dose[19]. No subsequent studies have been conducted. Merck has investigated multiple selective $M_1$ PAMs, which failed to show cognitive improvement in patients with Alzheimer's disease[20]. The $M_1$ selective PAMs of Pfizer were associated with gastrointestinal and cardiovascular adverse events in pre-clinical studies[21]. The $M_1$ bitopic agonistic ligand GSK1034702 improved episodic memory in a nicotine abstinence cognitive impairment model[22], but further development was discontinued due to observed side effects. $M_1/M_4$ receptor agonist xanomeline showed improvements in verbal learning and memory, but unfortunately had an unfavourable side effect profile[23,24]. Clearly, finding a $M_1$ receptor agonist that leads to cognitive improvement and with a favourable side effect profile continues to be a significant development challenge.



## Prospects for (new) symptomatic treatments of neurodegenerative diseases

This dissertation focussed on the development of symptomatic drugs. Alzheimer's disease, Lewy bodies disease and schizophrenia have an immense impact on the quality of life of the patients and their relatives, on global health and costs. In the Netherlands, Alzheimer's disease has a prevalence of approximately 300.000 and this number will likely increase to 690.000 by 2050. Currently 800.000 caregivers take care of their relatives for on average 40 hours a week. With 9 billion euros, the costs take 9.5% of the health care budget of the Netherlands[25]. According to the WHO, around 50 million people worldwide have dementia, of which 60-70% is caused by Alzheimer's disease. The total number of people with dementia is projected to reach 152 million in 2050. Approximately 50.000 patients with Parkinson's disease and parkinsonism are being treated by a neurologist in the Netherlands. This number is expected to increase to 68.500 by 2025[26]. The prevalence of schizophrenia is much lower (prevalence of 0.5% of the Dutch citizens), but the disease has a profound impact on functioning and the quality of life of these patients[27]. A change in the prevalence and course of these devastating diseases is urgently needed. Therefore, not only the symptomatic treatments but also disease modifying therapies are in development[28].

Most agents for Alzheimer's disease in phase 2 (85%, n=55) and phase 3 (59%, n=17) of development are potentially disease modifying[28]. A wide range of the pathological processes and proteins are targeted. These include preventing amyloid β from clumping into plaques and removing β-amyloid plaques (eg solanezumab, Aducanumab, verubecestat), preventing tau from forming tangles (eg JNJ-63733657, TRX0237), reducing inflammation (eg sargramostin, Mastinib, ALZT-OP1), improving synaptic function (eg AGB101, ANAVEX2-73), reducing vascular risks (eg with losartan, amlodipine, atorvastatin and exercise), neurogenesis, and epigenetics. In addition, a high percentage of the new agents developed as treatment for Parkinson's disease is potentially disease modifying (49%)[29].

Now that disease modifying treatments are on their way, is there still a need for (new) symptomatic treatments? There are multiple reasons supporting the need. First, without an approved disease modifying treatment, the need for improved symptomatic treatments remains high. As stated before, the current symptomatic treatments have modest beneficial effects and modest side effects[7-9]. The development of new drugs is a long and uncertain trajectory and even though multiple disease modifying products are in phase 3 of the clinical development, there is no guarantee that there will be an approved drug soon. The clinical failure rate for

disease-modifying treatments for neurodegenerative diseases is nearly 100%[30,31]. As long as there is no disease modifying treatment approved, there is need for improved symptomatic treatments. Second, when a disease modifying drug is approved, it is highly unlikely that the neurodegenerative diseases will be cured and eradicated in the foreseeable future, even if the number of approved disease modifying treatments increases. Consequently symptomatic treatments remain necessary. Third, the new disease modifying treatments will hopefully slow down or stop further disease progression. The moment of starting the treatment will decide which symptoms already emerged and which symptoms can still be prevented or slowed down. Currently, there is no population screening to identify Alzheimer's disease at a prodromal stage and hence development of symptoms cannot be prevented. Often the earliest clinical manifestation of Alzheimer's disease is memory impairment and even when this is not the primary complaint, memory deficits can be detected in most patients with Alzheimer's disease at the time of presentation. A diagnosis is made on average one year after the onset of symptoms[25]. Consequently, the start of disease modifying therapies will be after the onset of cognitive dysfunction. As complete recovery is not expected from disease modifying treatments, symptomatic treatment of the cognitive symptoms remain needed. Fourth, the efficacy of the disease modifying drugs will also influence the need for symptomatic treatment. The efficacy might very between patients and consequently millions of patients worldwide will experience progressive cognitive and behavioural symptoms requiring symptomatic treatment. Finally, considering the wide variety in patient characteristics such as age, comorbidity, amyloid β and tau protein levels, not all patients will meet the criteria to receive the treatment. Again, symptomatic treatment might be useful in this situation. Development of the $M_1$ receptor agonists HTL0009936 and HTL0018318, and the pro-drug of galantamine Gln-1062 presented in this dissertation are in line with the expected continued need for symptomatic treatments.

Due to the complexity of the disease, it is not expected that the optimal treatment will consist of a single drug. The future treatment of Alzheimer's disease might be a tailored combination therapy, based on multisystemic approach as suggested by Hampel et al[32]. Targeting multiple processes, such as amyloid β accumulation, inflammatory mechanisms and vascular insufficiency, may treat the disease at multiple levels during its course. Future research might reveal more treatment options (e.g. targeting mitochondrial dysfunction or epigenetic factors), allowing an even more variable and personalized treatment. Improving cholinergic neuronal functioning can be expected to play an crucial role in any combination therapy and improvement of cholinergic drugs therefore remains of importance in the years ahead.



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# SAMENVATTING EN DISCUSSIE

## IN DIT PROEFSCHRIFT

In Hoofdstuk 1 is het cholinerge systeem beschreven. Dit bestaat uit neuronen die reageren op de neurotransmitter acetylcholine, danwel neuronen die acetylcholine verspreiden. Het cholinerge systeem is betrokken bij cruciale functies in zowel het centrale als het perifere zenuwstelsel. In het centrale zenuwstelsel zijn de voornaamste cholinerge structuren 1) de nucleus basalis (van Meynert), die de signalen projecteert op de cerebrale cortex en de hippocampus, 2) de pedunculopontine nucleus en laterodorsale tegmentale nucleus die thalamische nuclei innerveren, en 3) de cholinerge neuronen die binnen het striatum communiceren. Doordat cerebrale cortex en de hippocampus betrokken bij cognitieve functies zoals geheugen, leren en aandacht, is de cholinerge structuur nucleus basalis van Meynert ook indirect betrokken bij deze functies. Het staat er immers nauw mee in verbinding. De cholinerge neuronen in het striatum dragen bij aan de balans tussen de neurotransmitters dopamine en acetylcholine. Dopamine, en vooral de balans tussen dopamine en acetylcholine, speelt een belangrijke rol in de motorische vaardigheden. Cholinerge neuronen in het striatum zijn indirect betrokken bij motorische vaardigheden door hun rol in deze balans. In het perifere zenuwstelsel zijn cholinerge neuronen betrokken bij parasympatische activiteiten zoals het reguleren van de bloeddruk en contracties van het gladde spierweefsel in hart, blaas en maagdarmstelsel. Daarbij zijn ze onderdeel van de sympathische innervatie van de zweetklieren.

Dysfunctie van het cholinerge systeem in het centrale zenuwstelsel wordt gezien in de neurodegeneratieve aandoeningen de ziekte van Alzheimer, Lewy body dementie en Parkinson dementie en de psychiatrische ziekte schizofrenie. Behandeling van deze dysfunctie kan op dit moment alleen met medicijnen die de symptomen verminderen. De goedgekeurde middelen hiervoor zijn de cholinesterase remmers rivastigmine, donepezil en galantamine. De werking hiervan berust op het remmen van het enzym cholinesterase waardoor de afbraak van acetylcholine geremd wordt en hierdoor de concentratie acetylcholine in de synapsspleten hoger blijft. De gunstige effecten van deze cholinesterase remmers zijn helaas matig en veel patiënten ervaren bijwerkingen zoals misselijkheid, braken en diarree. Deze bijwerkingen zijn het gevolg van het effect op meerdere soorten receptoren. Immers wordt overal in het lichaam de concentratie acetylcholine hooggehouden en dus worden ook overal de neuronen geactiveerd. Verbetering van de symptomatische behandeling is hard nodig. Nieuwe medicijnen met een selectievere werking zijn in ontwikkeling. Deze nieuwe middelen grijpen aan op specifieke receptoren zoals de nicotinerge α7 receptor of de muscarinerge $M_1$ receptor. Beide receptoren blijven relatief veel aanwezig in mensen met de ziekte van Alzheimer in tegenstelling tot andere cholinerge receptoren. Daarbij zijn deze twee type receptoren aanwezig in de hersenstructuren die betrokken zijn bij geheugen, leren en aandacht. De $M_4$ receptor is een potentieel doelwit om de verstoorde dopamine balans in patiënten met Lewy bodies gerelateerde ziekten te herstellen. Tevens wordt de $M_4$ receptor in verband gebracht met psychotische symptomen die optreden bij patiënten met dementie of schizofrenie, wat het een interessant doelwit maakt.

**AGONIST SELECTIEF VOOR DE M1 RECEPTOR** In dit proefschrift worden twee agonisten beschreven die selectief zijn voor de $M_1$ receptor: HTL0009936 en HTL0018318

In Hoofdstuk 2 is het onderzoek naar $M_1$ receptor agonist HTL0009936 beschreven. We hebben de veiligheid, tolerantie, farmacokinetiek en farmacodynamiek van HTL0009936 onderzocht in gezonde oudere proefpersonen met een beneden gemiddeld cognitief functioneren. HTL0009936 werd middels een infuus toegediend beginnend met een oplaaddosis. Hiermee werd de plasmaconcentratie tot het beoogde niveau gebracht. Met de hierop volgende onderhoudsdosering werd de plasmaconcentratie op het gewenste niveau gehouden zodat de hersenen voldoende blootgesteld zouden worden aan HTL0009936 tijdens het uitvoeren van cognitieve testen. De resultaten van alle metingen werden vergeleken met placebo en met 'vergelijker' fysostigmine, een cholinesterase remmer. De belangrijkste bevindingen waren dat de toediening voldoende veilig bleek en een gebrek aan farmacodynamische effecten van HTL0009936, op een toename van de P300 amplitude na. Dit effect op de P300 zou een aanwijzing kunnen zijn voor een verbetering in de vroege fase van het aandachtsproces. De derde bevinding was een verbetering in de uitvoering van de adaptive tracking test na toediening van fystigmine. De adaptive tracking test wordt gebruikt om volgehouden aandacht te meten.

Hoofdstuk 3 beschrijft het onderzoek dat eenmalige toedieningen van verschillende dosisniveaus HTL0018318 bestudeert in gezonde jong volwassenen en oudere mensen. HTL0081318 is een partiële agonist selectief voor de $M_1$ receptor en we hebben hiervan de veiligheid, tolerantie, farmacokinetiek en farmacodynamiek onderzocht. Dit onderzoek resulteerde in een duidelijk beeld van de farmacokinetische eigenschappen en van de bijwerkingen van het middel. De bijwerkingen waren mild, dosis-gerelateerd en kwamen beperkt voor bij zowel jongeren als ouderen. De bloeddruk steeg licht na toediening. Er werden geen significante effecten gevonden op het cognitief functioneren.

We hebben ook onderzoek gedaan naar meerdere toediening van verschillende dosisniveaus HTL0018318 in gezonde jong volwassenen en oudere mensen. Dit staat beschreven in Hoofdstuk 4. Net als in Hoofdstuk 3, is ook hier de veiligheid,

farmacokinetiek en farmacodynamiek onderzocht. De resultaten met oog op de veiligheid komen overeen met de bevindingen in Hoofdstuk 3. In dit Hoofdstuk werden er echter ook verbeteringen in de uitvoering van de cognitieve testen n-back test (werkgeheugen) en Milner maze test (leren en geheugen) geobserveerd.

In Hoofdstuk 5 is een studie beschreven die onderzoekt of er een interactie is tussen HTL0018318 en cholinesterase remmer donepezil in gezonde proefpersonen. Als HTL0018318 aan patiënten met Alzheimer toegediend wordt, zal het hoogstwaarschijnlijk in combinatie zijn met de bestaande behandeling (cholinesterase remmers). Omdat HTL0018318 en cholinesterase remmers beide de cholinerge activiteit verhogen, was het doel van dit onderzoek om te bestuderen of die verhoogde activiteit als gevolg van de gecombineerde behandeling veilig is en goed verdragen wordt. Daarbij werd onderzocht of de middelen elkaars farmacokinetiek beïnvloeden. Na analyse van de gegevens konden we concluderen dat de combinatie van HTL0018318 en donepezil goed verdragen werd. Er waren geen zorgen over de veiligheid of farmacokinetiek. De farmacodynamiek werd niet onderzocht in dit onderzoek.

**REMMING VAN ACETYLCHOLINESTERASE** Naast onderzoek naar agonisten selectief voor de $M_1$ receptor, hebben we ook een prodrug van cholinesterase remmer galantamine onderzocht. Deze prodrug heet Gln-1062.

In Hoofdstuk 6 beschrijven we het onderzoek naar Gln-1062. Gln-1062 is een inactieve prodrug van galantamine dat wordt toegediend als neusspray. Wanneer dit gesplitst wordt door de enzymen carboxy-esterase en butyrylcholinesterase, blijft het actieve galantamine over. We onderzoeken de veiligheid, verdraagzaamheid, farmacokinetiek en farmacodynamiek van Gln-1062. Een belangrijke bevinding was dat de proefpersonen de adaptive tracking test beter uit konden voeren na toediening van Gln-1062. De adaptive tracking test werd gebruikt om volgehouden aandacht te meten. Ook ervaarden de proefpersonen minder bijwerkingen na Gln-1062 dan na het originele medicijn galantamine. Er werden wel veel meer nasale symptomen waargenomen na Gln-1062 dan na galantamine.

**VERBETEREN VAN DE METHODE OM FARMACODYNAMISCHE EFFECTEN TE METEN** Zoals beschreven in Hoofdstuk 1 is het gebruik van biomarkers in de ontwikkeling van een geneesmiddel essentieel om farmacologische effecten aan te tonen en het therapeutische venster te bepalen. Het therapeutische venster wordt aan de ene kant begrenst door het dosisniveau waarop farmacologische effecten worden waargenomen en aan de andere kant het dosisniveau waarop onacceptabele bijwerkingen optreden. Het optimale dosisniveau om patiënten mee te behandelen wordt gebaseerd op dit therapeutische venster. Tijdens het uitvoeren van de onderzoeken die beschreven zijn in Hoofdstuk 2-4 en 6, zijn een groot deel van de dosisniveaus bepaald met hulp van data over veiligheid, farmacokinetiek en farmacodynamiek. Om farmacodynamische effecten te meten in deze studies hebben we gebruik gemaakt van neuropsychologische en neurofysiologische testen, allen onderdeel van de test batterij genaamd Neurocart. Er werden verschillende testen gecombineerd zodat effecten waargenomen konden worden die verwacht werden bij het ingrijpen op het cholinerge systeem. Effecten werden verwacht op de functies volgehouden aandacht (adaptive tracking test), werkgeheugen (n-back test) en geheugen (Milner maze test en/of visual verbal learning test).

Elk van deze studies laat zien wat de toegevoegde waarde is van het meten van farmacologische effecten en/of de bijkomende uitdagingen van deze metingen in gezonde proefpersonen. In Hoofdstuk 3 werden er geen consistente farmacodynamische effecten aangetoond na eenmalige toediening van HTL0018318. Deze werden echter wel gezien naar herhaalde toedieningen van HTL0018318. Het onderzoeken van farmacodynamische effecten in gezonde proefpersonen is lastig omdat er plafond effecten zijn, maar deze data kon wel gebruikt worden bij het bepalen van de te onderzoeken dosisniveaus in het onderzoek naar meerdere toedieningen van HTL0018318. In Hoofdstuk 2 hebben we geprobeerd om het plafond effect te omzeilen door het onderzoeksmiddel te onderzoeken in gezonde proefpersonen met een beneden gemiddeld cognitief functioneren. Deze onderzoekspopulatie dient niet verward te worden met mensen met een milde cognitieve stoornis. Zover we weten is het de eerste keer dat een dergelijke onderzoekspopulatie is gebruikt voor onderzoek. Er werd een toename in de P300 amplitude gezien na toediening van 13.5 mg HTL0009936 en een verbetering in de uitvoering van de adaptive tracking test na toediening van fysostigmine. De uitdaging was wederom de mogelijke plafond effecten in deze populatie omdat nog onbekend was hoeveel ruimte er was voor verbetering van het cognitieve functioneren. Er was immers geen sprake van cholinerge tekortkomingen bij deze mensen. Het gebruik van biomarkers in dit onderzoek bevestigde de effectiviteit van 'vergelijker' fysostigmine. Zoals beschreven in Hoofdstuk 6, werden er effecten van Gln-1062 op de uitvoer van de adaptive tracking test waargenomen, wat ook bevestigd is met PK-PD analyse. Deze bevinding heeft de ontwikkelaar aangemoedigd om het product verder te ontwikkelen.

Hoewel het lastig kan zijn om in gezonde mensen verbetering in cognitief functioneren aan te tonen, is het wel degelijk van toegevoegde waarde om biomarkers te gebruiken in vroege fase geneesmiddelen onderzoek. In Hoofdstuk 7 en 8 hebben we gekeken hoe we het onderzoek naar farmacodynamische effecten kunnen verbeteren.

In Hoofdstuk 7 is het onderzoek naar een farmacologisch challenge model dat gebruikt maakt van biperideen onderzocht in gezonde ouderen. Biperideen is een $M_1$ receptor antagonist die tijdelijk dosis gerelateerde cognitieve achteruitgang induceert. Deze achteruitgang werd waargenomen op de gebieden volgehouden aandacht, verbaal geheugen en werkgeheugen. De verwachting is dat er ten tijde van deze tijdelijke cognitieve achteruitgang ruimte is om het cognitief functioneren te verbeteren. Bij het testen van een nieuw experimenteel middel, bijvoorbeeld een $M_1$ receptor agonist, in dit challenge model zullen er dan dus minder of geen plafond effecten aanwezig zijn. Dit farmacologische model kan gebruikt worden om de farmacologie van een nieuw experimenteel middel te bewijzen en om verbetering van cognitie door het nieuwe experimentele middel aan te tonen.

Het literatuur review beschreven in Hoofdstuk 8 geeft een overzicht van biomarkers die zijn gebruikt om de effecten van pro- en anticholinergica bij gezonde proefpersonen te onderzoeken. We hebben het vermogen van de biomarkers om de effecten van medicijnen te detecteren geëvalueerd. In totaal zijn 132 relevante artikelen geïncludeerd, waarin 223 individuele tests beschreven werden. De meest prominente effecten werden gevonden in de geneesmiddelengroep muscarine receptor antagonisten, die consistent een verslechtering van de uitvoering van leer- en geheugentests veroorzaakten. Onder de biomarkers die gebruikt zijn om effecten van nicotine receptor antagonisten te onderzoeken waren er minder testen die effect aantoonden op leren en geheugen. Nicotine receptor agonisten produceerde matige verbeteringen op cognitief gebied. Cholinesterase remmers lieten geen consistente effecten op testen zien bij gezonde proefpersonen.

## Huidige status van middelen aangrijpend op de muscarine receptoren

Op het moment van schrijven zijn er drie onderzoeken uitgevoerd met HTL0009936, waarvan de in Hoofdstuk 2 beschreven studie de laatste was. Alle drie behoren tot de zogenaamde fase 1-onderzoeken van de geneesmiddelenontwikkeling. Het is nog onduidelijk of het ontwikkelingsprogramma van de middel voortgezet zal worden. Na de studies beschreven in Hoofdstuk 3-5, is HTL0018318 onderzocht bij patiënten met de ziekte van Alzheimer (fase 1b en fase 2 studies). De verdere ontwikkeling werd stopgezet vanwege nieuwe bevindingen in een toxicologisch onderzoek bij niet-menselijke primaten. In deze toxicologische studie werd een zeldzame tumor waargenomen bij een dosisniveau en tijdsduur die hoger en langer waren dan die tot nu toe klinisch bij mensen werden toegediend. Deze toxicologische bevinding wordt onderzocht om inzicht te krijgen in onderliggende relevante



mechanismen zodat het klinische ontwikkelingsprogramma met HTL0018318 hopelijk voortgezet kan worden. Gln-1062 is verder ontwikkeld als een maagsapresistente tablet in plaats van neusspray. Deze nieuwe formulering is onderzocht in een fase 2-studie en zal naar verwachting worden bestudeerd in een cruciale fase 3-studie die start in het derde kwartaal van 2021.

Naast de onderzoeksmiddelen die in dit proefschrift beschreven zijn, zijn er meer middelen met als doelwit de $M_1$-receptor de klinische ontwikkelingsfase ingegaan. TAK-071 is een positieve allosterische modulator (PAM) dat oraal wordt toegediend. Dit middel is onderzocht bij gezonde proefpersonen en patiënten met milde cognitieve stoornissen (NCT02769065). De resultaten van deze studie moeten nog worden gepubliceerd. PAM VU319 werd getest in een fase 1-studie waarin het middel eenmalig werd toegediend in de proefpersonen. De gepubliceerde samenvatting stelt dat er geen bijwerkingen werden waargenomen die het dosisniveau beperken, maar gedetailleerde informatie werd niet verstrekt. De farmacodynamiek is niet onderzocht in dit onderzoek. Een fase 2-studie met VU319 bij patiënten met milde cognitieve stoornissen wordt gepland. De $M_1$-receptor agonist NGX267 werd bestudeerd in een studie waarin meerdere dosisniveaus eenmalig werden toegediend om het maximaal getolereerde dosisniveau in te schatten. Er zijn geen vervolgonderzoeken uitgevoerd. Merck heeft meerdere PAM's selectief voor de $M_1$ receptor onderzocht, die geen cognitieve verbetering lieten zien bij patiënten met de ziekte van Alzheimer. De $M_1$ receptor selectieve PAM's van Pfizer werden in verband gebracht met gastro-intestinale en cardiovasculaire bijwerkingen in preklinische studies. Het middel GSK1034702, een bitopische agonist selectief voor de $M_1$ receptor verbeterde het episodisch geheugen in een challenge model gebaseerd op nicotine-onthouding, maar de verdere ontwikkeling werd stopgezet vanwege bijwerkingen. $M_1/M_4$-receptor agonist xanomeline vertoonde verbeteringen in verbaal leren en geheugen, maar had helaas een ongunstig bijwerkingenprofiel. Het is duidelijk dat het vinden van een $M_1$-receptor agonist die cognitieve verbetering kan bewerkstelligen en een gunstig bijwerkingenprofiel heeft een belangrijke, maar moeilijke uitdaging blijft.

## Vooruitzichten voor (nieuwe) symptomatische behandelingen van neurodegeneratieve ziekten

Dit proefschrift richt zich op de ontwikkeling van symptomatische therapieën. De ziekte van Alzheimer, Lewy-bodies gerelateerde ziekten en schizofrenie hebben een enorme impact op de kwaliteit van leven van de patiënten en hun familieleden, op de wereldwijde gezondheid en de hiermee gepaard gaande kosten. In Nederland hebben ongeveer 300.000 mensen ziekte van Alzheimer en dit aantal zal waarschijnlijk

toenemen tot 690.000 mensen in 2050. Momenteel zorgen 800.000 zorgverleners gemiddeld 40 uur per week voor hun familieleden. Met 9 miljard euro beslaan de kosten 9,5% van het zorgbudget van Nederland. Volgens de wereld gezondheidsorganisatie (WHO) hebben wereldwijd ongeveer 50 miljoen mensen dementie, waarvan 60-70% wordt veroorzaakt door de ziekte van Alzheimer. Het totale aantal mensen met dementie zal naar verwachting 152 miljoen mensen bereiken in 2050. Er worden momenteel in Nederland ongeveer 50.000 patiënten met de ziekte van Parkinson en parkinsonisme behandeld door een neuroloog. Dit aantal zal naar verwachting toenemen tot 68.500 patenten in 2025. De prevalentie van schizofrenie is veel lager (0,5% van de Nederlanders), maar ook deze ziekte heeft een grote invloed op het functioneren en de kwaliteit van leven van deze patiënten. Een verandering in de prevalentie en het verloop van deze verwoestende ziekten is dringend nodig. Daarom zijn niet alleen de symptomatische behandelingen, maar ook ziekte modificerende therapieën in ontwikkeling.

De meeste middelen tegen de ziekte van Alzheimer die zich bevinden in fase 2 (85%, n=55) en fase 3 (59%, n=17) van de ontwikkeling zijn potentieel ziekte modificerend. Een breed scala aan pathologische processen en eiwitten vormen het doelwit. Hieronder vallen onder andere het voorkomen dat amyloïde bèta samenklontert tot plaques en het verwijderen van bèta-amyloïde plaques (bijv. Solanezumab, Aducanumab, verubecestat), het voorkomen dat tau eiwitkluwen vormt (bijv. JNJ-63733657, TRX0237), en het verminderen van ontstekingen (bijv. Sargramostin, Mastinib, ALZT-OP1). Ook wordt er gericht op het verbeteren van de synaptische functie (bijv. AGB101, ANAVEX2-73), vermindering van vasculaire risico's (bijv. met losartan, amlodipine, atorvastatine en lichaamsbeweging), de neurogenese en epigenetica. Van de nieuwe middelen die als behandeling van Parkinson worden ontwikkeld is eveneens een hoog percentage potentieel ziekte modificerend.

Nu ziekte modificerende behandelingen in ontwikkeling zijn, is er nog behoefte aan (nieuwe) symptomatische behandelingen? Het antwoord is ja en wel om de volgende redenen. Ten eerste blijft de behoefte aan verbeterde symptomatische behandelingen aanwezig zolang er geen ziekte modificerende behandeling is goedgekeurd. Zoals eerder vermeld hebben de huidige symptomatische behandelingen in beperkt mate gunstige effecten en een ongunstig bijwerkingenprofiel, waardoor er ruimte is voor verbetering. De ontwikkeling van nieuwe geneesmiddelen is een lang en onzeker traject en hoewel er zich meerdere ziekte modificerende producten in fase 3 van de ontwikkeling bevinden, is er geen garantie dat er binnenkort ook een ziekte modificerend middel zal worden goedgekeurd. Het percentage van ziekte modificerende behandelingen voor neurodegeneratieve ziekten dat faalt in de klinische ontwikkelingsfase is bijna 100%. Tot de goedkeuring van een ziekte



modificerende behandeling is er behoefte aan een betere symptomatische behandeling. Ten tweede, wanneer een ziekte modificerend medicijn wordt goedgekeurd, is het hoogst onwaarschijnlijk dat de neurodegeneratieve ziekten op afzienbare termijn zullen worden genezen en uitgeroeid, zelfs als het aantal goedgekeurde ziekte modificerende behandelingen toeneemt. Voor de patiënten blijft er dan dus behoefte aan symptomatische behandelingen. Ten derde, de nieuwe ziekte modificerende behandelingen zullen hopelijk de verdere ziekteprogressie vertragen of stoppen. Het moment waarop de behandeling gestart wordt bepaald welke symptomen er al zijn opgetreden en welke symptomen nog kunnen worden voorkomen of vertraagd. Momenteel is er geen bevolkingsonderzoek waarmee de ziekte van Alzheimer in een prodromaal stadium geïdentificeerd kan worden. Hierdoor is op dit moment het voorkomen van symptomen niet mogelijk. De vroegste uiting van de ziekte van Alzheimer wordt vaak getekend door geheugenstoornissen en zelfs als dit niet de primaire klacht is, kunnen bij de meeste patiënten met de ziekte van Alzheimer geheugenstoornissen worden aangetoond op het moment dat de patiënt zich voor het eerst presenteert. De diagnose wordt gemiddeld een jaar na het begin van de symptomen gesteld. Met de huidige tijdlijnen voor het diagnosticeren van de ziekte, zal de start van ziekte modificerende therapie na de aanvang van cognitieve dysfunctie zijn. Omdat volledig herstel niet wordt verwacht, zal daarom symptomatische behandeling van de cognitieve symptomen nodig blijven. Ten vierde, naast de timing van het starten van de behandeling, zal de mate van werkzaamheid van de ziekte modificerende geneesmiddelen ook de behoefte aan symptomatische behandeling beïnvloeden. De therapie zal niet bij iedereen even goed werkzaam zijn en als gevolg zullen miljoenen patiënten wereldwijd progressieve cognitieve stoornissen en gedragssymptomen ervaren die om symptomatische behandeling vragen. Tot slot, gezien de grote verscheidenheid in patiëntkenmerken zoals leeftijd, co-morbiditeit, amyloïde bèta- en tau-eiwitniveaus, zullen niet alle patiënten voldoen aan de criteria om de behandeling te krijgen. Ook in deze situatie zal symptomatische behandeling een goede optie zijn. De ontwikkeling van de $M_1$-receptor agonisten HTL0009936 en HTL0018318, en de prodrug van galantamine Gln-1062 die in dit proefschrift worden gepresenteerd, zijn in lijn met de verwachte voortdurende behoefte aan symptomatische behandeling.

Vanwege de complexiteit van de ziekte wordt niet verwacht dat de toekomstige behandeling bestaat uit één medicijn. De toekomstige behandeling van de ziekte van Alzheimer is waarschijnlijk een op maat gemaakte combinatie van therapieën, waarbij meerdere pathologische processen of eiwitten het doel vormen, zoals voorgesteld door Hampel et al.. Het aanpakken van meerdere processen, zoals accumulatie van amyloïde bèta, ontstekingsmechanismen en vasculaire insufficiëntie, kan de

ziekte tijdens het beloop op meerdere niveaus behandelen. Toekomstig onderzoek zou meer behandelingsopties aan het licht kunnen brengen (bijvoorbeeld gericht op mitochondriale dysfunctie of epigenetische factoren), waardoor een nog meer variabele en gepersonaliseerde behandeling mogelijk wordt. Verbetering van het functioneren van het cholinerge systeem zal naar verwachting een cruciale rol spelen in de combinatietherapie en verbetering van cholinerge geneesmiddelen blijft daarom van belang de komende jaren.

## CURRICULUM VITAE

Charlotte Bakker (Leiden, 1985) graduated from secondary school in 2003 (Zwijsen College Veghel) and started medical school at Erasmus University Rotterdam in the same year. During her study she performed internships at the department of gynaecology (Berlin, Germany) and primary health care (Calcutta, India). She obtained her medical degree in 2010 after which she started working as physician, initially at the paediatric department of St Franciscus Gasthuis in Rotterdam, followed by the intensive care unit of the Maasstad Ziekenhuis in Rotterdam. In 2015, she changed careers to follow her long-standing interest in science. Her scientific career started as researcher at the Nutrition and Movement Sciences department of Maastricht University. From 2016 on, Charlotte worked as project leader and research physician at Centre for Human Drug Research (CHDR). Here she was involved in early phase clinical trials and studies related to drug development methods under supervision of prof. dr. Geert Jan Groeneveld and prof. dr. Joop van Gerven. Whilst working at CHDR, she was trained as clinical pharmacologist. As of September 2020, Charlotte is seconded to the European Medicines Agency (EMA) in Amsterdam and works as national expert on clinical pharmacology and translational sciences related topics.

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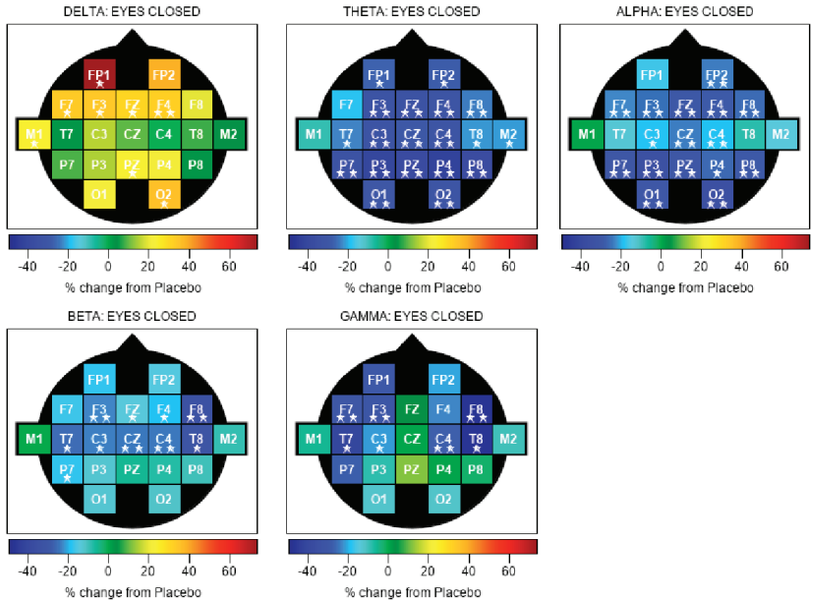
PAGE 154 CHAPTER VII – FIGURE 3A Heatplots showing the effects of 4 mg biperiden on EEG eyes closed condition. For each frequency band and each electrode (representing a cortical area) the % of change in power compared with placebo is shown. * = p<0.05; **=p<0.01




PAGE 154 CHAPTER VII – FIGURE 3B Heatplots showing the effects of 4 mg biperiden on EEG eyes open condition. For each frequency band and each electrode (representing a cortical area) the% of change in power compared with placebo is shown. * = p<0.05; **=p<0.01