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ORIGINAL ARTICLE

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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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 and other dementias. We investigated safety, tolerability, pharmacokinetics 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. Pharmacodynamic assessments were performed using a battery of neurocognitive tasks and electrophysiological measurements. Cerebrospinal fluid concentrations of HTL0018318 and food effects on pharmacokinetics 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 mmHg in younger adults (95% CI [4.2–16.3], 35-mg dose) and up to 11.9 mmHg 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.

Conclusion: HTL0018318 showed well-characterized pharmacokinetics and following single doses were generally well tolerated in the dose range studied. These

The authors confirm that the PI for this paper is Geert Jan Groeneveld and that he had direct clinical responsibility for patients.

provide encouraging data in support of the development for HTL0018318 for Alzheimer's disease and other dementias.

KEYWORDS
Alzheimer's disease, healthy subjects, muscarinic, pharmacokinetics, safety

1 | 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 N-methyl-D-aspartate 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 presynaptic cholinergic neurons with reduced ACh synthesis capacity with disease progress.

An alternative and potentially more effective strategy is to target postsynaptic **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), general clinical status (measured using the Clinician's Interview-Based Impression of Change),and behavioural symptoms such as delusions, hallucinations, agitation (measured using the Alzheimer's Disease Symptomatology Scale).[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 nonselective 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 (ethnyl (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

What is already known about this subject

- Damage of acetylcholine receptors and neurons contribute to cognitive dysfunction in patients with Alzheimer's disease and dementia with Lewy bodies.
- The M_1 receptor plays a key role in cognitive function and is relatively preserved in Alzheimer's disease and dementia with Lewy bodies. Therefore, the M_1 receptor is a potential therapeutic target.

What this study adds

- Information on the safety, pharmacokinetics and exploratory pharmacodynamics of the selective M_1 receptor partial agonist HTL0018318.
- HTL0018318 was tolerated well by healthy younger adult and elderly subjects up to single doses of 35 mg.
- HTL0018318 doses showed rapid absorption and dose-dependent exposures. The mean half-life was between 12 and 16 hours.

agonist that is being developed to treat the symptomatic decline of cognitive function in dementias associated with cholinergic degeneration including AD and DLB. Preclinical studies demonstrated that HTL0018318 has approximately a 2-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 procognitive 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.

2 | METHODS

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

2.1 | Design

This study consisted of 3 parts. Part A used a double-blind, placebo controlled, randomized, single ascending dose design and consisted of 5 cohorts of 8 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 2 to 4 weeks. A single cerebrospinal fluid (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 5 cohorts of 12 healthy elderly subjects, both male and female (9 active and 3 placebo per cohort).

2.2 | Participants

Younger adult subjects aged 18–55 years, inclusive, and elderly subjects aged ≥65 years 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 45–100 beats/min 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.

2.3 | Materials

HTL0018318 was administered as an oral aqueous solution in 100 mL. Dose levels in Part A were 1, 3, 9, 20 and 35 mg, in Part B 20 mg, and in Part C 9, 15, 23, 30 and 35 mg. The 1-mg dose level is the human equivalent to the no-effect level 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 maximum plasma concentration (C_{max}) of 267 ng/mL in humans due to observed increases in blood pressure and change in heart rate in the preclinical 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 1 minute before and after the administration of the oral solution.

2.4 | 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).

2.5 | PK assessments

In all parts, blood samples for determination of plasma HTL0018318 levels were collected at predose and 15 and 30 min, 1, 1.5, 2, 3, 4, 6, 8, 9, 12, 24, 30, 48 and 72 hours, and at follow-up (5–7 days postdose). Urine was collected at predose, up to 72 hours postdose and at follow-up. Plasma and urine samples were analysed 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 predefined acceptance criteria for quality control (± 15% of the nominal concentration) and calibration standards (± 15% except ± 20% at the lower limit of quantification). The quantification range was 0.5–1000 ng/mL. The following PK parameters were estimated from the plasma and urine concentration for HTL0018318 by noncompartmental analysis: the area under the plasma

concentration–time curve (AUC) calculated from 0 to the last measurement point ($AUC_{0\text{-last}}$), from 0 to 24 hours (AUC_{0-24}) and AUC to infinity ($AUC_{0\text{-inf}}$), 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}, $AUC_{0\text{-}t}$ and $t_{1/2}$.

CSF samples were collected only in Part B at 2, 4, 6 and 8 hours postdose. 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.

2.6 | Exploratory PD 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 central nervous system (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 3 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 3 types of trials in the MMT: Immediate for imprinting (5 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 electroencephalogram (EEG; power in δ, θ, α, β and γ bands) and event-related potential (ERP) P300 and mismatch negativity. Other PD measurements included the Leeds Sleep Evaluation Questionnaire 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 3 Salivettes dental rolls that were put into the oral cavity for 3 min) were also examined.

In Parts A and C, all tests were performed twice at baseline and repeated at 1, 3, 5, 6 and 9 hours after administration of HTL0018318 or placebo. The only exceptions were EEG/ERP measurements, which was also performed 2.5 hours postdose, and the MMT, which was not performed 6 hours postdose. 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.

2.7 | 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 analysed 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 analysed with a 1-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 mismatch negativity) were excluded from statistical analysis due to data quality and technical issues with stimuli timing and recording. Hence only resting state EEG power data are reported.

3 | RESULTS

3.1 | 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 2 additional subjects who were enrolled because CSF-sampling could not be performed in 2 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

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)

24.7 kg/m^2 (range 19.4–31.6) and 33.3% were female. In the 30 mg cohort only 9 subjects were included (7 active: 2 placebo) due to recruitment difficulties.

3.2 | 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 probably 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 SBP, DBP or pulse rate measured in supine position were observed in the 1–30 mg dose range. However, following the 35-mg dose, there was a 10.3-mmHg (95% CI [4.2–16.3], *P* = .0015) increase in mean SBP, a 9.2-mmHg (95% CI [3.2–15.1], *P* = .0038) increase in mean DBP, and a 9.8-beats/min increase in mean pulse rate (95% CI [4.4–15.2], *P* = .0008) relative to placebo (Figure 1). Hypertension was considered an TEAE in 1 subject following a 9-mg dose and 3 subjects who received the 35-mg dose. In these 4 subjects, the SBP increased between 14 and 40 mmHg from baseline, and the DBP increased between 0 and 27 mmHg from baseline between 25 minutes and 2 hours postdose. The highest SBP considered to be an TEAE was 145 mmHg post dose which was 105 mmHg at baseline. The highest DBP was 90 mmHg postdose, which was 63 mmHg at baseline.

In elderly subjects in Part C, the mean SBP was significantly higher than placebo following 15 mg HTL0018318 (difference of 11.9 mmHg, 95% CI [4.9–18.9], *P* = .0012), 23 mg (difference of 9.3 mmHg, 95% CI [2.2–16.5], *P* = .0114) and 30 mg (difference of 7.8 mmHg, 95% CI [0.3–15.4], *P* = .0430). The mean DBP was significantly higher following 15 mg (difference of 6.1 mmHg, 95% CI [1.4–10.8], *P* = .0118) and 23 mg (difference of 5.0 mmHg, 95% CI [0.2–9.7], *P* = .04). Hypertension was considered an TEAE in 1 subject following 9 mg HTL0018318, 1 subject following 15 mg, and 3 subjects following 35 mg administration. In these 5 subjects, the SBP increased by 14–51 mmHg from baseline and the DBP increased by 10–31 mmHg between 25 minutes and 3 hours postdose. The highest blood pressure considered to be an TEAE was 181/98 mmHg; this was 156/82 mmHg at baseline.

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

3.3 | 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 postdose (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) compared with elderly subjects (5-8 L/h). The mean $t_{1/2}$ was 12 hours in younger adults and

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)

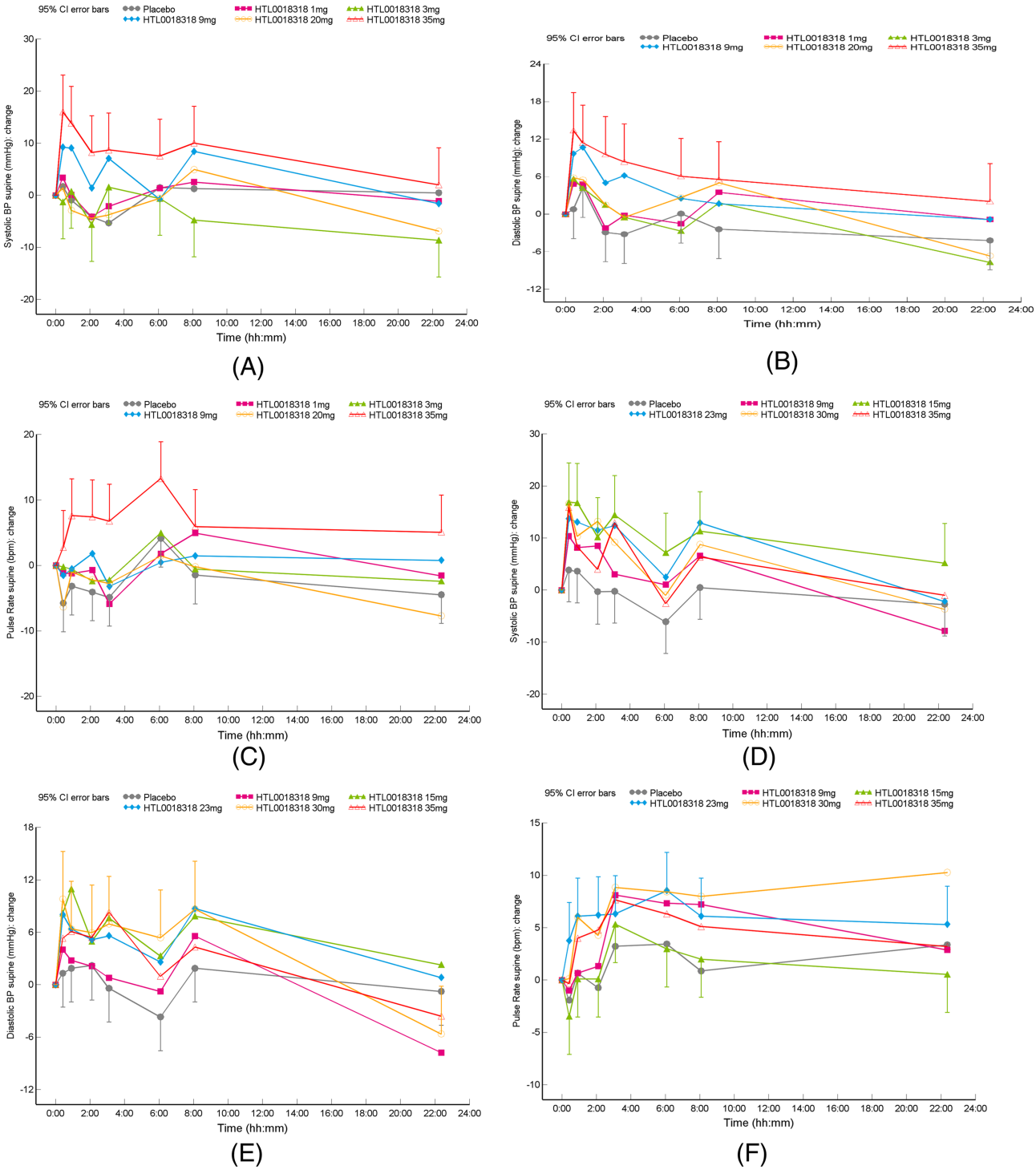

FIGURE 1 Vital signs in adult subjects (A, B, C) and elderly subjects (D, E, F) presented as change from baseline

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 Cmax 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 in younger adults and 260 ng/mL 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 postdose and remained at approximately the same (mean 22.6–30.3 ng/mL) to the last sampling point at 9 hours postdose, with the rise in apparent unbound partition coefficient being primarily a function of decreasing plasma HTL0018318 concentration.

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

Matrix	C_{max} (ng/mL)	T_{max} (h)	C_{last} (ng/mL)	T_{last} (h)	AUC_{0-last} (ng.h/mL)	CSF/plasma(u) ratio (%)	
						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.
C_{max}, maximum plasma concentration; AUC_{0-last}, area under the plasma concentration–time curve calculated from 0 to the last measurement point; T_{max}, time of C_{max}; C_{last}, final plasma concentration; T_{last}, time of C_{last}; CSF, cerebrospinal fluid.

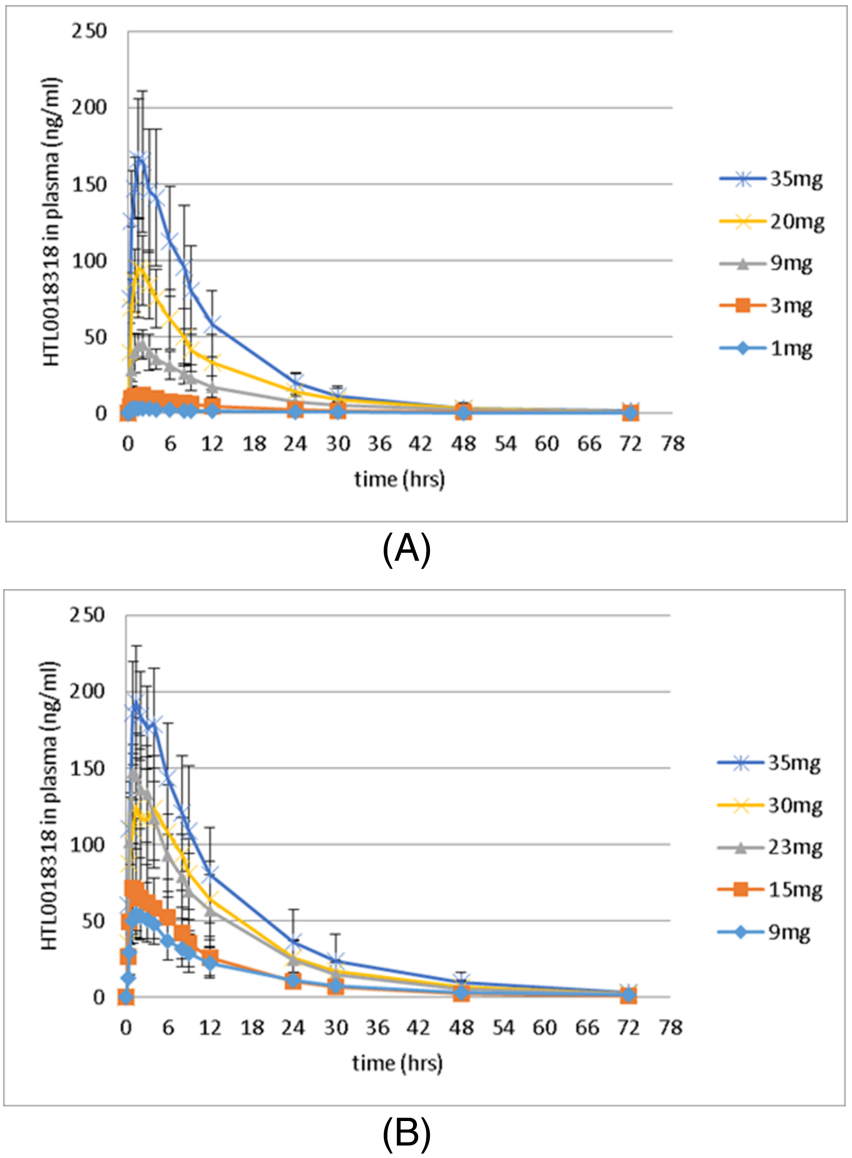

FIGURE 2 HTL0018318 arithmetic mean (± standard deviation) plasma concentration against time after dose following single oral doses of HTL0018318 in healthy younger adults (A) and elderly (B) subjects

Dosing an oral solution of HTL0018318 with an Food and Drug Administration-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]).

3.4 | 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 procognitive effects of HTL0018318, selective statistically significant effects were noted for

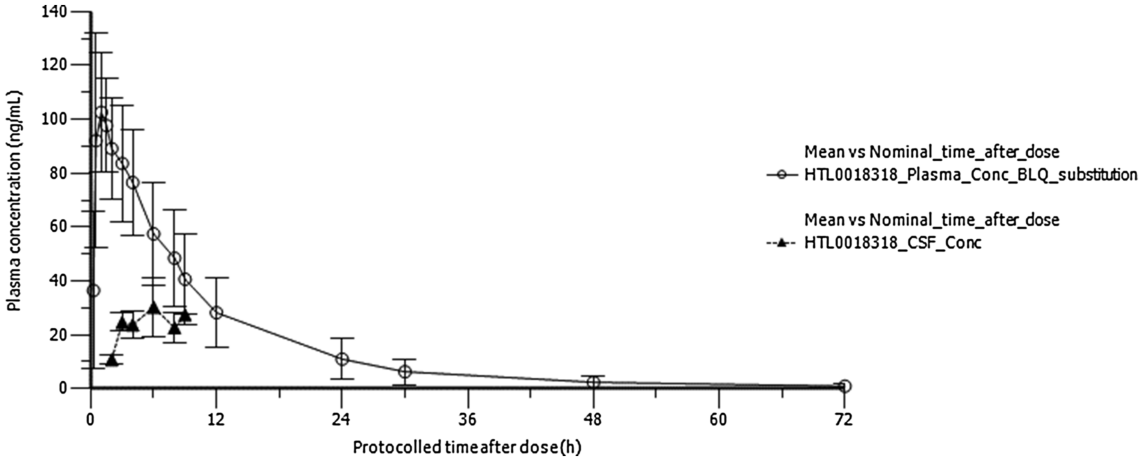

FIGURE 3 HTL0018318 plasma and cerebrospinal fluid (CSF) concentration–time profile after 20 mg HTL0018318 in fasted state. Group mean ± standard deviation

some endpoints (Tables S2 and S3). 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 <.2) in certain cognitive processes including memory/executive function (MMT) 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 Leeds Sleep Evaluation Questionnaire outcomes between HTL0018318 and placebo treatment (Tables S2 and S3). These differences were inconsistent and the magnitude of the change were <5 mm 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 the left and right eyes. In the 15-, 23-, 30- and 35-mg cohorts, a significant increase in pupil/iris ratio (left eye) was observed compared to placebo, and in the 15-, 23- 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.

4 | 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- and 35--mg doses (elderly). In younger adult subjects, doses up to 20 mg were not associated with changes in SBP and DBP and heart rate. However, the 35-mg dose was associated with an increase in mean SBP and DBP (up to 10 mmHg) and mean heart rate (up to 9.8 beats/min). In elderly subjects, significant increases in mean SBP and DBP (up to 11.9 mmHg) and mean heart rate (up to 6.3 beats/min) 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× 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 <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 >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 ≤35 mg are better tolerated following repeat dosing in healthy subjects as well as patients with AD 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 postdose 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 in younger adult and 12–17 L/h 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 h, 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 postadministration and 25.97% 24 hours postadministration, 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 procognitive 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 hours in younger adult subjects and 16 hours 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, $t_{½}$) was modest, with interindividual 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) compared with elderly subjects (5–8 L/h). 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 a Food and Drug Administration-style high calorie breakfast caused a trend towards delay in T_{max} (group median 0.75–2.25 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 Tables S2 and S3; 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 <.2) were noted on certain cognitive processes including memory/executive function (MMT) 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-, 23-, 30- and 35-mg cohorts, a significant increase in pupil/iris ratio (left eye) was observed compared to placebo, and in the 15-, 23- 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, ECGs and 24-hour Holter registrations were observed in both young and elderly subjects.

In summary, HTL0018318 showed well-characterized PK 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 PD data in support of the development of HTL0018318 as a symptomatic treatment for cognitive impairment in dementia including AD and DLB.

4.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[12]

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COMPETING INTERESTS

T.T., J.L., G.B., A.B., M.C., M.W., F.M. and P.N. all are currently, or have been, paid employees of SH and have owned stock in the company, D.C. is a paid independent consultant for SH.

CONTRIBUTORS

G.A.B., A.B., M.C., M.W. and F.H.M. developed the compound. T.T., J.L., E.P.H., S.P. and G.J.G. contributed to designing of the study. S.P., T.F.D., E.P.H. and C.B. performed the study. E.S.K. and D.M.C. contributed to data analysis. T.T., J.L., G.JG., C.B., D.M.C. and P.J.N. contributed to writing and critical revision of this manuscript. All authors have read and approved the final version of this manuscript.

DATA AVAILABILITY STATEMENT

Availability of data and material: The datasets generated during and/or analysed during the current study are filed in EudraCT and are not publicly available [in accordance with the regulations for Phase 1 data]. Further information is available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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