

RESEARCH ARTICLE

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 compared to oral galantamine.

Methods: Forty-eight healthy elderly subjects were randomized 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% confidence interval [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 gastrointestinal side effects than oral galantamine. Gln-1062 behaved pharmacokinetically as expected and improved performance on cognitive tests.

KEYWORDS

Alzheimer's disease, cholinesterase inhibitor, dementia, galantamine, pharmacodynamics, pharmacokinetics, pharmacology, randomized controlled trial, safety, side effects

1 | 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 diarrhea, which reduce the likelihood of the drugs' cognitive enhancing effects.⁴ As there is no curative treatment for AD yet, it is important to optimize the symptomatic treatment.

Gln-1062 (MEMOGAIN) was developed as an augmented form of galantamine, a reversible, competitive ChEI. Galantamine is a

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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 butyryl-cholinesterase. 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 and 20.0 mg/kg resulted in blood-to-brain ratios of 8.1 and 10.2 mg/kg, respectively,⁶ which is higher than the brain-to-blood ratio of galantamine 4 mg/kg in mice of 2.1⁷. 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. In addition, 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.

2 | METHODS

2.1 | Trial design and subjects

This was a randomized, double-blind, placebo-controlled study with multiple intranasal doses of Gln-1062 in healthy subjects (≥ 65 years, n = 48). Subjects were randomized to Gln-1062 or placebo with a ratio of 12:4 per cohort. All 12 placebo subjects (cohorts 1–3) 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 seven 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 of 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 provided 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

HIGHLIGHTS

- Gln-1062 up to 22 mg, b.i.d., for 7 days is considered to be safe and well tolerated
- Gln-1062 caused less nausea and vomiting than orally administered galantamine
- Gln-1062 improved sustained attention in healthy elderly subjects

RESEARCH IN CONTEXT

1. **Systematic review:** The authors searched PubMed for publications regarding the effectiveness and side effects of cholinesterase inhibitors (ChEIs) in patients with Alzheimer's disease (AD).
2. **Interpretation:** ChEIs can reduce symptoms in patients with mild to moderate AD; however, the efficacy is moderate and the daily recommended doses often induce side effects that lead to discontinuation of medication. The effects of Gln-1062, a newly developed prodrug of galantamine, have been investigated before in a first in human study.
3. **Future directions:** This study provides information to be used for the further development of Gln-1062. Long-term safety and efficacy in patients with AD remain to be determined.

Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. The trial was registered in the Netherlands Trials Register (NL5557).

2.2 | 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, electrocardiography (ECG), and safety blood sampling (supplementary Table S1).

2.3 | Pharmacokinetic assessment

To assess PK characteristics of Gln-1062, venous blood samples were obtained from 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 three 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. 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 (AUC_{0-last}) and apparent elimination half-life ($t_{1/2}$) are presented in this paper.

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.

2.4 | 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 three conditions with increasing working memory load. The adaptive tracking test measured attention and eye-hand coordination.^{6,12,13} 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. Pharmacoelectroencephalography (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.

2.5 | 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%-point 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 analyzed with a mixed model analysis of variance with treatment, time, and treatment by time as fixed factors, and subject as a 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 end points. All calculations were performed using SAS for Windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

2.6 | PK-PD analysis

The relationship between galantamine plasma and CSF levels and PD was investigated by analyzing the PK-PD data using nonlinear mixed-effect modeling (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 were 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 was evaluated using a backward reduction method. The least significant covariate not resulting in an increase of $P < 0.001$ was removed from the model.

3 | RESULTS

3.1 | Subjects

A total of 48 healthy elderly subjects were enrolled in this study. The mean (\pm SD) age of the subjects was 70.0 ± 3.4 years, the mean weight (\pm SD) was 72.0 ± 10.6 kg, and the mean body mass index (\pm SD) was

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)
Diarrhea	-7h	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 hemorrhage	1 (8.3)	7 (58.3)	9 (75.0)	-
Intranasal paresthesia	-	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)	-	-
Rhinorrhea	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)
Diarrhea	-	-	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.

25.0 ± 2.6 kg/m². 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 diarrhea after the first dose on the first dosing day. Data from these subjects were included in the analysis sets.

3.2 | 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 in intensity and were self-limiting. There were no serious adverse events. (For an overview of the most frequent occurring TEAEs see 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.

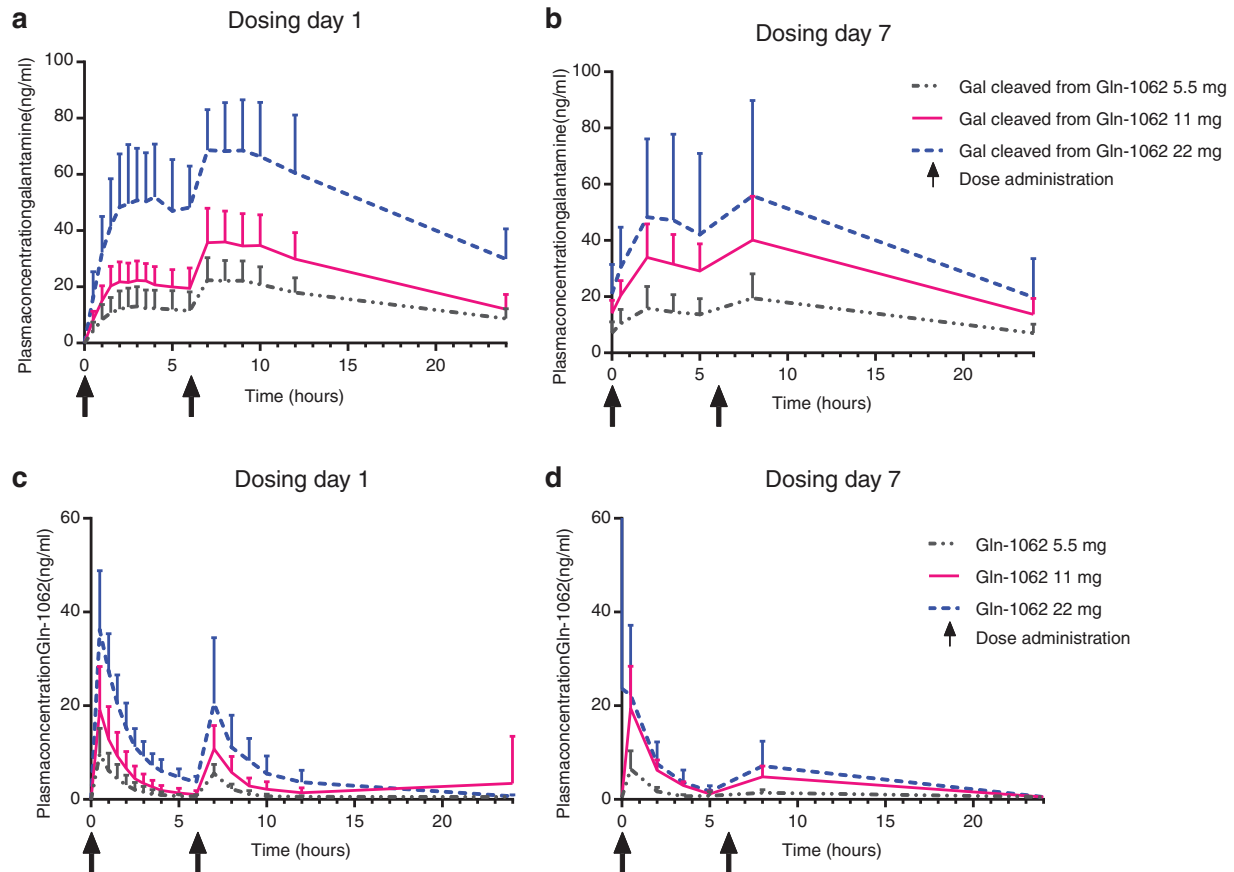


FIGURE 1 A and B, Plasma concentrations of galantamine cleaved from Gln-1062 on dosing days 1 and 7 after administration of Gln-1062 5.5 mg, 11 mg, or 22 mg, b.i.d. C and D, Plasma Gln-1062 concentrations on dosing days 1 and 7 after administration of Gln-1062 5.5 mg, 11 mg, or 22 mg, b.i.d.

3.3 | Pharmacokinetics

Gln-1062 plasma concentrations increased immediately following dosing with a median T_{max} of 0.5 hour (range 0.5 to 1.0 hour). Plasma concentrations of Gln-1062 declined in a monophasic manner following C_{max} . The mean (\pm SD) $t_{1/2}$ of 5.5 mg Gln-1062 was 1.16 (\pm 0.37) hour, of 11 mg Gln-1062 was 1.57 (\pm 1.74) hour, and of 22 mg Gln-1062 was 2.09 (\pm 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).

3.4 | Pharmacodynamics

The PD effects of Gln-1062 compared with placebo are summarized 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.

3.5 | PK-PD analysis

PK data were fit best by a two-compartment model for Gln-1062 in plasma; one compartment for galantamine in plasma and one 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.

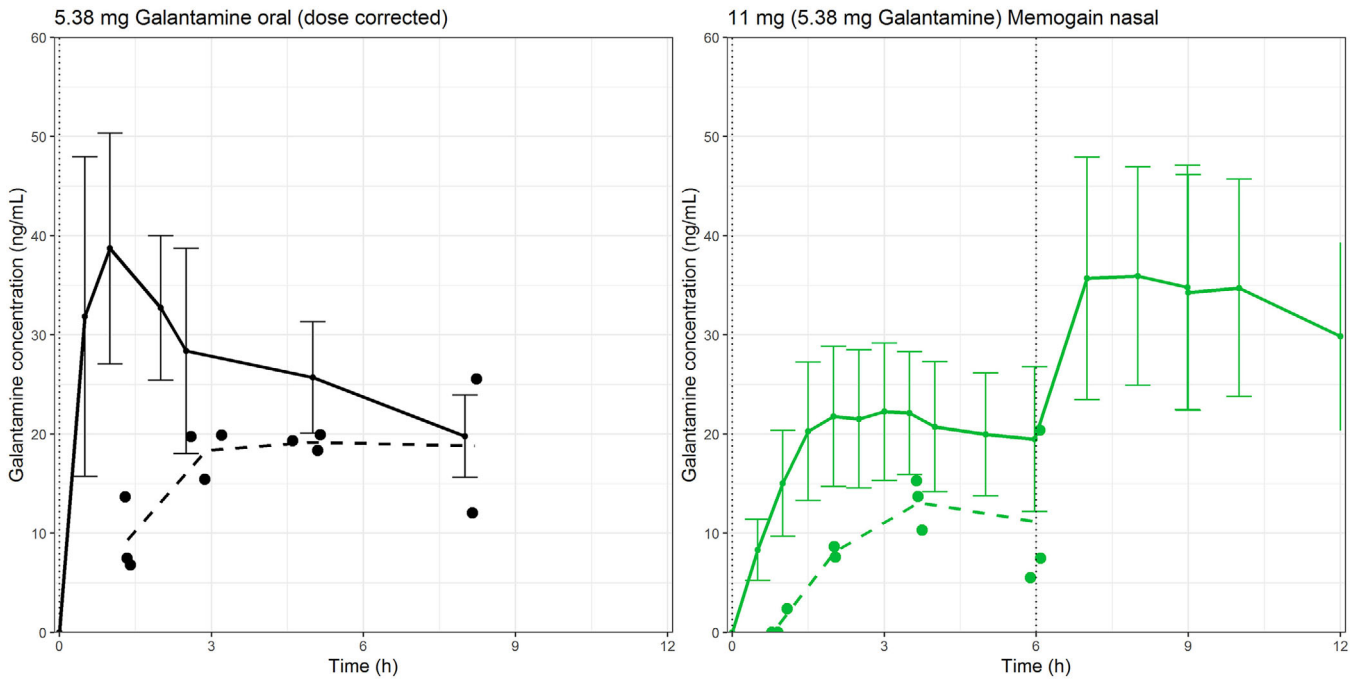


FIGURE 2 Galantamine plasma and CSF concentrations after oral galantamine, dose corrected (left) and 11 mg Gln-1062 (right). Dots/dotted line = CSF galantamine concentration. Solid line = plasma galantamine concentration

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 1.15–5.90) and a concentration resulting in half of the maximum (EC_{50} ; mg/L) of 0.0231 (95% CI –0.0005–0.134) for Gln-1062 and 0.172 (95% CI –0.584–5.01) for oral galantamine.

4 | DISCUSSION

This was a randomized, 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 those of 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. Of interest, 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 seven 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 the 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

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 placebo	Gln-1062 11 mg placebo	Gln-1062 22 mg placebo
Smooth Pursuit (%)	-1.28 (-5.10, 2.53) P = 0.5011	-0.10 (-4.04, 3.84) P = 0.9597	2.00 (-1.88, 5.88) P = 0.3039
Saccadic Inaccuracy (%)	0.59 (-0.46, 1.64) P = 0.2627	0.20 (-0.89, 1.30) P = 0.7099	0.51 (-0.56, 1.58) P = 0.3393
Saccadic Peak Velocity (deg/s)	-0.06 (-21.97, 21.85) P = 0.9958	5.03 (-17.82, 27.87) P = 0.6588	8.63 (-13.65, 30.91) P = 0.4379
Saccadic Reaction Time (sec)	-0.0112 (-0.0224, -0.0001) P = 0.0481	-0.0018 (-0.0134, 0.0098) P = 0.7546	-0.0013 (-0.0126, 0.0101) P = 0.8228
Adaptive tracking (%-point)	0.520 (-0.779, 1.818) P = 0.4240	0.318 (-0.992, 1.627) P = 0.6266	1.954 (0.630, 3.279) P = 0.0055
VAS Alertness (mm)	-1.09 (-3.74, 1.57) P = 0.4134	0.63 (-2.15, 3.41) P = 0.6514	-0.21 (-2.86, 2.44) p = 0.8735
VAS Calmness (mm)	0.44 (-2.59, 3.48) P = 0.7694	0.27 (-2.86, 3.40) P = 0.8615	0.44 (-2.61, 3.49) P = 0.7734
VAS Mood (mm)	-0.92 (-3.98, 2.13) P = 0.5462	-0.49 (-3.59, 2.62) P = 0.7530	-0.68 (-3.66, 2.29) P = 0.6455
VAS Nausea log(mm)	0.0282 (-0.0517, 0.1082) P = 0.4800	0.0485 (-0.0330, 0.1301) P = 0.2366	0.0447 (-0.0359, 0.1253) P = 0.2697
N-back mean RT 0 back (msec)	8.2 (-18.3, 34.6) P = 0.5374	5.8 (-21.2, 32.8) P = 0.6665	-21.7 (-47.5, 4.0) P = 0.0959
N-back mean RT 1 back (msec)	-48.5 (-91.0, -6.0) P = 0.0264	6.1 (-39.6, 51.8) P = 0.7893	-24.4 (-67.1, 18.3) P = 0.2558
N-back mean RT 2 back (msec)	-45.6 (-99.3, 8.2) P = 0.0944	-41.1 (-99.7, 17.5) P = 0.1641	-71.4 (-126.4, -16.3) P = 0.0123
N-back corr-incorr/total 0	0.003 (-0.023, 0.029) P = 0.7985	0.006 (-0.020, 0.032) P = 0.6664	0.007 (-0.019, 0.033) P = 0.5978
N-back corr-incorr/total 1	0.026 (-0.026, 0.078) P = 0.3186	0.027 (-0.023, 0.076) P = 0.2811	0.020 (-0.029, 0.069) P = 0.4242
N-back corr-incorr/total 2	-0.002 (-0.068, 0.064) P = 0.9575	-0.016 (-0.081, 0.049) P = 0.6231	0.036 (-0.028, 0.099) P = 0.2661
VVLT Word recall correct 1	0.36 (-1.31, 2.03) P = 0.6635	0.62 (-1.07, 2.32) P = 0.4608	-0.12 (-1.79, 1.54) P = 0.8816
VVLT Word recall correct 2	-0.45 (-2.44, 1.55) P = 0.6545	-0.63 (-2.65, 1.40) P = 0.5370	-0.01 (-2.00, 1.99) P = 0.9959
VVLT Word recall correct 3	-0.12 (-2.42, 2.17) P = 0.9136	-0.89 (-3.23, 1.44) P = 0.4444	0.99 (-1.30, 3.29) P = 0.3883
VVLT Delayed word recall correct	0.36 (-2.02, 2.73) P = 0.7624	-0.73 (-3.15, 1.70) P = 0.5486	0.48 (-1.89, 2.86) P = 0.6838
VVLT Delayed word recognition correct	-2.60 (-6.60, 1.40) P = 0.1967	-2.19 (-6.24, 1.87) P = 0.2825	-0.43 (-4.43, 3.57) P = 0.8291
VVLT Delayed word recognition RT correct (msec)	-49.93 (-166.97, 67.10) P = 0.3944	-30.91 (-150.24, 88.41) P = 0.6041	-120.03 (-237.07, -3.00) P = 0.0447
Systolic BP supine (mmHg)	5.0 (-0.3, 10.4) P = 0.0644	1.2 (-4.1, 6.5) P = 0.6528	1.8 (-3.6, 7.2) P = 0.5098
Diastolic BP supine (mmHg)	2.5 (-0.6, 5.6) P = 0.1105	0.9 (-2.2, 4.1) P = 0.5566	1.2 (-1.9, 4.3) P = 0.4437
Pulse Rate supine (bpm)	-2.5 (-6.4, 1.4) P = 0.2044	-1.0 (-4.9, 2.9) P = 0.6030	0.5 (-3.5, 4.4) P = 0.8169
EEG Delta Fz-Cz closed (uV ² /Hz)	-1.8% (-30.4%, 38.5%) P = 0.9140	-14.8% (-37.6%, 16.4%) P = 0.3023	-19.8% (-35.7%, -0.1%) P = 0.0495

(Continues)

TABLE 3 (Continued)

Parameter	Gln-1062 5.5 mg placebo	Gln-1062 11 mg placebo	Gln-1062 22 mg placebo
EEG Delta Fz-Cz open (uV ² /Hz)	-13.6% (-35.0%, 14.8%) P = 0.3010	-2.5% (-25.2%, 27.0%) P = 0.8435	-30.1% (-48.3%, -5.3%) P = 0.0235
EEG Delta Pz-O1 closed (uV ² /Hz)	-3.1% (-36.1%, 46.9%) P = 0.8765	-12.4% (-41.5%, 31.2%) P = 0.5079	-27.4% (-46.1%, -2.1%) P = 0.0369
EEG Delta Pz-O1 open (uV ² /Hz)	-12.9% (-39.4%, 25.1%) P = 0.4391	-5.4% (-33.6%, 34.6%) P = 0.7474	-29.1% (-45.3%, -8.0%) P = 0.0126
EEG Delta Pz-O2 closed (uV ² /Hz)	-13.4% (-39.2%, 23.5%) P = 0.4151	-0.4% (-28.5%, 38.7%) P = 0.9799	-21.5% (-35.1%, -5.0%) P = 0.0154
EEG Delta Pz-O2 open (uV ² /Hz)	3.1% (-23.8%, 39.4%) P = 0.8382	3.7% (-21.2%, 36.6%) P = 0.7851	-16.5% (-33.4%, 4.8%) P = 0.1128

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

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. In addition, 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 modeling, 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 EC₅₀ 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 In addition, the improve-

ment 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 EC₅₀ 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-cholinergic⁹ and pro-cholinergic compounds.⁶ Furthermore the study was powered on the adaptive tracking test, and 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. Because 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 Ref 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 in EEG delta power at frontal and parietal locations was observed. This is consistent with the decrease in delta power after ChEI administration in healthy subjects³⁷ 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

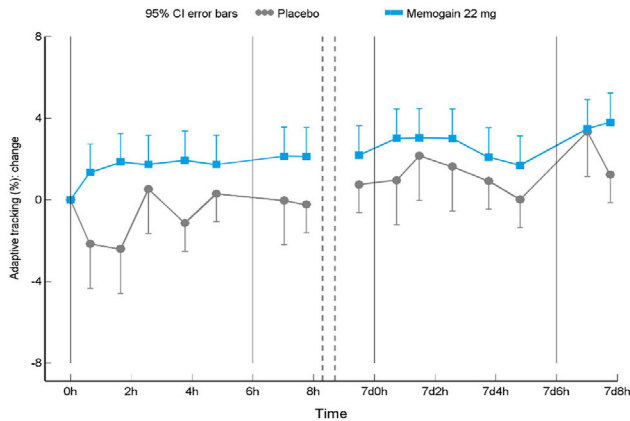


FIGURE 3 The change in adaptive tracking test performance (%-point) from baseline after 22 mg Gln-1062 on the first and seventh dosing days

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 first in human 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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CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

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

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