Innovation in cholinergic enhancement for Alzheimer's Disease

Baakman, A.C.

CHAPTER 5

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

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

A.C. Baakman[1], E. 't Hart[1], D.G. Kay[2], J. Stevens[1], E.S. Klaassen[1], A. Maelicke[2,3], G.J. Groeneveld[1]

1 Centre for Human Drug Research, Leiden, NL
2 Neurodyn Life Sciences, Charlottetown, CA
3 Galantos Pharma, Nieder-Olm, DE

ABSTRACT

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

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

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

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

INTRODUCTION

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

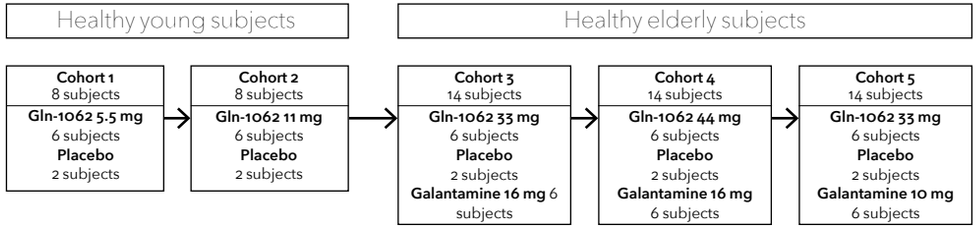
Due to its more favourable brain-to-blood ratio, Gln-1062 is expected to have fewer peripheral side effects than galantamine and other ChEIs and a comparable, or possibly an improved, effectiveness in cognition enhancement. In this study, safety, pharmacokinetic and pharmacodynamic effects of Gln-1062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

METHODS

Trial design and subjects

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

FIGURE 1 Schematic overview of cohorts



Dosing Rationale

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

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

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

Pharmacokinetic assessment

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

Pharmacodynamic assessments

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

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

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

Safety assessments

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

Statistics

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

RESULTS

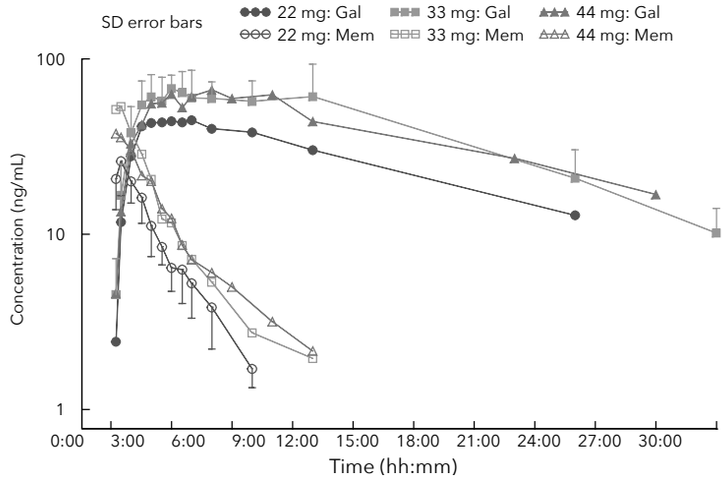
Subjects

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

Pharmacokinetics

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

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



Mem: Gln-1062; Gal: galantamine

TABLE 2 Pharmacokinetic parameters.

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

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

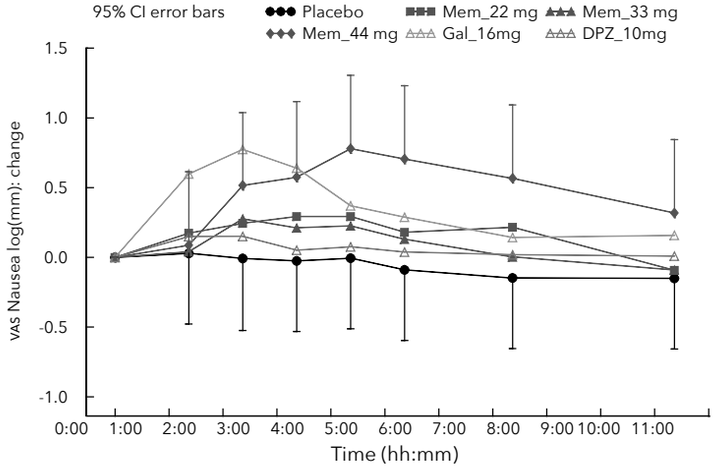
The 44 mg Gln-1062 dose led to a mean exposure that was comparable to the 33 mg dose, however, in view of the considerable inter-individual variability in exposure, the number of subjects per dose level (n=6) and the limited increase in dose from 33 mg to 44 mg (+33%), it cannot be concluded from these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

Safety

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

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

FIGURE 3 Scores on VAS nausea in cohort 3-5



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

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

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

Number of subjects; percentage in parentheses.

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

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

Pharmacodynamics

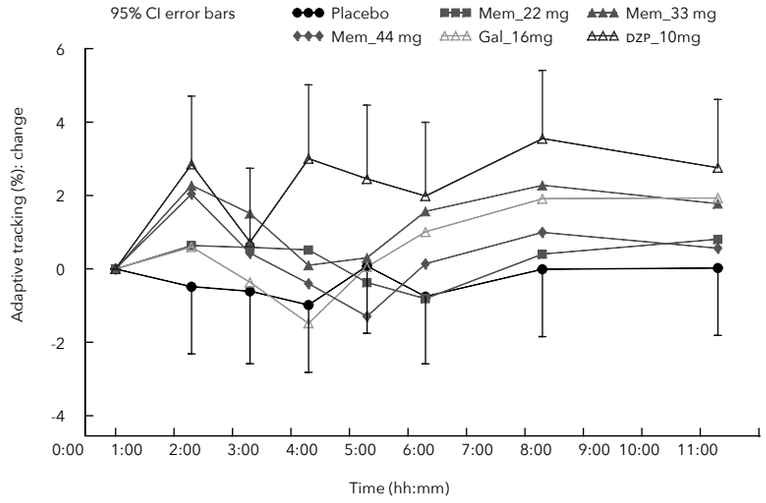
Pharmacodynamic effects of Gln-1062 compared to placebo are summarized in table 3. An improvement on the adaptive tracking performance was seen in the healthy young subjects receiving Gln-1062 11 mg and healthy elderly subjects receiving Gln-1062 33 mg or 44 mg (figure 4).

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

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

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

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



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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

REFERENCES

1 Mikulca JA, Nguyen V, Gajdosik DA, Teklu SG, Giunta EA, Lessa EA, Tran CH, Terak EC, Raffa RB. Potential novel targets for Alzheimer pharmacotherapy: II. Update on secretase inhibitors and related approaches. J Clin Pharm Ther 2014; 39: 25-37.

2 Prins ND, Visser PJ, Scheltens P. Can novel therapeutics halt the amyloid cascade? Alzheimers.Res Ther 2010; 2: 5.

3 Prins ND, Scheltens P. Treating Alzheimer's disease with monoclonal antibodies: current status and outlook for the future. Alzheimers.Res Ther 2013; 5: 56.

4 Patel KR. Biogen's aducanumab raises hope that Alzheimer's can be treated at its source. Manag.Care 2015; 24: 19.

5 Siemers ER, Sundell KL, Carlson C, Case M, Sethuraman G, Liu-Seifert H, Dowsett SA, Pontecorvo MJ, Dean RA, Demattos R. Phase 3 solanezumab trials: Secondary outcomes in mild Alzheimer's disease patients. Alzheimers Dement. 2015.

6 Wallin AK, Wattmo C, Minthon L. Galantamine treatment in Alzheimer's disease: response and long-term outcome in a routine clinical setting. Neuropsychiatr.Dis Treat. 2011; 7: 565-576.

7 Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev 2006; CD005593.

8 Birks J, Grimley EJ, Iakovidou V, Tsolaki M, Holt FE. Rivastigmine for Alzheimer's disease. *Cochrane Database Syst Rev* 2009; CD001191.

9 Olin J, Schneider L. Galantamine for Alzheimer's disease. Cochrane Database Syst Rev 2002; CD001747.

10 Rosler M, Anand R, Cicin-Sain A, Gauthier S, Agid Y, Dal-Bianco P, Stahelin HB, Hartman R, Gharabawi M. Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. BMJ 1999; 318: 633-638.

11 Tariot PN, Solomon PR, Morris JC, Kershaw P, Lilienfeld S, Ding C. A 5-month, randomized, placebo-controlled trial of galantamine in AD. The Galantamine USA-10 Study Group. Neurology 2000; 54: 2269-2276.

12 Knopman DS. Donepezil 23 mg: An empty suit. Neurol Clin Pract. 2012; 2: 352-355.

13 Loy C, Schneider L. Galantamine for Alzheimer's disease and mild cognitive impairment. *Cochrane Database Syst Rev* 2006; CD001747.

14 Riemann D, Gann H, Dressing H, Muller WE, Aldenhoff JB. Influence of the cholinesterase inhibitor galanthamine hydrobromide on normal sleep. Psychiatry Res 1994; 51: 253-267.

15 Zhao Q, Brett M, Van ON, Huang F, Raoult A, Van PA, Verhaeghe T, Hust R. Galantamine pharmacokinetics, safety, and tolerability profiles are similar in healthy Caucasian and Japanese subjects. J Clin Pharmacol 2002; 42: 1002-1010.

16 Mihara M, Ohnishi A, Tomono Y, Hasegawa J, Shimamura Y, Yamazaki K, Morishita N. Pharmacokinetics of E2020, a new compound for Alzheimer's disease, in healthy male volunteers. Int J Clin Pharmacol Ther Toxicol. 1993; 31: 223-229.

17 Lim HK, Juh R, Pae CU, Lee BT, Yoo SS, Ryu SH, Kwak KR, Lee C, Lee CU. Altered verbal working memory process in patients with Alzheimer's disease: an fMRI investigation. Neuropsychobiology 2008; 57: 181-187.

18 Rombouts SA, Barkhof F, Van Meel CS, Scheltens P. Alterations in brain activation during cholinergic enhancement with rivastigmine in Alzheimer's disease. J Neurol Neurosurg. Psychiatry 2002; 73: 665-671.

19 Sweet LH, Rao SM, Primeau M, Durgerian S, Cohen RA. Functional magnetic resonance imaging response to increased verbal working memory demands among patients with multiple sclerosis. Hum Brain Mapp. 2006; 27: 28-36.

20 Laeng B, Lag T, Brennen T. Reduced Stroop interference for opponent colors may be due to input factors: evidence from individual differences and a neural network simulation. J Exp Psychol Hum Percept.Perform. 2005; 31: 438-452.

21 Borland RG, Nicholson AN. Comparison of the residual effects of two benzodiazepines (nitrazepam and flurazepam hydrochloride) and pentobarbitone sodium on human performance. *Br J Clin Pharmacol* 1975; 2: 9-17.

22 Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. *Br J Clin Pharmacol* 1984; 18 Suppl 1: 69S-72S.

23 Gijsman HJ, van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, Cohen AF, Van Kempen GM. Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. J Clin Psychopharmacol. 1998; 18: 289-295.

24 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-180.

25 van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MS, Kroon JM, Breimer DD, Cohen AF. Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. Psychopharmacology (Berl) 1993; 110: 471-478.

26 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol. 1999; 13: 10-17.

27 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003; 55: 39-50.

28 Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971; 10: 181-191.

29 Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. Neurology 1975; 25: 1065-1070.

30 Bittencourt PR, Wade P, Smith AT, Richens A. Benzodiazepines impair smooth pursuit eye movements. *Br J Clin Pharmacol* 1983; 15: 259-262.

31 van Steveninck AL. A microcomputer based system for recording and analysis of smooth pursuit and saccadic eye movements. Br.J Clin Pharmacol 1989; 27 (5): 712P-713P.

32 Wright BM. A simple mechanical ataxia-meter. J Physiol. 1971; 218 Suppl: 27P-28P.

33 Lezak MD, Howieson DB, Loring DW. Neuropsychological Assessment, 4th edition Edition, New York: Oxford University Press, 2004.

34 de Haas SL, Franson KL, Schmitt JA, Cohen AF, Fau JB, Dubruc C, van Gerven JM. The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A alpha2,3 selective agonist, in comparison with lorazepam in healthy volunteers. J Psychopharmacol. 2009; 23: 625-632.

35 Moser, E. B. Repeated Measures Modeling With Proc Mixed. Sugi 29. P188-29. 2004. Ref Type: Conference Proceeding

36 Merkus P, Guchelaar HJ, Bosch DA, Merkus FW. Direct access of drugs to the human brain after intranasal drug administration? *Neurology* 2003; 60: 1669-1671.

37 Farlow MR. Clinical pharmacokinetics of galantamine. *Clin Pharmacokinet* 2003; 42: 1383-1392.

38 Repantis D, Laisney O, Heuser I. Acetylcholinesterase inhibitors and memantine for neuroenhancement in healthy individuals: a systematic review. *Pharmacol Res* 2010; 61: 473-481.

39 Pepeu G, Giovannini MG, Bracco L. Effect of cholinesterase inhibitors on attention. *Chem.Biol Interact.* 2013; 203: 361-364.

40 Drachman DA, Leavitt J. Memory impairment in the aged: storage versus retrieval deficit. *J Exp Psychol* 1972; 93: 302-308.

41 Drachman DA, Leavitt J. Human memory and the cholinergic system. A relationship to aging? *Arch Neurol* 1974; 30: 113-121.

42 Bracco L, Bessi V, Padiglioni S, Marini S, Pepeu G. Do cholinesterase inhibitors act primarily on attention deficit? A naturalistic study in Alzheimer's disease patients. *J Alzheimers.Dis* 2014; 40: 737-742.

43 Samochocki M, Hoffle A, Fehrenbacher A, Jostock R, Ludwig J, Christner C, Radina M, Zerlin M, Ullmer C, Pereira EF, Lubbert H, Albuquerque EX, Maelicke A. Galantamine is an allosterically potentiating ligand of neuronal nicotinic but not of muscarinic acetylcholine receptors. *J Pharmacol Exp Ther* 2003; 305: 1024-1036.