Innovation in cholinergic enhancement for Alzheimer's Disease

Baakman, A.C.

CHAPTER 2

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

Alzheimers Dement (NY). 2019 Apr 1;5:89-98

A.C. Baakman[1], R. Alvarez-Jimenez[1], G. Loewen[2], M.L. de Kam[1], K. Broekhuizen[1], D. C. Hilt[2], G.J. Groeneveld[1]

1 Centre for Human Drug Research, Leiden, NL
2 Forum Pharmaceuticals, Waltham , USA

ABSTRACT

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

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

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

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

INTRODUCTION

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

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

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

METHODS

Trial design and subjects

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

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

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

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

Dosing rationale

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

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

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

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

Pharmacokinetic assessment

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

Pharmacodynamic assessment

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

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

Safety assessments

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

Sample size calculation and statistics

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

RESULTS

Subjects

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

Safety

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

TABLE 2 Subject demographics and baseline characteristics

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

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

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

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

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

Pharmacodynamics

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

TABLE 4 Pharmacodynamic effects compared to placebo

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

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

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

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

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

Pharmacokinetics

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

DISCUSSION

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

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

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

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

TABLE 5 Pharmacokinetic parameters.

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

Means ± SD are presented; DZP=donepezil

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

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

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

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

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

REFERENCES

1 Dementia: a public health priority. World Health Organization; 2012. Report No.: 9241564458.
2 R. Brookmeyer, E. Johnson, K. Ziegler-Graham, H.M. Arrighi. Forecasting the global burden of Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association 2007;3(3):186-91.
3 J. Birks. Cholinesterase inhibitors for Alzheimer's disease. The Cochrane database of systematic reviews 2006(1):CD005593.
4 E. Scarpini, P. Scheltens, H. Feldman. Treatment of Alzheimer's disease: current status and new perspectives. The Lancet Neurology 2003;2(9):539-47.
5 M. Hashimoto, H. Kazui, K. Matsumoto, Y. Nakano, M. Yasuda, E. Mori. Does donepezil treatment slow the progression of hippocampal atrophy in patients with Alzheimer's disease? The American journal of psychiatry 2005;162(4):676-82.
6 M.R. Weed, J. Polino, L. Signor, M. Bookbinder, D. Keavy, Y. Benitex, et al. Nicotinic alpha 7 receptor agonists EVP-6124 and BMS-933043, attenuate scopolamine-induced deficits in visuo-spatial paired associates learning. PloS one 2017;12(12):e0187609.
7 W.J. Deardorff, A. Shobassy, G.T. Grossberg. Safety and clinical effects of EVP-6124 in subjects with Alzheimer's disease currently or previously receiving an acetylcholinesterase inhibitor medication. Expert review of neurotherapeutics 2015;15(1):7-17.
8 J. Prickaerts, N.P. van Goethem, R. Chesworth, G. Shapiro, F.G. Boess, C. Methfessel, et al. EVP-6124, a novel and selective alpha7 nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of alpha7 nicotinic acetylcholine receptors. Neuropharmacology 2012;62(2):1099-110.
9 Clinicaltrials.gov. Safety, Tolerability, and Pharmacokinetic Study of EVP-6124 in Patients With Alzheimer's Disease https://clinicaltrialsgov/ct2/show/NCT00766363.
10 Clinicaltrials.gov. Safety and Cognitive Function Study of EVP-6124 in Patients With Mild to Moderate Alzheimer's Disease https://clinicaltrialsgov/ct2/show/NCT01073228.
11 Clinicaltrials.gov. Study of the Safety and Effectiveness of Two Doses of Investigational Study Drug EVP-6124 in Subjects With Alzheimer's Disease https://clinicaltrialsgov/ct2/show/NCT01969123.
12 Clinicaltrials.gov. Study of the Safety and Effectiveness of Two Doses of Investigational Study Drug EVP-6124 in Subjects With Alzheimer's Disease https://clinicaltrialsgov/ct2/show/NCT01969136.
13 https://www.alzforum.org/news/research-news/rare-severe-side-effects-sideline-some-phase-3-encenicline-trials. Rare but Severe Side Effects Sideline Some Phase 3 Encenicline Trials 2015.
14 M. Liem-Moolenaar, P. de Boer, M. Timmers, R.C. Schoemaker, J.G. van Hasselt, S. Schmidt, et al. Pharmacokinetic-pharmacodynamic relationships of central nervous system effects of scopolamine in healthy subjects. British journal of clinical pharmacology 2011;71(6):886-98.
15 P.J. Snyder, M.M. Bednar, J.R. Cromer, P. Maruff. Reversal of scopolamine-induced deficits with a single dose of donepezil, an acetylcholinesterase inhibitor. Alzheimer's & dementia : the journal of the Alzheimer's Association 2005;1(2):126-35.
16 A.J. Barbier, M. Hilhorst, A. Van Vliet, P. Snyder, M.G. Palfreyman, M. Gawryl, et al. Pharmacodynamics, pharmacokinetics, safety, and tolerability of encenicline, a selective alpha7 nicotinic receptor partial agonist, in single ascending-dose and bioavailability studies. Clinical therapeutics 2015;37(2):311-24.
17 U. Ebert, M. Siepmann, R. Oertel, K.A. Wesnes, W. Kirch. Pharmacokinetics and pharmacodynamics of scopolamine after subcutaneous administration. Journal of clinical pharmacology 1998;38(8):720-6.
18 E. Thomas, P.J. Snyder, R.H. Pietrzak, C.E. Jackson, M. Bednar, P. Maruff. Specific impairments in visuospatial working and short-term memory following low-dose scopolamine challenge in healthy older adults. Neuropsychologia 2008;46(10):2476-84.
19 U.D. Renner, R. Oertel, W. Kirch. Pharmacokinetics and pharmacodynamics in clinical use of scopolamine. Therapeutic drug monitoring 2005;27(5):655-65.
20 G.J. Groeneveld, J.L. Hay, J.M. Van Gerven. Measuring blood-brain barrier penetration using the NeuroCart, a CNS test battery. Drug discovery today Technologies 2016;20:27-34.
21 A. Feinstein, R. Brown, M. Ron. Effects of practice of serial tests of attention in healthy subjects. Journal of clinical and experimental neuropsychology 1994;16(3):436-47.
22 H.K. Lim, R. Juh, C.U. Pae, B.T. Lee, S.S. Yoo, S.H. Ryu, et al. Altered verbal working memory process in patients with Alzheimer's disease: an fMRI investigation. Neuropsychobiology 2008;57(4):181-7.
23 M.J. Mattila, M.E. Mattila-Evenden. Effects of alcohol and hypnosedative drugs on digit-symbol substitution: comparison of two different computerized tests. Journal of psychopharmacology (Oxford, England) 1997;11(4):313-7.
24 T. Reed, D. Carmelli, G.E. Swan, J.C. Breitner, K.A. Welsh, G.P. Jarvik, et al. Lower cognitive performance in normal older adult male twins carrying the apolipoprotein E epsilon 4 allele. Archives of neurology 1994;51(12):1189-92.
25 S.A. Rombouts, F. Barkhof, C.S. Van Meel, P. Scheltens. Alterations in brain activation during cholinergic enhancement with rivastigmine in Alzheimer's disease. Journal of neurology, neurosurgery, and psychiatry 2002;73(6):665-71.
26 L.H. Sweet, S.M. Rao, M. Primeau, S. Durgerian, R.A. Cohen. Functional magnetic resonance imaging response to increased verbal working memory demands among patients with multiple sclerosis. Human brain mapping 2006;27(1):28-36.
27 B. Laeng, T. Lag, T. Brennen. Reduced Stroop interference for opponent colors may be due to input factors: evidence from individual differences and a neural network simulation. Journal of experimental psychology Human perception and performance 2005;31(3):438-52.
28 R.G. Borland, A.N. Nicholson. Visual motor co-ordination and dynamic visual acuity. British journal of clinical pharmacology 1984;18 Suppl 1:69s-72s.
29 R.G. Borland, A.N. Nicholson. Comparison of the residual effects of two benzodiazepines (nitrazepam and flurazepam hydrochloride) and pentobarbitone sodium on human performance. British journal of clinical pharmacology 1975;2(1):9-17.
30 H.J. Gijsman, J.M. Van Gerven, M.C. Tieleman, R.C. Schoemaker, M.S. Pieters, M.D. Ferrari, et al. Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. Journal of clinical psychopharmacology 1998;18(4):289-95.
31 A.L. van Steveninck, R. Gieschke, H.C. Schoemaker, M.S. Pieters, J.M. Kroon, D.D. Breimer, et al. Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. Psychopharmacology 1993;110(4):471-8.
32 A.L. van Steveninck, H.C. Schoemaker, M.S. Pieters, R. Kroon, D.D. Breimer, A.F. Cohen. 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. Clinical pharmacology and therapeutics 1991;50(2):172-80.
33 A.L. van Steveninck, B.N. van Berckel, R.C. Schoemaker, D.D. Breimer, J.M. van Gerven, A.F. Cohen. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. Journal of psychopharmacology (Oxford, England) 1999;13(1):10-7.
34 E. Wezenberg, B.G. Sabbe, W. Hulstijn, G.S. Ruigt, R.J. Verkes. The role of sedation tests in identifying sedative drug effects in healthy volunteers and their power to dissociate sedative-related

impairments from memory dysfunctions. Journal of psychopharmacology (Oxford, England) 2007;21(6):579-87.
35 J.M. Andrew. Delinquents and the Tapping Test. Journal of clinical psychology 1977;33(3):786-91.
36 S.J. de Visser, J.P. van der Post, P.P. de Waal, F. Cornet, A.F. Cohen, J.M. van Gerven. Biomarkers for the effects of benzodiazepines in healthy volunteers. British journal of clinical pharmacology 2003;55(1):39-50.
37 H. Norris. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971;10(21):181-91.
38 R.W. Baloh, A.W. Sills, W.E. Kumley, V. Honrubia. Quantitative measurement of saccade amplitude, duration, and velocity. Neurology 1975;25(11):1065-70.
39 P.R. Bittencourt, P. Wade, A.T. Smith, A. Richens. Benzodiazepines impair smooth pursuit eye movements. British journal of clinical pharmacology 1983;15(2):259-62.
40 B.M. Wright. A simple mechanical ataxia-meter. The Journal of physiology 1971;218 Suppl:27p-28p.
41 S.L. de Haas, K.L. Franson, J.A. Schmitt, A.F. Cohen, J.B. Fau, C. Dubruc, et al. The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A alpha2,3 selective agonist, in comparison with lorazepam in healthy volunteers. Journal of psychopharmacology (Oxford, England) 2009;23(6):625-32.
42 M. Liem-Moolenaar, R.W. Zoethout, P. de Boer, M. Schmidt, M.L. de Kam, A.F. Cohen, et al. The effects of the glycine reuptake inhibitor R213129 on the central nervous system and on scopolamine-induced impairments in psychomotor and cognitive function in healthy subjects. Journal of psychopharmacology (Oxford, England) 2010;24(11):1671-9.
43 M. Liem-Moolenaar, R.W. Zoethout, P. de Boer, M. Schmidt, M.L. de Kam, A.F. Cohen, et al. The effects of a glycine reuptake inhibitor R231857 on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. Journal of psychopharmacology (Oxford, England) 2010;24(11):1681-7.
44 I. Prohovnik, S.E. Arnold, G. Smith, L.R. Lucas. Physostigmine reversal of scopolamine-induced hypofrontality. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 1997;17(2):220-8.
45 K.R. Siegfried. Pharmacodynamic and early clinical studies with velnacrine. Acta neurologica Scandinavica Supplementum 1993;149:26-8.
46 L. Preda, M. Alberoni, S. Bressi, C. Cattaneo, J. Parini, N. Canal, et al. Effects of acute doses of oxiracetam in the scopolamine model of human amnesia. Psychopharmacology 1993;110(4):421-6.
47 R.W. Jones, K.A. Wesnes, J. Kirby. Effects of NMDA modulation in scopolamine dementia. Annals of the New York Academy of Sciences 1991;640:241-4.
48 W. Cho, P. Maruff, J. Connell, C. Gargano, N. Calder, S. Doran, et al. Additive effects of a cholinesterase inhibitor and a histamine inverse agonist on scopolamine deficits in humans. Psychopharmacology 2011;218(3):513-24.
49 R. Alvarez-Jimenez, G.J. Groeneveld, J.M. van Gerven, S.C. Goulooze, A.C. Baakman, J.L. Hay, et al. Model-based exposure-response analysis to quantify age related differences in the response to scopolamine in healthy subjects. British journal of clinical pharmacology 2016;82(4):1011-21.