Featured Article

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

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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 exploratory study is designed to determine the potentiation of the effects of donepezil by cotreatment with EVP-6124, an alpha-7 nicotinic agonist, to 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 randomized, single-center, placebo-controlled, double-blind, five-way, partial crossover study was performed with donepezil 2.5, 5 mg or placebo combined with EVP-6124 0.3, 1, 2, 4 mg or placebo in three 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. Donepezil pharmacokinetic parameters were similar with and without EVP-6124. Effective dose combinations were donepezil/EVP-6124(5/2 mg) and donepezil/EVP-6124 (5/0.3 mg) and showed improvements of the delayed recall of the Visual Verbal Learning Test (1.2; CI = 0.1–2.3) and reaction time during the two-back condition of the N-back (−42; CI = −77, −8), respectively. Overall, no marked reversal of scopolamine effects was observed.

Discussion: This study shows no synergistic effect of subtherapeutic 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.

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Keywords: Alpha-7 nicotinic agonist; EVP-6124; Cholinesterase inhibitor; Donepezil; Alzheimer's disease

1. 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 [1,2]. 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, whereas central AChE inhibition is suboptimal.

Table 1
Overview of study cohorts and treatment periods

Treatment period*	Cohort 1 (n = 12)		Cohort 2 (n = 12)		Cohort 3 (n = 12)	
	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

Abbreviation: DPZ = donepezil.

*The order of the treatment periods was randomized for each subject. Each treatment period was separated by a 14-day washout period; all subjects received scopolamine 0.3 mg i.v.

The nicotinic acetylcholine receptor agonist (nAChR) EVP-6124 might to 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. Coadministration 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 procognitive effect even if (presynaptic) cholinergic neurons are mostly degenerated. In a preclinical animal model, Prickaerts and colleagues indicated a potential synergistic effect of donepezil and EVP-6124 because coadministration of subtherapeutic 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 because of gastrointestinal adverse events [11–13]. Since then, evidence on the suggested synergistic effects of donepezil and EVP-6124 has not been pursued.

This exploratory study was designed to determine whether the strong potentiation of the effects of donepezil by cotreatment with EVP-6124 that was observed in rats can also be observed in healthy elderly volunteers during cognition deficits induced by scopolamine administration. Because it is difficult to demonstrate improvement of cholinergic neuronal functioning in healthy volunteers, scopolamine hydrobromide, a muscarinic acetylcholine receptor antagonist, was administered 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.

2. Methods

2.1. Trial design and subjects

A randomized, single-center, placebo-controlled, double-blind, five-way partial crossover 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 nonsmoking, 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 randomized 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 randomized order. Each treatment period was separated by a 14-day washout period. The study cohorts and treatment periods are summarized in Table 1. All subjects received scopolamine 0.3 mg intravenously on each occasion. 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).

2.2. Dosing rationale

2.2.1. 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 preclinical animal model, Prickaerts and colleagues reported a potential synergistic effect of donepezil and EVP-6124 because coadministration of subtherapeutic 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 because of 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 has not been pursued. As the combination of subtherapeutic doses of EVP-6124 and donepezil is expected to lead to enhanced efficacy, a 2.5 mg dose of donepezil was chosen in the present study to determine enhancement of the donepezil effect in the presence of EVP-6124. In addition, 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.

2.2.2. EVP-6124

Single oral doses ranging from 1 to 180 mg showed linear pharmacokinetics with C_{max} values from 0.6 to 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 present 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 preclinical studies showed a synergistic effect of donepezil and EVP-6124, when given at subtherapeutic dosages (0.3 and 1.0 mg/kg) [8].

2.2.3. 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].

2.3. 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. Pharmacokinetic noncompartmental data analysis was performed to determine T_{max}, 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 ($t_{1/2}$) were also calculated.

2.4. 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 digit symbol 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, 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 behavior (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. Predose 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.

2.5. 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

Table 2
Subject demographics and baseline characteristics

Variable	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/m^2)	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)

NOTE. Means and ranges are presented.

Abbreviations: BMI, body mass index; MMSE, Mini–Mental State Examination.

using monitoring of adverse events (AEs), vital signs, ECG and safety chemistry and hematology blood sampling.

2.6. Sample size calculation and statistics

A sample size of 36 patients was defined to have 80% power to detect an 80% reduction of scopolamine effects due to the combination of donepezil and EVP-6124. This calculation was based on a previous study with scopolamine and a glycine reuptake inhibitor where the effect of scopolamine alone reduced adaptive tracking scores with 42% [42,43]. Based on the estimated standard deviation of the difference, which was about 33% of the scopolamine effect, we assumed that with 12 subjects, the added effect of EVP-6124 over donepezil should be about 30%. If donepezil alone would reduce the scopolamine effect with 50% (to 21%), then the combination of donepezil and EVP-6124 should reduce the scopolamine effect with 80% (to 8.5%) to have 80% power with a two sided alpha of 0.05 and 12 subjects per cohort using a paired t-test. Pharmacodynamic end points were summarized (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. The complexity of the data with repeated measures in time and crossover for treatment led to the choice of a mixed model analysis of variance to analyze the data, separately for each cohort. Within the model, treatment and time were the fixed factors, and the interaction of time and treatment was also added. To account for the repeated measures over time and for the repeated measures over treatment, a random factor subject and the interactions subject by time and subject by treatment were added. The average prevalue per treatment, before scopolamine was given, was used as a covariate. In each of the random factors, a compound symmetry variance/covariance structure is fitted. The Kenwood Rogers degrees of freedom methods were chosen, to correct for the relative small amount of data within the factors of the analysis. Within the model, four estimated differences with 95% confidence interval and the P value of the difference were estimated for each cohort. For cohort 1, the estimated differences were as follows: each dose group versus placebo. For cohort 2, the four estimated differences were as follows: donepezil alone versus placebo and three doses of EVP in combination with donepezil versus placebo. For cohort 3,

the same estimated differences were calculated as for cohort 2. The change compared to the scopolamine challenge alone (with double oral placebo) was analyzed. Average prevalues were added to the model as a covariate. The carryover effect was not implemented in the statistical model as the washout period was considered ample and the randomization was balanced for first-order carryover effects. Analyses were performed on both the data as is and the change from baseline. A $P < .05$ (two-sided) was considered statistically significant. Noncompartmental pharmacokinetic analyses were performed on the plasma concentration data after 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).

3. Results

3.1. 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 postdose measurements. Data of this dropout subject were 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 hypertropia. All 37 dosed subjects were included in the safety analyses; 36 subjects were analyzed for pharmacokinetic and pharmacodynamic outcomes. Subject demographics and baseline characteristics are summarized 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.

3.2. Safety

All but one subject who received at least one dose of study medication (n = 36, 97.3%) reported at least one treatment related 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

Table 3
Most frequently occurring treatment-related adverse events for all dose combinations

DPZ	EVP-6124	N*	Somnolence	Dry mouth	Dizziness	Headache	Disturbance in attention	Gait disturbance
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%)

Abbreviation: DPZ = donepezil.
*All subjects received scopolamine 0.3 mg i.v. on each occasion.

receiving EVP-6124 placebo and donepezil placebo, because of urinary retention due to prostate hypertropia, 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.

3.3. Pharmacodynamics

Pharmacodynamic effects for all different combinations of donepezil and EVP-6124 are summarized 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. Furthermore, 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 affected by any combination of EVP-6124 and donepezil.

EVP-6124 alone, particularly at 4 mg, had a dose-dependent positive effect on the 0-back accuracy. EVP-6124 2 mg had a positive effect on 1-back accuracy (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 improvement 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.

3.4. Pharmacokinetics

Table 5 shows the pharmacokinetic parameters of donepezil and EVP-6124. Based on the noncompartmental 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.

4. Discussion

Preclinical experiments have shown a synergistic effect of EVP-6124 and donepezil in reducing the deleterious effects of scopolamine on short-term memory observed in rats using the Morris water maze task. A complete reversal

Table 4
Pharmacodynamic effects compared to placebo

Outcome	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) $P = .1200$	0.03 (−1.56, 1.63) $P = .9671$	−0.83 (−2.33, 0.67) $P = .2691$	−0.26 (−1.76, 1.24) $P = .7281$	1.49 (0.20, 2.77) $P = .0247$	−1.12 (−2.41, 0.17) $P = .0855$	−1.00 (−2.31, 0.31) $P = .1312$	−1.14 (−2.43, 0.14) $P = .0796$	0.72 (−0.76, 2.20) $P = .3297$	−0.23 (−1.74, 1.28) $P = .7616$	−0.02 (−1.52, 1.48) $P = .9777$	−0.00 (−1.49, 1.48) $P = .9985$
Body sway (mm)*	7.1 (3.2, 18.6) $P = .1796$	−0.3 (10.1, 10.5) $P = .9471$	−1.7 (−11.3, 8.8) $P = .7290$	11.6 (0.7, 23.6) $P = .0363$	1.9 (−10.9, 16.6) $P = .7767$	−4.4 (−16.0, 8.7) $P = .4792$	−0.0 (−12.3, 13.9) $P = .9957$	4.7 (−8.0, 19.1) $P = .4786$	−7.6 (−16.7, 2.5) $P = .1296$	2.6 (−7.7, 14.1) $P = .6260$	5.4 (−5.1, 17.2) $P = .3152$	−3.0 (−12.6, 7.7) $P = .5561$
VVLT delayed word recall (number of words)	0.4 (−0.9, 1.7) $P = .5508$	0.1 (−1.2, 1.4) $P = .8959$	−1.1 (−2.3, 0.2) $P = .0970$	−0.4 (−1.6, 0.9) $P = .5755$	−0.4 (−1.5, 0.7) $P = .4707$	0.1 (−1.0, 1.2) $P = .654$	0.5 (−0.6, 1.6) $P = .3988$	0.3 (−0.7, 1.4) $P = .5417$	−0.2 (−1.3, 0.8) $P = .6466$	0.2 (−0.9, 1.3) $P = .7362$	0.4 (−0.7, 1.5) $P = .4469$	1.2 (0.1, 2.3) $P = .0327$
N-back, 0-back accuracy (%)	0.02 (−0.04, 0.08) $P = .5073$	0.04 (−0.03, 0.10) $P = .2318$	0.05 (−0.01, 0.11) $P = .0818$	0.06 (0.00, 0.12) $P = .0470$	0.03 (−0.05, 0.11) $P = .4512$	0.04 (−0.04, 0.12) $P = .3346$	0.06 (−0.02, 0.14) $P = .1629$	0.01 (−0.07, 0.09) $P = .8116$	0.04 (−0.02, 0.10) $P = .1614$	−0.05 (−0.11, 0.01) $P = .1030$	0.01 (−0.05, 0.07) $P = .7496$	−0.03 (−0.10, 0.03) $P = .2730$
N-back, 0-back reaction time (ms)	8 (−12, 29) $P = .4004$	−17 (−37, 4) $P = .1085$	−5 (−25, 15) $P = .6209$	−16 (−36, 4) $P = .1151$	−24 (−48, 1) $P = .0615$	−9 (−31, 13) $P = .3889$	13 (−9, 35) $P = .2366$	11 (−11, 33) $P = .3189$	−18 (−36, −1) $P = .0386$	−7 (−24, 11) $P = .4388$	−6 (−24, 12) $P = .5046$	3 (−15, 20) $P = .7511$
N-back, 1-back accuracy (%)	0.02 (−0.05, 0.10) $P = .5558$	0.04 (−0.04, 0.12) $P = .3062$	0.08 (0.01, 0.16) $P = .0337$	0.05 (−0.03, 0.12) $P = .2385$	−0.00 (−0.12, 0.11) $P = .9598$	0.04 (−0.08, 0.16) $P = .5014$	0.01 (−0.11, 0.12) $P = .9269$	−0.01 (−0.13, 0.10) $P = .8351$	0.03 (−0.05, 0.11) $P = .4286$	−0.04 (−0.12, 0.04) $P = .3158$	−0.03 (−0.11, 0.05) $P = .5004$	−0.09 (−0.17, −0.01) $P = .0273$
N-back, 2-back accuracy (%)	−0.02 (−0.11, 0.07) $P = .6878$	0.01 (−0.08, 0.10) $P = .8796$	0.03 (−0.06, 0.13) $P = .4557$	−0.02 (−0.11, 0.07) $P = .7181$	0.04 (−0.08, 0.17) $P = .5034$	0.01 (−0.11, 0.14) $P = .8346$	−0.06 (−0.19, 0.06) $P = .3162$	−0.14 (−0.26, −0.01) $P = .0336$	0.02 (−0.08, 0.11) $P = .7469$	−0.03 (−0.12, 0.07) $P = .5790$	−0.04 (−0.14, 0.05) $P = .3887$	−0.08 (−0.18, 0.01) $P = .0800$
N-back, 2-back reaction time (ms)	−5 (−36, 25) $P = .7210$	−3 (−33, 28) $P = .8693$	1 (−29, 31) $P = .9305$	−8 (−39, 22) $P = .5733$	−27 (−70, 16) $P = .2115$	27 (−16, 70) $P = .2057$	35 (−8, 79) $P = .1070$	41 (−2, 85) $P = .0625$	35 (−1, 70) $P = .0541$	−42 (−77, −8) $P = .0187$	−20 (−55, 15) $P = .2513$	−15 (−49, 19) $P = .3722$
Simple reaction time test (%)*	3.3 (−7.0, 14.6) $P = .5380$	−1.5 (−11.5, 9.8) $P = .7844$	−1.1 (−10.9, 9.8) $P = .8319$	5.2 (−5.2, 16.7) $P = .3279$	−14.6 (−26.2, −1.3) $P = .0336$	2.7 (−10.5, 17.9) $P = .6933$	2.5 (−10.9, 17.8) $P = .7246$	6.4 (−7.6, 22.6) $P = .3786$	−3.1 (−9.2, 3.4) $P = .3379$	0.0 (−6.3, 6.8) $P = .9947$	−0.2 (−6.5, 6.6) $P = .9632$	0.0 (−6.3, 6.7) $P = .9999$
EEG alpha Fz-Cz (uV)*	6.8 (−1.4, 15.7) $P = .1053$	12.0 (3.1, 21.7) $P = .0087$	7.3 (−0.9, 16.2) $P = .0818$	5.3 (−2.8, 14.0) $P = .1987$	5.8 (−7.5, 21.0) $P = .4030$	−4.4 (−16.5, 9.5) $P = .5065$	3.1 (−10.1, 18.3) $P = .6505$	−6.6 (−18.4, 6.7) $P = .3051$	4.0 (−13.2, 24.6) $P = .6615$	−9.6 (−25.2, 9.4) $P = .2909$	−5.3 (−21.4, 14.1) $P = .5556$	−15.2 (−29.3, 1.8) $P = .0759$
Saccadic inaccuracy (%)	0.6 (−0.2, 1.4) $P = .1224$	0.0 (−0.8, 0.8) $P = .9525$	−0.1 (−0.9, 0.6) $P = .7196$	0.3 (−0.5, 1.1) $P = .4213$	−1.4 (−2.7, −0.1) $P = .0311$	1.3 (0.1, 2.6) $P = .0388$	1.4 (0.0, 2.7) $P = .0474$	0.8 (−0.5, 2.0) $P = .2132$	0.0 (−0.9, 1.0) $P = .9433$	−0.2 (−1.2, 0.8) $P = .7336$	0.5 (−0.5, 1.5) $P = .3449$	0.2 (−0.7, 1.2) $P = .6470$
Saccadic reaction time (ms)	−4 (−13, 5) $P = .4018$	−2 (−12, 7) $P = .5871$	−3 (−12, 6) $P = .4605$	3 (−6, 11) $P = .5707$	−4 (−20, 11) $P = .5539$	8 (−6, 23) $P = .2495$	3 (−12, 18) $P = .6847$	11 (−4, 26) $P = .1466$	−11 (−20, −2) $P = .0190$	−4 (13, 5) $P = .3548$	12 (3, 22) $P = .0190$	1 (−8, 10) $P = .8674$

Abbreviations: DPZ, donepezil; VVLT, Visual Verbal Learning Test.

*Back translated from log; effect parameter and 95% confidence intervals are presented; effect parameters represent differences compared to placebo estimated by the statistical model that includes random factors for subject, time, subject by time and treatment, and adjusts for pre-values.

Table 5
Pharmacokinetic parameters

Treatment	Treatment group	AUC_{0-t} (ng·hr·mL-1)	T_{max} (hr)	C_{max} (ng·mL-1)
DZP 2.5 mg	EVP-6124 placebo + DZP 2.5 mg	16.08 ± 6.097	3.61 ± 0.989	2.237 ± 0.8462
	EVP-6124 0.3 mg + DZP 2.5 mg	13.87 ± 4.740	4.50 ± 2.333	1.912 ± 0.6547
	EVP-6124 1 mg + DZP 2.5 mg	13.50 ± 4.782	4.72 ± 1.675	1.807 ± 0.6085
	EVP-6124 2 mg + DZP 2.5 mg	14.05 ± 5.035	5.17 ± 2.389	1.963 ± 0.8078
DZP 5.0 mg	EVP-6124 placebo + DZP 5 mg	41.51 ± 21.780	4.30 ± 2.359	6.035 ± 2.997
	EVP-6124 0.3 mg + DZP 5 mg	45.26 ± 16.280	4.13 ± 1.423	6.144 ± 2.800
	EVP-6124 1 mg + DZP 5 mg	37.68 ± 13.740	3.90 ± 0.879	5.284 ± 1.6190
	EVP-6124 2 mg + DZP 5 mg	43.52 ± 13.810	3.50 ± 0.737	5.424 ± 1.5420
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	14,600 ± 3310.0	5.49 ± 0.911	1671.0 ± 360.20
	DZP 2.5 mg + EVP-6124 2 mg	11,220 ± 2002.0	5.92 ± 1.35	1402.0 ± 252.70
	DZP 5 mg + EVP-6124 2 mg	12,920 ± 4474.0	6.25 ± 1.919	1493.0 ± 447.10
EVP-6124 4 mg	DZP placebo + EVP-6124 4 mg	27,960 ± 5020.0	5.99 ± 1.122	3249.00 ± 680.200

NOTE. Means ± SD are presented.
Abbreviation: DZP, donepezil.

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 present study was designed to reproduce the synergistic effect in humans observed in the animal model where subtherapeutic 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 coadministered. However, this study did not demonstrate synergy between donepezil and EVP-6124 when these drugs were given at subtherapeutic dose levels.

The dose combinations of donepezil/EVP-6124 (5 mg/ 2 mg) and donepezil/EVP-6124 5 mg/0.3 mg showed improvements of the delayed recall of the VVLT and reaction time during the 2-back condition of the N-back, respectively. It is important to note, however, that we should be reserved in our considerations about these pharmacodynamic effects, which most likely are standalone effects that are no prove of synergy between donepezil and EVP-6124. Because of a lacking dose response relationship, no conclusions should be drawn about treatment efficacy of donepezil and EVP-6124. In addition, we should keep in mind that this study was exploratory in nature, aiming for the determination of synergy between donepezil and EVP-6124, whereas the exploration of pharmacodynamic effects was a secondary objective of this study.

Synergy between donepezil and EVP-6124 was excluded as pharmacokinetic parameters suggest that the pharmacokinetic profile of EVP-6124 did not affect the profile of donepezil and vice versa. If the donepezil/EVP-6124 combination in our study turned out to be synergistic of origin, this would indicate that EVP-6124 in combination

with lower (subtherapeutic) dose levels of donepezil would have been at least equally efficacious and could have led to the prescription of lower dosages of donepezil in AD patients, with equal efficacy and fewer adverse events. 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 $AUC_{0 \to inf}$ 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. Although 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 preclinical studies showed a synergistic effect of donepezil and EVP-6124, when given at subtherapeutic dosages. These studies also indicated that desensitization would occur at higher doses [8–10]. In the present study, only the two highest doses of 2 mg and 4 mg EVP-6124 without coadministration 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.

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. Overall, treatment with subtherapeutic 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% 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. Most adverse events had an anticholinergic nature and were therefore most likely related to the administration of scopolamine.

In conclusion, although 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. With the results of this study, an important step is taken toward the understanding of the synergic effects of AChEIs and nAChRs for the treatment of AD patients. We believe that conducting translational studies, wherein preclinical experiments are adequately translated to humans, is highly relevant and informative, for the treatment of AD patients and for knowledge gaps with regard to translatability, for example, the use of the scopolamine model.

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.trci.2019.02.002.

RESEARCH IN CONTEXT

1. Systematic review: The authors searched PubMed and found that preclinical experiments have shown a synergistic effect of the nicotinic acetylcholine receptor agonist EVP-6124 and donepezil in reducing the deleterious effects of scopolamine on short-term memory observed in rats using the Morris water maze task. Full reversal of scopolamine-induced effects was observed when both donepezil and EVP-6124 were given at subtherapeutic dose levels, approximately 1/10th of the dose at which each of the compounds alone fully reversed the scopolamine effects.

2. Interpretation: With this study we aimed to reproduce the synergistic effect observed in the animal model in healthy elderly subjects. Results demonstrated that treatment with subtherapeutic dose levels of donepezil and EVP-6124, in combination with scopolamine, was well tolerated.

2. Future directions: While administration of EVP-6124 alone and donepezil alone led to some reduction of scopolamine-induced effects in some of the pharmacodynamic variables, this study shows no 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] Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. Alzheimer's Demen: J Alzheimer's Assoc 2007;3:186–91.

[3] Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev 2006:Cd005593.

[4] Scarpini E, Scheltens P, Feldman H. Treatment of Alzheimer's disease: current status and new perspectives. Lancet Neurol 2003; 2:539–47.

[5] Hashimoto M, Kazui H, Matsumoto K, Nakano Y, Yasuda M, Mori E. Does donepezil treatment slow the progression of hippocampal atrophy in patients with Alzheimer's disease? Am J Psychiatry 2005; 162:676–82.

[6] Weed MR, Polino J, Signor L, Bookbinder M, Keavy D, Benitex Y, 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:e0187609.

[7] Deardorff WJ, Shobassy A, Grossberg GT. Safety and clinical effects of EVP-6124 in subjects with Alzheimer's disease currently or previously receiving an acetylcholinesterase inhibitor medication. Expert Rev Neurotherapeutics 2015;15:7–17.

[8] Prickaerts J, van Goethem NP, Chesworth R, Shapiro G, Boess FG, Methfessel C, 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; 62:1099–110.

[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] Effects Sideline Some Phase 3 Encenicline Trials, https://www.alzforum.org/news/research-news/rare-severe-side-effects-sideline-some-phase-3-encenicline-trials. Rare but Severe Side, 2015.

[14] Liem-Moolenaar M, de Boer P, Timmers M, Schoemaker RC, van Hasselt JC, Schmidt S, et al. Pharmacokinetic-pharmacodynamic relationships of central nervous system effects of scopolamine in healthy subjects. Br J Clin Pharmacol 2011;71:886–98.

[15] Snyder PJ, Bednar MM, Cromer JR, Maruff P. Reversal of scopolamine-induced deficits with a single dose of donepezil, an acetylcholinesterase inhibitor. Alzheimer's Demen: J Alzheimer's Assoc 2005;1:126–35.

[16] Barbier AJ, Hilhorst M, Van Vliet A, Snyder P, Palfreyman MG, Gawryl M, et al. Pharmacodynamics, pharmacokinetics, safety, and tolerability of encenicline, a selective alpha7 nicotinic receptor partial agonist, in single ascending-dose and bioavailability studies. Clin Ther 2015;37:311–24.

[17] Ebert U, Siepmann M, Oertel R, Wesnes KA, Kirch W. Pharmacokinetics and pharmacodynamics of scopolamine after subcutaneous administration. J Clin Pharmacol 1998;38:720–6.

[18] Thomas E, Snyder PJ, Pietrzak RH, Jackson CE, Bednar M, Maruff P. Specific impairments in visuospatial working and short-term memory following low-dose scopolamine challenge in healthy older adults. Neuropsychologia 2008;46:2476–84.

[19] Renner UD, Oertel R, Kirch W. Pharmacokinetics and pharmacodynamics in clinical use of scopolamine. Ther Drug Monit 2005; 27:655–65.

[20] Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood-brain barrier penetration using the NeuroCart, a CNS test battery. Drug Discov Today Tech 2016;20:27–34.

[21] Feinstein A, Brown R, Ron M. Effects of practice of serial tests of attention in healthy subjects. J Clin Exp Neuropsychol 1994; 16:436–47.

[22] Lim HK, Juh R, Pae CU, Lee BT, Yoo SS, Ryu SH, et al. Altered verbal working memory process in patients with Alzheimer's disease: an fMRI investigation. Neuropsychobiology 2008;57:181–7.

[23] Mattila MJ, Mattila-Evenden ME. Effects of alcohol and hypnosedative drugs on digit-symbol substitution: comparison of two different computerized tests. J Psychopharmacol (Oxford, England) 1997; 11:313–7.

[24] Reed T, Carmelli D, Swan GE, Breitner JC, Welsh KA, Jarvik GP, et al. Lower cognitive performance in normal older adult male twins carrying the apolipoprotein E epsilon 4 allele. Arch Neurol 1994; 51:1189–92.

[25] 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; 73:665–71.

[26] 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.

[27] 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 Perception Perform 2005;31:438–52.

[28] Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol 1984;18:69s–72.

[29] 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.

[30] Gijsman HJ, Van Gerven JM, Tieleman MC, Schoemaker RC, Pieters M, Ferrari MD, et al. Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. J Clin Psychopharmacol 1998;18:289–95.

[31] van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MSM, Kroon JM, Breimer DD, et al. Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. Psychopharmacology 1993;110:471–8.

[32] 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;50:172–80.

[33] van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JMA, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol (Oxford, England) 1999; 13:10–7.

[34] Wezenberg E, Sabbe BG, Hulstijn W, Ruigt GS, Verkes RJ. The role of sedation tests in identifying sedative drug effects in healthy volunteers and their power to dissociate sedative-related impairments from memory dysfunctions. J Psychopharmacol (Oxford, England) 2007; 21:579–87.

[35] Andrew JM. Delinquents and the Tapping Test. J Clin Psychol 1977; 33:786–91.

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

[37] Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971;10:181–91.

[38] Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. Neurology 1975; 25:1065–70.

[39] Bittencourt PR, Wade P, Smith AT, Richens A. Benzodiazepines impair smooth pursuit eye movements. Br J Clin Pharmacol 1983; 15:259–62.

[40] Wright BM. A simple mechanical ataxia-meter. J Physiol 1971; 218:27p–8.

[41] de Haas SL, Franson KL, Schmitt JA, Cohen AF, Fau JB, Dubruc C, et al. The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A alpha2,3 selective agonist, in comparison with lorazepam in healthy volunteers. J Psychopharmacol (Oxford, England) 2009;23:625–32.

[42] Liem-Moolenaar M, Zoethout RW, de Boer P, Schmidt M, de Kam ML, Cohen AF, 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. J Psychopharmacol (Oxford, England) 2010; 24:1671–9.

[43] Liem-Moolenaar M, Zoethout RW, de Boer P, Schmidt M, de Kam ML, Cohen AF, 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. J Psychopharmacol (Oxford, England) 2010;24:1681–7.

[44] Prohovnik I, Arnold SE, Smith G, Lucas LR. Physostigmine reversal of scopolamine-induced hypofrontality. J Cereb Blood flow Metab: Official J Int Soc Cereb Blood Flow Metab 1997; 17:220–8.

[45] Siegfried KR. Pharmacodynamic and early clinical studies with velnacrine. Acta Neurol Scand Supplementum 1993;149:26–8.

[46] Preda L, Alberoni M, Bressi S, Cattaneo C, Parini J, Canal N, et al. Effects of acute doses of oxiracetam in the scopolamine model of human amnesia. Psychopharmacology 1993;110:421–6.

[47] Jones RW, Wesnes KA, Kirby J. Effects of NMDA modulation in scopolamine dementia. Ann New York Acad Sci 1991;640:241–4.

[48] Cho W, Maruff P, Connell J, Gargano C, Calder N, Doran S, et al. Additive effects of a cholinesterase inhibitor and a histamine inverse agonist on scopolamine deficits in humans. Psychopharmacology 2011;218:513–24.

[49] Alvarez-Jimenez R, Groeneveld GJ, van Gerven JM, Goulooze SC, Baakman AC, Hay JL, et al. Model-based exposure-response analysis to quantify age related differences in the response to scopolamine in healthy subjects. Br J Clin Pharmacol 2016;82:1011–21.